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**Antibody fragments for the treatment of biofilm-related disorders**

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**A61K 39/00** (2006.01)      **A61K 39/104** (2006.01)  
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**(56) Related Art**

**WO 2011/123396 A1**

**WO 2015/048484 A2**

**WO 2014/201305 A1**

**US 2016/0194384 A1**

**LAURA A. NOVOTNY ET AL, EBIOMEDICINE, vol. 10, 1 August 2016 (2016-08-01),  
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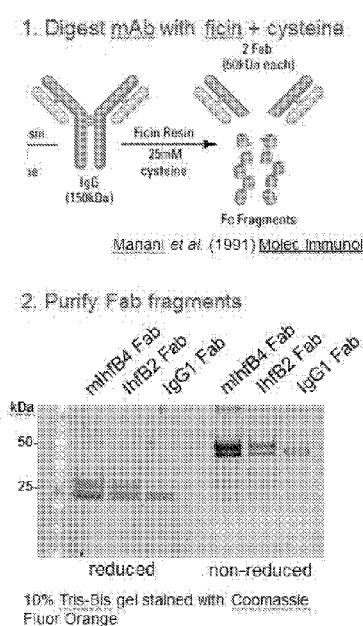
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## (54) Title: ANTIBODY FRAGMENTS FOR THE TREATMENT OF BIOFILM-RELATED DISORDERS

FIG. 1



**(57) Abstract:** This disclosure provides antibody fragments and isolated polypeptides comprising the antibody fragments that are useful as a therapeutic for those with an existing infection characterized by the formation of biofilms. Antibody fragments and isolated polypeptides comprising the antibody fragments can be administered to detect, treat, or prevent infection and/or remediate biofilms. Bacteria that cannot form functional biofilms are more readily cleared by the remainder of the host's immune system and/or traditional antibiotics.



**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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- *with international search report (Art. 21(3))*

## **ANTIBODY FRAGMENTS FOR THE TREATMENT OF BIOFILM-RELATED DISORDERS**

### **CROSS-REFERENCE TO RELATED APPLICATIONS**

**[0001]** This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 62/453,921, filed February 2, 2017, and 62/442,307, filed January 4, 2017, the contents of each of which are incorporated herein by reference in their entireties.

### **STATEMENT OF GOVERNMENT RIGHTS**

**[0002]** This invention was made with government support under Grant No. DC011818 awarded by the National Institutes of Health. The government has certain rights in the invention.

### **FIELD**

**[0003]** The present disclosure generally relates to methods and compositions to lessen and/or cure bacterial biofilms and treat diseases or disorders associated with biofilms using novel antibody fragments and compositions containing these fragments.

### **BACKGROUND**

**[0004]** At least one protein from the DNABII family of proteins is found in all known eubacteria and are naturally found outside of the bacterial cell. While the family elicits a strong innate immune response, host subjects fail to naturally produce specific protective antibody to family members as a result of infection. The DNABII protein and extracellular DNA (eDNA) contribute to the lattice structure of a “biofilm.” The major problem with bacterial biofilms is the inability of the host immune system and/or antibiotics and other antimicrobials to gain access to the bacteria protected within the biofilm.

**[0005]** Biofilms are present in an industrial setting as well. For example, biofilms are implicated in a wide range of petroleum process problems, from the production field to the gas station storage tank. In the field, sulfate reducing biofilm bacteria produce hydrogen sulfide (soured oil). In the process pipelines, biofilm activity develops slimes which impede filters and orifices. Biofilm and biofilm organisms also cause corrosion of pipeline and petroleum process equipment. These problems can be manifested throughout an oil or gas production facility to the point where fouling and corrosive biofilm organisms have even been found on the surfaces of final product storage tanks.

**[0006]** In the home, biofilms are found in or on any surface that supports microbial growth, e.g., in drains, on food preparation surfaces, in toilets, and in swimming pools and spas. Biofilms are implicated in a wide range of water processes, both domestic and industrial. They can grow on the surface of process equipment and impede the performance of the equipment, such as degradation of heat transfer or plugging of filters and membranes. Biofilms growing on a cooling tower fill can add enough weight to cause collapse of the fill. Biofilms cause corrosion of even highly specialized stainless steels. Biofilms in a water process can degrade the value of a final product such as biofilm contamination in a paper process or the attachment of even a single cell on a silicon chip. Biofilms growing in drinking water distribution systems can harbor potential pathogenic organisms, corrosive organisms or bacteria that degrade the aesthetic quality of the water.

#### PARTIAL SEQUENCE LISTING

SEQ ID NO. 1: Full Length Wild type (wt) 86-028NP *Haemophilus influenzae* IhfA; Genbank Accession No.: AAX88425.1, last accessed Mar. 21, 2011:

MATITKLDIIEYLSDKYHLSKQDTKNVVENFLEEIRLSLESGQDVKLSGFGNFELRDK  
SSRPGRNPKTGDPVPVSARRVVTKPGQKLRARVEKIK.

SEQ ID NO. 2: Full Length Wild type (wt) 86-028NP *Haemophilus influenzae* IhfB; Genbank Accession No.: AAX88699.1, last accessed May 13, 2015:

MTKSELMEKLSAKQPTLSAKEIENMVKDILEFISQSLENGDRVEVRGFGSFLHHRQP  
RLGRNPKTGDSVNLSAKSVPYFKAGKELKARV DVQA.

SEQ ID NO. 3: Full Length wt 86-028NP *Haemophilus influenzae* HU, Genbank Accession No.: YP\_248142.1, last accessed Mar. 21, 2011:

MRFVTIFINHAFNSSQVRLSFAQFLRQIRKDTFKESNFLFNRRYKFMNKTDLIDAIAAN  
AAELNKKQAKAALEATLDAITASLKEGEPVQLIGFTFKVNERAARTGRNPQTGAEI  
QIAASKVPAFVSGKALKDAIK.

SEQ ID NO. 4: Full Length wt R2846 *Haemophilus influenzae* IhfA, Genbank Accession No.: ADO96375, last accessed Mar. 21, 2011:

MATITKLDIIEYLSDKYHLSKQDTKNVVENFLEEIRLSLESGQDVKLSGFGNFELRDK  
SSRPGRNPKTGDPVPVSARRVVTKPGQKLRARVEKTK.

SEQ ID NO. 5: Full Length wt Rd *Haemophilus influenzae* IhfA; Genbank Accession No.: AAC22959.1, last accessed Mar. 21, 2011:

MATITKLDIIEYLSDKYHLSKQDTKNVENFLEEIRLSLESGQDVKLSGFGNFELRDK  
SSRPGRNPKTGDVVPVSARRVVTFKPGQKLRARVEKTK.

SEQ ID NO. 6: Full Length wt *E. coli* K12 IhfA; Genbank Accession No.: AAC74782.1, last accessed Mar. 21, 2011:

MALTKAEMSEYLFDKLGLSKRDAKELVELFFEEIRRALENGEQVKLSGFGNFDLRDK  
NQRPGRNPKTGEDIPITARRVVTFRPGQKLKSRVENASPKDE; DNA Genbank No. NC\_000913.

SEQ ID NO. 7: Full Length wt *E. coli* K12 IhfB; Genbank Accession No.: BAA35656, last accessed May 19, 2015:

MTKSELIERLATQQSHIPAKTVEDAVKEMLEHMASTLAQGERIEIRGFGSFLHYRAP  
RTGRNPKTGDKVELEGKYVPHFKPGKELRDRANIYG.

SEQ ID NO. 8: *E. coli* hupA, Genbank Accession No.: AP\_003818, Last accessed Mar. 21, 2011:

MNKTQLIDVIAEKAELSKTQAKAALESTLAAITESLKEGDAVQLVGFGTFKVNHRAE  
RTGRNPQTGKEIKIAAAANVPAFVSGKALKDAVK.

SEQ ID NO. 9: *E. coli* hupB, Genbank Accession No.: AP\_001090.1, Last accessed Mar. 21, 2011:

MNKSQOLIDKIAAGADISKAAGRALDAIIASVTESLKEGDDVALVGFGTFAVKERA  
RTGRNPQTGKEIAAKVPSFRAGKALKDAVN.

SEQ ID NO. 10: Full Length wt *P. aeruginosa* PA 01 IhfA; Genbank Accession No.: AAG06126.1, last accessed Mar. 21, 2011:

MGALTKAEIARLYEELGLNKREAKELVELFFEEIRQALEHNEQVKLSGFGNFDLRD  
KRQRPGRNPKTGEEIPITARRVVTFRPGQKLKARVEAYAGTKS.

SEQ ID NO. 11: Full Length wt *P. aeruginosa* PA 01 IhfB; Genbank Accession No.: AAF72950.1, last accessed May 19, 2015:

MTKSELIERIVTHQQLSAKDVELAIKTMLEQMSQALATGDRIEIRGFGSFLHYRAP  
RVGRNPKTGESVRLDGKFVPHFKPGKELRDRVNEPE.

SEQ ID NO. 12: *Haemophilus influenzae* IhfA, A-3 fragment:  
FLEEIRLSLESGQDVKLSGF.

SEQ ID NO. 13: *Haemophilus influenzae* IhfA, A5 fragment:  
RPGRNPKTGDVVPVSARRVV.

SEQ ID NO. 14: *Haemophilus influenzae* HU, A5 fragment: RTGRNPQTGAEIQIAASKVP.

SEQ ID NO. 15: *Haemophilus influenzae* IhfB, B2 fragment: TLSAKEIENMVKDILEFISQ.

SEQ ID NO. 16: *Haemophilus influenzae* IhfB, B4 fragment:

RGFGSFSLHHRQPRRLGRNPK.

SEQ ID NO. 17: *Haemophilus influenzae* IhfB, modified B4 (mB4) fragment:

FSLHHRQPRRLGRNPKTGDSV.

SEQ ID NO. 18: *Haemophilus influenzae* IhfA, A-1 fragment:

MATITKLDIIEYLSDKYHLS.

SEQ ID NO. 19: *Haemophilus influenzae* IhfA, A2 fragment:

KYHLSKQDTKNVVENFLEEI.

SEQ ID NO. 20: *Haemophilus influenzae* IhfA, A4 fragment:

KLSGFGNFELRDKSSRPGRN.

SEQ ID NO. 21: *Haemophilus influenzae* IhfA, A6 fragment:

ARRVVTFKPGQKLRARVEKTK.

SEQ ID NO. 22: *Haemophilus influenzae* IhfB, B1 fragment:

MTKSELMEKLSAKQPTLSAK.

SEQ ID NO. 23: *Haemophilus influenzae* IhfB, B3 fragment:

EFISQSLENGDRVEVRGFGS.

SEQ ID NO. 24: *Haemophilus influenzae* IhfB, B5 fragment:

GRNPKTGDSVNLSAKSVPYF.

SEQ ID NO. 25: *Haemophilus influenzae* IhfB, B6 fragment:

SVPYFKAGKELKARVDVQA.

SEQ ID NO. 26: *Haemophilus influenzae* IhfA, A tip fragment:

NFELRDKSSRPGRNPKTGDVV.

SEQ ID NO. 27: *Haemophilus influenzae* IhfB, B tip fragment:

SLHHRQPRRLGRNPKTGDSVNL.

SEQ ID NO. 28 *Haemophilus influenzae* HU, fragment:

MNKTDLIDAIAANAAELNKKQAK.

SEQ ID NO. 29 *Haemophilus influenzae* HU, fragment: KKQAKAALEATLDAITASLKEG.

SEQ ID NO. 30 *Haemophilus influenzae* HU, fragment: SLKEGEPVQLIGFTFKVNERA.

SEQ ID NO. 31 *Haemophilus influenzae* HU, fragment: VNERAARTGRNPQTGAEIQIAA.

SEQ ID NO. 32 *Haemophilus influenzae* HU, fragment: IQIAASKVPAFVSGKALKDAIK.

SEQ ID NO. 33: *Haemophilus influenzae* HU, A3 fragment:

KKQAKAALEATLDAITASLKEG.

SEQ ID NO. 34: Human IgD constant region, Uniprot: P01880:

APTKAPDVFPPISGCRHPKDNSPVVLACLITGYHPTSVTVWYMGTSQSPQRTFPEIQ  
 RRDSYYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVPTA  
 QPQAEGSLAKATTAPATTRNTGRGGEEKKEKEKEEQQEERETKTPECPHTQPLGVY  
 LLTPAVQDLWLRDKATFTCFVVGSVLKDAHLTWEVAGKVPTGGVEEGLLERHSNG  
 SQSQHSRLTLPRSLWNAGTSVTCTLNHPSSLPPQRLMALREPAAQAPVKLSLNLLASS  
 DPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVL  
 RVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDHGPMK.

SEQ ID NO. 35: Human IgG1 constant region, Uniprot: P01857:

ASTKGPSVFLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHCPCPAP  
 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE  
 PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDG  
 SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

SEQ ID NO. 36: Human IgG2 constant region, Uniprot: P01859:

ASTKGPSVFLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
 SSGLYSLSSVVTVPSSNFGTQTYTCNVVDHKPSNTKVDKTVERKCCVECPCPAPPVA  
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT  
 KPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKKGQPREPQVY  
 TLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTPVLDSDGSFFL  
 YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

SEQ ID NO. 37: Human IgG3 constant region, Uniprot: P01860:

ASTKGPSVFLAPCSRSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
 QSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKVELKPLGDTTHCPRC  
 PEPKSCDTPPPCCPRCPEPKSCDTPPPCCPRCPEPKSCDTPPPCCPRCPAPEELLGGPSVFLFPP  
 KPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKT  
 KPREEQYNSTFR

VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKGQPREPVYTLPPSREEM  
TKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLSDGSFFLYSKLTVDKS  
RWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK.

SEQ ID NO. 38: Human IgM constant region, Uniprot: P01871:

GSASAPTLFPLVSCENSPSDTSSAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPSV  
LRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVS  
FVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESG  
PTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPS  
FASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKHTHNISESHPNATFSAVGEAS  
ICEDDWNSGERFTCTVHTDLPPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESA  
TITCLVTGFSPADVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEE  
WNTGETYTCVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY.

SEQ ID NO. 39: Human IgG4 constant region, Uniprot: P01861:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTKYTCNCVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLG  
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR  
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPVY  
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFL  
YSRLTVDKSRWQEGNVFSCSVMHEALHNYTQKSLSLSLGK.

SEQ ID NO. 40: Human IgA1 constant region, Uniprot: P01876:

ASPTSPKVFPPLSLCSTQPDGNVVIACLVQGFPQEPLSVTWSESGQGVTARNFPPSQD  
ASGDLYTTSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCTPVPSTPPTPSPSTPP  
TPSPSCCHPRLSLHRALEDELLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGP  
PERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKPLTATLSKGNTFRPEVH  
LLPPPSEELALNELVLTCLARGFSPKDVLVRWLQGSQELPREKYLTwASRQEPSQG  
TTTFAVTSILRVAEAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVV  
MAEVDGTCY.

SEQ ID NO. 41: Human IgA2 constant region, Uniprot: P01877:

ASPTSPKVFPPLSLDSTPQDGNNVVACLVQGFPQEPLSVTWSESGQNVTARNFPPSQD  
ASGDLYTTSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPCTPVPFFFFCCHPRLSL  
HRALEDELLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGP PERDLCGCYSVS  
SVLPGCAQPWNHGETFTCTAAHPELKPLTANITKSGNTFRPEVHLLPPPSEELALNE

LVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTwASRQEPSQGTTFAVTSILRVA  
AEDWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDGTCY.

SEQ ID NO. 42: Human Ig kappa constant region, Uniprot: P01834:

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
QDSKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

SEQ ID NO. 43: Non-limiting exemplary linker: GPSLKL.

SEQ ID NO. 44: Non-limiting exemplary linker: GGG.

SEQ ID NO. 45: Non-limiting exemplary linker: GPSL.

SEQ ID NO. 46: Non-limiting exemplary linker: GPS.

SEQ ID NO. 47: Non-limiting exemplary linker: PSLK.

SEQ ID NO. 48: Non-limiting exemplary linker: GPSLK.

SEQ ID NO. 49: Non-limiting exemplary linker: SLKL.

SEQ ID NO. 50: Non-limiting exemplary heavy chain variable region nucleotide sequence,  
IhfA5 fragment:

GAGGTGCAGCTGCAGGAGTCTGGACCTGGCCTGGTGACGCCCTCACAGAGCCTG  
TCCATGACTTGCACTGTCTCTGGGTTTCATTAACCAGCTATAGTGTACACTGGGT  
TCGCCAGCCTCCAGGAAAGAGTCTGGAGTGGCTGGGAGTAATATGGGCTGGTGG  
AAGCACAAATTATAATTGGCTCTCATGTCCAGACTGAGCATCAGCAAAGACAA  
CTCCAAGAGCCAAGTTTCTAAAAATGGACAGTCTGCAAACGTGATGACACAGCC  
ATATACTACTGTGCCAGAGAGGACTCCTGGGTCAAGGAACCTCAGTCACCGTCT  
CCTCA.

SEQ ID NO. 51: Non-limiting exemplary heavy chain variable region amino acid sequence,  
IhfA5 fragment:

EVQLQESGPLVTPSQSLSMTCVSGFSLTSYSVHWVRQPPGKSLEWLGVIWAGGST  
NYNSALMSRLSISKDNSKSQVFLKMDSLQTDDTAIYYCAREDSWGQGTSVTVSS.

SEQ ID NO. 52: Non-limiting exemplary heavy chain variable region nucleotide sequence,  
IhfmB4 fragment:

GAGGTGCAGCTGCAGGAGTCTGGGGCAGAGCTTGTGAGGTCAAGGGCCTCAGTC  
AAGTTGTCCTGCACAGCTTCTGGCTTCAACATTAAAGACTACTATATGCACTGGG  
TGAAGCAGAGGCCTGAACAGGGCCTGGAGTGGATTGGATTGATCCTGAAA

ATGATGATACTGAATATGTCCCGAAGTTCCAGGGCAAGGCCAGTATGACTGCAG  
ACACATCCTCCAACACACAGCCTACCTGCAGCTCAGCAGCCTGACATCTGAGGACA  
CTGCCGTCTATTACTGTACAGAGCTCGGAGCTTACTGGGCCAGGGACTCTGGT  
C.

SEQ ID NO. 53: Non-limiting exemplary heavy chain variable region amino acid sequence, IhfmB4 fragment:

EVQLQESGAELVRSGASVQLSCTASGFNIKDYMMHWVKQRPEQGLEWIGWIDPEND  
DTEYVPKFQGKASMTADTSSNTAYLQLSSLTSEDTAVYYCTELGAYWGQQGTLV.

SEQ ID NO. 54: Non-limiting exemplary light chain variable region nucleotide sequence, IhfA5 fragment:

GACATTGTGATGACCCAGTCTAAAAATTATGTCCACATCAGTAGGAGACAGG  
GTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGGTACTAATGTAGCCTGGTATC  
AACAGAAACCAGGGCAATCTCCTAAAGCACTGATTACTCGGCATCCTACCGGTA  
CAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGACAGATTCACTCTC  
ACCATCAGCAATGTGCAGTCTGAAGACTTGGCAGAGTATTCTGTCAGCAATATA  
ACAGCTATCCCACGTTGGAGGGGGACCAAGTTGGAAATAAAA.

SEQ ID NO. 55: Non-limiting exemplary light chain variable region amino acid sequence, IhfA5 fragment:

DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRYS  
GVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYNSYPTFGGGTKLEIK.

SEQ ID NO. 56: Non-limiting exemplary light chain variable region nucleotide sequence, IhfmB4 fragment:

GATGTTGTGATGACCCAGATTCCACTCACTTGTGGTTACCATTGGACAACAG  
CCTCCATCTCTTGCAAGTCAGTCAGAGCCTCTAGATAGTAATGGAAAGACATA  
TTGAATTGGTTTTCAAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTG  
GTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGA  
CAGATTCACACTGAAAATCAGCAGAGTTGAGGCTGAGGATTGGAAATTATTA  
TTGCTGGCAAAGTACACATTTCCTCACACGTTGGAGGGGGACCAAGTTGGAA  
ATCAAA.

SEQ ID NO. 57: Non-limiting exemplary light chain variable region amino acid sequence, IhfmB4 fragment:

DVVMTQIPLTLSVTIGQPASISCKSSQSLLDSNGKTYLNWLFQRPGQSPKRЛИYLVSKL  
DSGVPDRFTGSGSGTDFTLKISRVEAEDLGIYYCWQSTHFPHTFGGGTKLEIK.

SEQ ID NO. 58: Partial non-limiting exemplary CDRH1 sequence, IhfA5 fragment: FSLTSYS.

SEQ ID NO. 59: Partial non-limiting exemplary CDRH1 sequence, IhfA5 fragment: FSLTSYSV.

SEQ ID NO. 60: Partial non-limiting exemplary CDRH1 sequence, IhfA5 fragment: FSLTSYSVH.

SEQ ID NO. 61: Partial non-limiting exemplary CDRH1 sequence, IhfA5 fragment: GFSLTSYS.

SEQ ID NO. 62: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: IWAGGST.

SEQ ID NO. 63: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: VIWAGGST.

SEQ ID NO. 64: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: GVIWAGGST.

SEQ ID NO. 65: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: LGVIWAGGST.

SEQ ID NO. 66: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: WLGVIWAGGST.

SEQ ID NO. 67: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: IWAGGSTN.

SEQ ID NO. 68: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: VIWAGGSTN.

SEQ ID NO. 69: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: GVIWAGGSTN.

SEQ ID NO. 70: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: LGVIWAGGSTN.

SEQ ID NO. 71: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: WLGVIWAGGSTN.

SEQ ID NO. 72: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: IWAGGSTNY.

SEQ ID NO. 73: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: VIWAGGSTNY.

SEQ ID NO. 74: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: GVIWAGGSTNY.

SEQ ID NO. 75: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: LGVIWAGGSTNY.

SEQ ID NO. 76: Partial non-limiting exemplary CDRH2 sequence, IhfA5 fragment: WLGVIWAGGSTNY.

SEQ ID NO. 77: Partial non-limiting exemplary CDRH3 sequence, IhfA5 fragment: REDS.

SEQ ID NO. 78: Partial non-limiting exemplary CDRH3 sequence, IhfA5 fragment: AREDS.

SEQ ID NO. 79: Partial non-limiting exemplary CDRL1 sequence, IhfA5 fragment: QNVGTN.

SEQ ID NO. 80: Partial non-limiting exemplary CDRL1 sequence, IhfA5 fragment: QNVGTNV.

SEQ ID NO. 81: Partial non-limiting exemplary CDRL1 sequence, IhfA5 fragment: QNVGTNVA.

SEQ ID NO. 82: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: SAS.

SEQ ID NO. 83: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: YSAS.

SEQ ID NO. 84: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: IYSAS

SEQ ID NO. 85: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: LIYSAS.

SEQ ID NO. 86: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: ALIYSAS.

SEQ ID NO. 87: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: SASY.

SEQ ID NO. 88: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: YSASY.

SEQ ID NO. 89: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: IYSASY.

SEQ ID NO. 90: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: LIYSASY.

SEQ ID NO. 91: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: ALIYSASY.

SEQ ID NO. 92: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: SASYR.

SEQ ID NO. 93: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: YSASYR.

SEQ ID NO. 94: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: IYSASYR.

SEQ ID NO. 95: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: LIYSASYR.

SEQ ID NO. 96: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: ALIYSASYR.

SEQ ID NO. 97: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: SASYRY.

SEQ ID NO. 98: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: YSASYRY.

SEQ ID NO. 99: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: IYSASYRY.

SEQ ID NO. 100: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: LIYSASYRY.

SEQ ID NO. 101: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: ALIYSASYRY.

SEQ ID NO. 102: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: SASYRYS.

SEQ ID NO. 103: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: YSASYRYS.

SEQ ID NO. 104: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: IYSASYRYS.

SEQ ID NO. 105: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: LIYSASYRYS.

SEQ ID NO. 106: Partial non-limiting exemplary CDRL2 sequence, IhfA5 fragment: ALIYSASYRYS.

SEQ ID NO. 107: Non-limiting exemplary linker: GGSGGS.

SEQ ID NO. 108: Partial non-limiting exemplary CDRL3 sequence, IhfA5 fragment: QQYNSYP.

SEQ ID NO. 109: Partial non-limiting exemplary CDRL3 sequence, IhfA5 fragment: QQYNSYPT.

SEQ ID NO. 110: Partial non-limiting exemplary CDRH1 sequence, IhfmB4 fragment: FNIKDYY.

SEQ ID NO. 111: Partial non-limiting exemplary CDRH1 sequence, IhfmB4 fragment: FNIKDYYM.

SEQ ID NO. 112: Partial non-limiting exemplary CDRH1 sequence, IhfmB4 fragment: FNIKDYYMH.

SEQ ID NO. 113: Partial non-limiting exemplary CDRH1 sequence, IhfmB4 fragment: GFNIKDYY.

SEQ ID NO. 114: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IDPENDDT.

SEQ ID NO. 115: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIDPENDDT.

SEQ ID NO. 116: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: GWIDPENDDT.

SEQ ID NO. 117: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IGWIDPENDDT.

SEQ ID NO. 118: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIGWIDPENDDT.

SEQ ID NO. 119: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IDPENDDTE.

SEQ ID NO. 120: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIDPENDDTE.

SEQ ID NO. 121: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: GWIDPENDDTE.

SEQ ID NO. 122: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IGWIDPENDDTE.

SEQ ID NO. 123: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIGWIDPENDDTE.

SEQ ID NO. 124: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IDPENDDTEY.

SEQ ID NO. 125: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIDPENDDTEY.

SEQ ID NO. 126: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: GWIDPENDDTEY.

SEQ ID NO. 127: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: IGWIDPENDDTEY.

SEQ ID NO. 128: Partial non-limiting exemplary CDRH2 sequence, IhfmB4 fragment: WIGWIDPENDDTEY.

SEQ ID NO. 129: Partial non-limiting exemplary CDRH3 sequence, IhfmB4 fragment: TELGAY.

SEQ ID NO. 130: Partial non-limiting exemplary CDRL1 sequence, IhfmB4 fragment: QSLLDSNGKTY.

SEQ ID NO. 131: Partial non-limiting exemplary CDRL1 sequence, IhfmB4 fragment: QSLLDSNGKTYL.

SEQ ID NO. 132: Partial non-limiting exemplary CDRL1 sequence, IhfmB4 fragment: QSLLDSNGKTYLN.

SEQ ID NO. 133: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LVS.

SEQ ID NO. 134: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: YLVS.

SEQ ID NO. 135: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: IYLV.

SEQ ID NO. 136: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LIYLVS.

SEQ ID NO. 137: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: RLIYLVS.

SEQ ID NO. 138: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LVSK.

SEQ ID NO. 139: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: YLVSK.

SEQ ID NO. 140: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: IYLVSK.

SEQ ID NO. 141: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LIYLVSK.

SEQ ID NO. 142: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: RLIYLVSK.

SEQ ID NO. 143: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LVSKL.

SEQ ID NO. 144: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: YLVSKL.

SEQ ID NO. 145: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: IYLVSKL.

SEQ ID NO. 146: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LIYLVSKL.

SEQ ID NO. 147: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: RLIYLVSKL.

SEQ ID NO. 148: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LVSKLD.

SEQ ID NO. 149: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: YLVSKLD.

SEQ ID NO. 150: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: IYLVSKLD.

SEQ ID NO. 151: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LIYLVSKLD.

SEQ ID NO. 152: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: RLIYLVSKLD.

SEQ ID NO. 153: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LVSKLDS

SEQ ID NO. 154: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: YLVSKLDS.

SEQ ID NO. 155: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: IYLVSKLDS.

SEQ ID NO. 156: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: LIYLVSKLDS.

SEQ ID NO. 157: Partial non-limiting exemplary CDRL2 sequence, IhfmB4 fragment: RLIYLVSKLDS.

SEQ ID NO. 158: Partial non-limiting exemplary CDRL3 sequence, IhfmB4 fragment: WQSTHFPH.

SEQ ID NO. 159: Partial non-limiting exemplary CDRL3 sequence, IhfmB4 fragment: WQSTHFPHT.

## DESCRIPTION OF TABLES

**[0007]** Table 1 is a listing of hybridomas producing non-limiting exemplary antibodies.

**[0008]** Table 2 is a non-limiting list of exemplary antibodies and their corresponding CDRs.

**[0009]** Table 3 is a non-limiting list of exemplary antibodies and their corresponding heavy chain and light chain variable regions.

**[0010]** Table 4 is a summary of the scoring methodology used in the otitis media model of Example 3 to generate a mucosal biomass score.

**[0011]** Table 5 is a summary of the efficacies of IgG Fab fragments versus intact rabbit polyclonal and mouse monoclonal IgG.

## SUMMARY OF THE DISCLOSURE

**[0012]** Within bacterial cells, the DNABII proteins are DNA binding proteins that necessarily bend DNA substrates upon binding. Similarly, DNA that is already in a bent conformation is an exemplary substrate as the energy required for bending is rendered unnecessary.

**[0013]** The DNABII family is a member of a class of proteins referred to as nucleoid associated proteins (NAPs), bacterial proteins that, in part, shape the intracellular bacterial nucleoid (Browning et al. (2010) Curr. Opin. Microbiol. 13:773-780). In addition, this family is ubiquitous, expressed by virtually all eubacteria. All characterized family members to date function as either a homodimer or heterodimer of subunits. The family is divided into two types, HU (histone-like protein) and IHF (integration host factor). The primary distinction between these family members is that HU binds DNA in a sequence independent manner, while IHF binds a consensus sequence (WATCAANNNTTR where W is A or T and R is a purine and N is any base (SEQ ID NO. 160)) conserved across genera (Swinger et al. (2004)

Curr. Opin. Struct. Biol. 14:28-35). All DNABII proteins bind to and bend DNA considerably, e.g., *E. coli* IHF can bend DNA into a virtual U-turn (Rice et al. (1996) Cell 87:1295-1306). In addition, all family members have a preference for pre-bent or curved DNA structures, e.g., Holliday junctions, a cruciform-like structure central to DNA recombination. In fact, DNABII proteins function as accessory factors facilitating all intracellular DNA functions, including gene expression, recombination, repair and replication (Swinger et al. (2004) Curr. Opin. Struct. Biol. 14:28-35).

**[0014]** In one aspect of this disclosure, Applicants provide an antibody or antigen binding fragments of antibodies that are specific to DNABII polypeptides. The antibody or antigen binding fragments, upon administration to the host, will reduce or eradicate an existing or pre-formed biofilm. Thus, in one aspect, this disclosure relate to one or more antibody or antigen bindng fragments that specifically bind to a DNABII polypeptide and their use in dissolving, remediating, or disrupting a biofilm *in vitro* or *in vivo*.

**[0015]** In another aspect, the antibody or antigen binding fragment is described as an isolated polypeptide that comprises, or alternatively consists essentially of, or yet further consists of a Fab fragment of an antibody that specifically recognizes and binds a conformational tip region of a DNABII binding polypeptide produced by any appropriate organism, e.g., an *Haemophilus influenzae* IHFalpha, IHF beta or an HU polypeptide. Non-limiting examples of such are selected from the group of the conformational tip regions designed an A5 or mB4 peptide fragment, or an equivalent of each thereof. In one aspect, the fragment is derived from an mIhfB4<sub>NTHI</sub> antibody or an equivalent of the antibody. In one aspect, the fragment is of derived from an IHF A5 antibody or an equivalent of the antibody.

**[0016]** In certain aspects, the disclosure relates to an isolated polypeptide comprising, or alternatively consisting essentially of a Fab fragment of an mIhfB4<sub>NTHI</sub> antibody or A5 antibody or an equivalent of each of these polypeptides. In other aspects, the disclosure relates to an isolated polypeptide consisting essentially of a Fab fragment of an IHF fragment B4, a mIhfB4 or A5 antibody or an equivalent of each of the polypeptide. In some embodiments, the antibody fragment or antigen binding fragment is derived from polyclonal sera. In other aspects, the antibody fragment or antigen binding fragment is not derived from a polyclonal antibody. In some embodiments, the antibody fragment or antigen binding fragment is derived from a monoclonal antibody. Table 1 lists non-limiting exemplary monoclonal antibodies that can derive the fragments and polypeptides containing the fragments of this disclosure and the hybridomas that produce them. For example, the

hybridoma cell lines that produce monoclonal antibodies that specifically recognize and bind *Haemophilus influenzae* IhfA fragment A5 (SEQ ID NO. 13) or IhfB fragment B4 (SEQ ID NO. 16), and IhfB fragment mB4 (SEQ ID NO. 17) were deposited with American Type Culture Collection (ATCC) under the listed Accession Numbers in Table 1 and pursuant to the provisions of the Budapest Treaty on July 30, 2015. Further non-limiting exemplary antibodies include those that specifically recognize and bind *Haemophilus influenzae* IhfA fragment A3 (SEQ ID NO. 12) or IhfB fragment B2 (SEQ ID NO. 15) produced by hybridoma cell lines IhfA3 NTHI 9B10.F2.H3, IhfB2 NTHI 7A4.E4.G4, and IhfB2 NTHI 7A4.E4.G11 (deposited with American Type Culture Collection (ATCC) under the Accession Numbers listed in Table 1 and pursuant to the provisions of the Budapest Treaty on August 1, 2016); and antibodies that specifically recognize or bind a polypeptide comprising a polypeptide selected from the group of: SEQ ID NO. 31, SEQ ID NO. 33, a DNA BII polypeptide comprising the amino acid sequence NPXT (wherein X is any amino acid or alternatively X is selected from the amino acids Q, R, K, S, or T), or an equivalent of each thereof.

**[0017]** The antibodies and fragments derived from the antibodies of this disclosure can be from a mammalian polyclonal antibody. In another aspect, the mammalian polyclonal antibody is selected from the group of a rabbit polyclonal antibody, a murine polyclonal antibody, a sheep polyclonal antibody, an equine polyclonal antibody, a canine polyclonal antibody, or a human polyclonal antibody. In another aspect, the antibody is monoclonal antibody. In another aspect, the antibody is a monoclonal antibody, a humanized antibody or a human antibody. In another aspect, mammalian monoclonal antibody is selected from the group of a rabbit monoclonal antibody, a murine monoclonal antibody, a sheep monoclonal antibody, an equine monoclonal antibody, or a canine monoclonal antibody. In another aspect, the antibody is a monoclonal antibody produced by a hybridoma listed in Table 1. The antibodies can be of any isotype, e.g., IgM or IgG.

**[0018]** In another aspect, the isolated antibody fragments further comprising a detectable and/or a purification label.

**[0019]** In a further aspect, also provided is a composition comprising, consisting essentially of, or yet further consisting of, the polypeptide as described herein and a carrier, non-limiting examples of such include a preservative or a stabilizer. In one aspect, the composition is processed for ease of transport, e.g., lyophilization.

**[0020]** In another aspect, also provided is an isolated polynucleotide encoding the antibody fragment or a polypeptide as described herein. In another aspect, the isolated polynucleotide further comprises a detectable and/ or a purificiaton label. In another aspect, the detectable label is not a conjugated naturally occurring flourescnet polynucleotide. In another aspect, the isolated polynucleotide is operably linked to one or more regulatory elements for replication and/or expression, that optionally is contained within an expression vector or a host cell.

**[0021]** In another aspect, also provided is a composition comprising, consisting essentially of, or yet further consisting of, the isolated polynucleotide as described herein and a carrier. In another aspect, the carrier comprises a preservative or stabilizer.

**[0022]** In another aspect, also provided is a vector and/or host cell comprising the isolated polynucleotide, that optionally comprises an expression or replication vecor as described herein. In one aspect, the host cell is a prokaryotic cell or a eukaryotic cell, non-limiting examples of such are disclosed herein.

**[0023]** In another aspect, provided is a method for producing a polypeptide as described herein comprising, or alternatively consisting essentiaall of, or yet further consisting of, culturing the isolated host cell disclosed herein under conditions that allow for the expression of polynucleotide to a polypeptide. In another aspect, the method further comprises isolating the polypeptide expressed from the polypeptide from the host cell or cell culture medium. The host cell can be a eukaryotic or a prokaryotic cell.

**[0024]** In another aspect, provided is a method to prevent formation of or to disrupt a biofilm associated with an industrial process comprising treating a surface susceptible to or containing a biofilm with the antibody fragment, antigen binding fragment, or polypeptide as described herein.

**[0025]** In another aspect, provided is a method to identify a binding moiety, e.g., an antibody, antigen binding fragment, small molecule or an other agent, that disrupts a biofilm, comprising contacting a candidate binding moiety with a biofilm component and with the at least one polypeptide as described herein and assaying for the binding of the binding moiety to the DNABII in the biofilm as compared to the binding of the polypeptide as described herein to the DNABII in the biofilm, wherein a binding moiety with substantially the same or greater binding as compared to the binding of the polypeptide is a binding moiety that disrupts a biofilm.

**[0026]** In another aspect, provided is a method to identify an agent, e.g., an antibody, an antigen binding agent, a small molecule, a polynucleotide or other therapeutic or diagnostic agent, that reverses drug resistance in multiple species of bacteria comprising evaluating an agent for activity in disrupting biofilms produced by multiple species, wherein an agent which disrupts the biofilms with substantial equivalent activity as an antibody fragment, antigen binding fragment or a polypeptide described herein (and optionally wherein an agent that binds a multiplicity of said proteins) is identified as an agent that reverses drug resistance. In one aspect, the agent will be effective against biofilms derived from at least two bacterial species, including gram positive and gram negative species, non-limiting examples of such include *S. aureus*, *P. aeruginosa*, and *B. cenocepacia*.

**[0027]** In another aspect, provided is a method to treat a condition in a subject characterized by the formation of a biofilm and/or to detect the formation of a biofilm in the subject, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject in need thereof or treating the subject in need thereof with the antibody fragment, antigen binding fragment or the polypeptide as described herein. The amount to be administered must be an effective amount to disrupt or detect the biofilm. In one aspect, when the biofilm is to be detected, the method further comprises imaging and/or monitoring biofilm formation and/or disruption in the subject before, during and after administration of the composition containing the antibody fragment, antigen binding fragment or polypeptide as described herein. In one aspect, when the biofilm is to be detected, the antibody fragment, antigen binding fragment or polypeptide is chemically modified. In one aspect, when the biofilm is to be detected, the antibody fragment, antigen binding fragment or polypeptide is conjugated to a diagnostic agent or detectable label or agent. In one aspect, when the biofilm is to be detected, the method further comprises observing complexation of the antibody fragment, antigen binding fragment or polypeptide as described herein with any biofilm present. In one aspect, the condition to be treated is selected from the group of: chronic non-healing wounds, including venous ulcers and diabetic foot ulcers, ear infections, sinus infections, urinary tract infections, pulmonary infections, cystic fibrosis, chronic obstructive pulmonary disease, catheter-associated infections, infections associated with implanted prostheses, or periodontal disease.

**[0028]** In another aspect, provided is a method to monitor the development or progression of a condition in a subject characterized by the formation of a biofilm comprising treating or administering to the subject the antibody fragment, antigen binding fragment or polypeptide

as described herein. In one aspect, the method further comprises observing complexation of the antibody fragment, antigen binding fragment or polypeptide as described herein with any biofilm present. In one aspect, the polypeptide is chemically modified. In one aspect, the polypeptide is conjugated to a diagnostic agent or detectable label or agent.

**[0029]** In another aspect, provided is a method to monitor the treatment of a condition in a subject characterized by the formation of a biofilm comprising, or alternatively consisting essentially of, or yet further consisting of, administering the subject the antibody fragment, antigen binding fragment or polypeptide as described herein. In one aspect, the method further comprises imaging and/or monitoring biofilm formation and/or disruption in the subject. In one aspect, the polypeptide is chemically modified. In one aspect, the polypeptide is conjugated to a detectable label or a diagnostic agent.

**[0030]** In another aspect, provided is a method to prevent formation of or to disrupt a biofilm in a subject in need thereof, comprising, or alternatively consisting essentially of, or yet further consisting of administering to the subject an effective amount of antibody fragment, antigen binding fragment or polypeptide as described herein.

**[0031]** In another aspect, provided is a non-physiological surface coated with the antibody fragment, antigen binding fragment or polypeptide of as described herein; and optionally, wherein the surface is in an industrial setting such as in a pipe

**[0032]** In one aspect, provided herein is one or method(s) such as: to treat a subject infected with a organism that produces a biofilm, to disrupt a biofilm in a subject, and/or to treat a subject suffering from a condition associated with the presence of a biofilm, comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of the antibody fragment, antigen binding fragment or polypeptide as described herein, and further wherein the antibody fragment, antigen binding fragment or polypeptide induces the production of anti-inflammatory cytokines in the subject. In another aspect, the anti-inflammatory cytokine comprises one or more of IL-4, IL-13, or IL-10. In another aspect, the subject is a mammal. In another aspect, the mammal is a human. In yet another aspect, the subject is a pediatric patient.

**[0033]** In another aspect, the above methods are combined with the administration of an effective amount of an antibiotic. In another aspect, the a method is provided to increase the the potency of an antibiotic by administering an effective amount of the antibody fragment, antigen binding fragment or polypeptide as described herein.

**[0034]** A kit is also provided comprising the isolated antibody fragment, antigen binding fragment or polypeptide as described herein and instructions for use. In one aspect, the kit may be used for diagnostic or therapeutic use and therefore comprise the isolated antibody fragment, antigen binding fragment or polypeptide as described herein and instructions for use. In another aspect, the kit may be used to monitor biofilm formation and/or disruption. In another aspect, the kit further comprises an effective amount of a detectable or diagnostic label and/or an antibiotic.

**[0034a]** Without being taken to limit the foregoing of subsequent disclosure, particularly provided herein is:

1. A method to treat or prevent an inflammatory response caused by an infection with biofilm-producing eubacteria that produce a DNABII protein in a subject, or treat a subject infected with a biofilm or suffering from a condition caused by the presence of a biofilm produced by a biofilm-producing eubacteria that produced the DNABII protein, the method comprising administering to the subject an effective amount of an antibody Fab fragment of an mIhfB4<sub>NTHI</sub> antibody.
2. The use of an antibody Fab fragment of an mIhfB4<sub>NTHI</sub> antibody in the manufacture of a medicament for treating or preventing an inflammatory response caused by an infection with biofilm-producing eubacteria that produce a DNABII protein in a subject, or treating a subject infected with a biofilm or suffering from a condition caused by the presence of a biofilm produced by a biofilm-producing eubacteria that produced the DNABII protein, by administering to the subject an effective amount of the antibody Fab fragment.
3. The method of 1 or use of 2,
  - a. wherein the mIhfB4<sub>NTHI</sub> antibody is derived from a mammalian polyclonal antibody, that is optionally selected from the group of a rabbit polyclonal antibody, a murine polyclonal antibody, a sheep polyclonal antibody, a canine polyclonal antibody, or a human polyclonal antibody;
  - b. wherein the mIhfB4<sub>NTHI</sub> antibody is derived from monoclonal antibody; or
  - c. wherein the mIhfB4<sub>NTHI</sub> antibody is a humanized antibody or a human antibody.

4. The method or use of any one of 1 to 3, wherein the mIhfB4<sub>NTHI</sub> antibody is derived from a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
5. The method or use of any one of 1 to 4, wherein the Fab fragment further comprises a detectable label.
6. The method or use of any one of 1 to 5, wherein the administration of the Fab fragment of the mIhfB4<sub>NTHI</sub> antibody induces the production of an anti-inflammatory cytokine response that comprises one or more of: IL-4, IL-13, or IL-10.
7. The method or use of any one of 1 to 6, wherein the subject is a mammal.
8. The method or use of 7, wherein the mammal is a human.
9. The method or use of 7 or 8, wherein the subject is a pediatric patient.
10. The method or use of any one of 1 to 9, further comprising administering an effect amount of an antibiotic and/or vaccine adjuvant.
11. An isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
12. The polypeptide of 11, further comprising a detectable label.
13. A composition comprising the polypeptide of 11 or 12 and a carrier.
14. The composition of 13, wherein the carrier comprises a preservative or stabilizer.
15. An isolated polynucleotide encoding the polypeptide of 11 or 12.
16. The isolated polynucleotide of 15, further comprising a detectable label.
17. The isolated polynucleotide of 16, wherein the detectable label is not a conjugated polynucleotide.
18. The isolated polynucleotide of any one of 15 to 17 that is operably linked to a regulatory element, that optionally is contained within an expression vector or a host cell
19. A composition comprising the isolated polynucleotide of any one of 15 to 17 and a carrier.
20. The composition of 19, wherein the carrier comprises a preservative or stabilizer.

21. A vector comprising the isolated polynucleotide of any one of 15 to 17.
22. An isolated host cell comprising the polypeptide of any one of 11 to 12.
23. An isolated host cell comprising the isolated polynucleotide of any one of 15 to 18.
24. The isolated host cell of 22 or 23, wherein the host cell is a prokaryotic cell or a eukaryotic cell.
25. A method for producing a polypeptide comprising culturing the isolated host cell of any one of 22 to 24 under conditions that allow for the expression of polynucleotide to a polypeptide.
26. The method of 25, further comprising isolating the polypeptide.
27. A method to prevent formation of or to disrupt a biofilm associated with an industrial process comprising treating a surface susceptible to or containing a biofilm caused by eubacteria that express IHF DNABII an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
28. A method of identifying if a binding moiety disrupts a biofilm, comprising contacting a biofilm component with the binding moiety and with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335 and assaying for the binding of the binding moiety to the DNABII in the biofilm as compared to the binding of the isolated polypeptide to the DNABII in the biofilm, wherein a binding moiety with substantially the same or greater binding as compared to the binding of the polypeptide disrupts a biofilm.
29. A method to treat a condition characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein in a subject or detect the formation of a biofilm caused by eubacteria that express IHF DNABII protein in the subject, comprising administering an effective amount of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.

30. Use of the polypeptide of claim 11 or claim 12 in the manufacture of a medicament for treating a condition in a subject or detecting a biofilm in the subject, wherein the condition is characterized by the formation of a biofilm, by administering an effective amount of the polypeptide.
31. The method of claim 29 or use of claim 30, wherein, when the biofilm is to be detected, the method further comprises imaging or monitoring biofilm formation or disruption in the subject.
32. The method or use of claim 31, wherein, when the biofilm is to be detected, the polypeptide is chemically modified.
33. The method or use of claim 31 or 32, wherein, when the biofilm is to be detected, the polypeptide is conjugated to a diagnostic agent.
34. The method or use of claim 31, wherein, when the biofilm is to be detected, the method further comprises observing complexation of the polypeptide of any one of claims 1 to 8 with any biofilm present.
35. The method or use of claim 31, wherein the condition is selected from the group of: chronic non-healing wounds, including venous ulcers and diabetic foot ulcers, ear infections, sinus infections, urinary tract infections, pulmonary infections, cystic fibrosis, chronic obstructive pulmonary disease, catheter-associated infections, infections associated with implanted prostheses, or periodontal disease.
36. A method to monitor the development or progression of a condition in a subject, wherein the condition is characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein, the method comprising treating the subject with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
37. The method of claim 36, wherein the method further comprises observing complexation of the isolated polypeptide with any biofilm present.

38. A method to monitor the treatment of a condition in a subject, wherein the condition is characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein, the method comprising administering the subject with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
39. The method of claim 38, wherein the method further comprises imaging or monitoring biofilm formation and/or disruption in the subject.
40. The method of claim 36 or 38, wherein the polypeptide is chemically modified.
41. The method of any one of claims 36 to 40, wherein the polypeptide is conjugated to a diagnostic agent.
42. A method to prevent formation of or to disrupt a biofilm caused by eubacteria that express IHF DNABII protein in a subject in need thereof, comprising administering to the subject an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
43. An anti-biofilm coating for a non-physiological surface comprising the polypeptide of claim 11 or 12, optionally wherein the surface is in an industrial setting.
44. A non-physiological surface coated with the polypeptide of claim 11 or 12; and optionally, wherein the surface is in an industrial setting.
45. A method to treat or prevent a condition associated with infection of eubacteria that produce a DNABII polypeptide in the subject in a subject, comprising administering to the subject an effective amount of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335 and an antibiotic and/or vaccine adjuvant.

46. Use of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335, in the manufacture of a medicament for treating or preventing a condition associated with infection of eubacteria that produce a DNABII polypeptide in a subject, the treating or preventing comprising administering to the subject an effective amount of the isolated polypeptide and an antibiotic and/or vaccine adjuvant.
47. The method of 22 or use of 23, wherein the polypeptide increases the potency of the antibiotic.
48. A kit comprising the isolated polypeptide of 11 or 12 and instructions for use.
49. The kit of 48, further comprising an anti-IhfB2<sub>NTHI</sub> antibody or fragment thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **FIG. 1** depicts the generation of Fabs by digestion of monoclonal antibodies. Fab fragments were generated by digestion of murine monoclonal antibodies with ficin in the presence of cysteine (see Mariani *et al.*, A new enzymatic method to obtain high-yield F(ab)2 suitable for clinical use from mouse IgG1, *Molec. Immunol.* (1991) Jan-Feb; 28(1-2):69-77. PMID: 2011130). Fab fragments were purified and the purity of each preparation was assessed using 10% Bis-Tris PAGE (BioRad) and Coomassie Fluor Orange.

[0036] **FIG. 2** depicts the disruption of biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP upon incubation with mAb mIhfB4<sub>NTHI</sub> Fab (also referred to in this disclosure as IHF mB4 or alternatively as mB4). NTHI biofilms were incubated with medium (control), mAb IgG1 Fab (control), mAb IhfB2<sub>NTHI</sub> Fab, and mAb mIhfB4<sub>NTHI</sub> Fab. [0037] **FIG. 3** depicts the disruption of biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP, *S. aureus* 29213, *P. aeruginosa* 27853, and *B. cenocepacia* K56-2 upon incubation with mAb mIhfB4<sub>NTHI</sub> Fab. Biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP, *S. aureus* 29213, *P. aeruginosa* 27853, and *B. cenocepacia* K56-2 were incubated with murine IgG1, Murine IgG1 Fab, mAb IhfB2<sub>NTHI</sub>, mAb IhfB2<sub>NTHI</sub> Fab, mAb mIhfB4<sub>NTHI</sub>, and mAb mIhfB4<sub>NTHI</sub> Fab.

[0038] **FIG. 4** depicts the method by which the disruption of biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP was analyzed in the middle ear of adult chinchillas. At day zero (0), 1000 colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP were inoculated into the middle ear of adult chinchillas. At day 4, 83-100% of ears develop a mucosal biofilm, as shown. At days 4 and 5, Fab fragments were administered by direct delivery into the middle ear (342 nM). On day 6, analysis was performed to assess the biofilms, using otoscopy; imaging of mucosal biofilms; collection of middle ear fluids, middle ear mucosa, and adherent bacterial biofilm; enumeration of

bacterial load; and assessment of cytokine levels in middle ear fluids. Experiments included cohorts using murine mAb IgG1 Fab fragments (4 chinchillas), murine mAb IhfB2<sub>NTHI</sub> Fab fragments (4 chinchillas), and murine mAb mIhfB4<sub>NTHI</sub> Fab fragments (4 chinchillas).

**[0039]** **FIG. 5(A)** depicts the mean otoscopy scores at days 4, 5, and 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab. Using a WelchAllyn MacroView otoscope, the tympanic membrane of each animal was visualized and blindly ranked on a 0 – 4+ scale, wherein a score of  $\geq 2.0$  indicated the presence of fluid within the middle ear space, i.e. otitis media. (Novotny *et al.*, (2006) *Vaccine* 24:4804). Administration of mIhfB4<sub>NTHI</sub> Fab demonstrated a lower mean otoscopy score compared to IgG1 Fab and IhfB2<sub>NTHI</sub> Fab controls. P values are shown: \* $P \leq 0.05$  and \*\*  $P \leq 0.01$ . **FIG. 5(B)** shows representative otoscopy images at day 0, day 4, and day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab. At day 4, middle ear fluid is seen behind the tympanic membrane. At day 6, middle ear fluid is seen in chinchillas administered IgG1 Fab (control) and thickening of the tympanic membrane can be seen in chinchillas administered IhfB2<sub>NTHI</sub> Fab, which indicate the presence of otitis media in these animals. At day 6, a non-inflamed tympanic membrane is seen in chinchillas administered mAb mIhfB4<sub>NTHI</sub> Fab.

**[0040]** **FIG. 6(A)** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of mucosal biofilm in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed on day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge (treatments were performed on days 4 and 5).

**[0041]** **FIG. 6(B)** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of middle ear fluid in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation.

**[0042]** **FIG. 7(A)** shows quantitations of mass (grams, g) of middle ear mucosa and mucosal biofilm collected from chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed on day 6 after *Haemophilus influenzae*

(NTHI) 86-028NP challenge (treatments were performed on days 4 and 5). **FIG. 7(B)** shows quantitations of volume (microliters, mL) of middle ear fluid (MEF) retrieved from chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed on day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge (treatments were performed on days 4 and 5).

**[0043]** **FIG. 8(A)** shows mucosal biomass scores reviewed blindly by 7 individuals for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. A mucosal biofilm score scale was used to rank the residual biofilm within the middle ear space. Using an established rubric, a score of 0 to 4 was assigned to each image, as follows: zero (0): no biofilm visible; 1: biofilm fills  $>0$  to  $\leq 25\%$  of middle ear space; 2: biofilm fills  $>25\%$  to  $\leq 50\%$  of middle ear space; 3: biofilm fills  $>50\%$  to  $\leq 75\%$  middle ear space; and 4: biofilm fills  $>75\%$  to  $\leq 100\%$  middle ear space. A significantly lower mucosal biomass score was observed in chinchillas treated with mIhfB4<sub>NTHI</sub> Fab compared to chinchillas administered IgG1 Fab or IhfB2<sub>NTHI</sub> Fab controls. P values are shown:  $*P \leq 0.05$  and  $***P \leq 0.001$ . **FIG. 8(B)** depicts representative middle ear images obtained by otoscopy 1 day after receipt of final dose of either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation.

**[0044]** **FIG. 9** shows the relative quantity of a panel of pro- and anti-inflammatory cytokines in clarified middle ear fluids (IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17A, IL-12p70, and TNF) in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitation was performed to provide picogram (pg) cytokine per milliliter (ml) MEF.

**[0045]** **FIG. 10** shows a histologic analysis of the middle ear in chinchillas. The image on the left shows the middle ear from an animal administered murine IhfB2<sub>NTHI</sub> Fab control. The image on the right shows the middle ear from an animal administered murine mIhfB4<sub>NTHI</sub> Fab. Staining was performed with hematoxylin and eosin stains. Images were obtained using a 10X objective. The scale bar represents 100  $\mu$ m.

**[0046]** **FIG. 11** depicts the method by which the disruption of biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP was analyzed in the middle ear of adult chinchillas using Fab fragments generated from polyclonal rabbit anti-IhfB2<sub>NTHI</sub>, anti-

mIhfB4<sub>NTHI</sub>, and naïve rabbit serum. Experiments included cohorts using naïve rabbit serum IgG Fab fragments, rabbit anti-IhfB2<sub>NTHI</sub> Fab fragments, and rabbit anti-mIhfB4<sub>NTHI</sub> Fab fragments. On day zero (0), *Haemophilus influenzae* (NTHI) 86-028NP was inoculated into the middle ear of chinchillas. Then, either on days 4 and 5 (two dose experiments) or on days 4, 5, and 6 (three dose experiments), Fab fragments were administered by direct delivery into the middle ear (342 nM). For those administered on days 4 and 5, three (3) chinchillas per cohort were sacrificed either on day 6 or day 12. For those administered on days 4, 5, and 6, three (3) chinchillas per cohort were sacrificed on day 13. Following sacrifice, chinchillas were imaged, middle ear mucosa was collected, adherent biofilm was assessed, middle ear fluids were collected, quantitation of bacteria was performed, and middle ear fluids were assessed using a cytokine multiplex assay. The purity of each Fab preparation is also shown (3 separate preparations: naïve, IhfB2, and mIhfB4), as confirmed by 10% Bis-Tris PAGE (BioRad) and SYPRO Orange Protein Gel stain (Invitrogen).

**[0047]** **FIG. 12** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of mucosal biofilm in chinchillas administered either rabbit IgG1 Fab, rabbit IhfB2<sub>NTHI</sub> Fab, or rabbit mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed in three different experiments, as follows: (1) two doses of the respective Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge. The P value is shown: \*\*  $P \leq 0.01$ .

**[0048]** **FIG. 13** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milliliter (ml) of middle ear fluid in chinchillas administered either rabbit IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed in three different experiments, as follows: (1) two doses of the respective Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge. P values are shown: \*  $P \leq 0.05$  and \*\*  $P \leq 0.01$ .

**[0049]** **FIG. 14** shows mean mucosal biofilm scores (7 blinded reviewers) for chinchillas administered either rabbit IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed in three different experiments, as follows: (1) two doses of the respective Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge. A mucosal biofilm score scale was used to rank the residual biofilm within the middle ear space. Using an established rubric, a score of 0 to 4 was assigned to each image, as follows: zero (0): no biofilm visible; 1: biofilm fills >0 to ≤25% of middle ear space; 2: biofilm fills >25% to ≤50% of middle ear space; 3: biofilm fills >50% to ≤75% middle ear space; and 4: biofilm fills >75% to 100% middle ear space. P values are shown: \* $P \leq 0.05$  and \*\* $P \leq 0.01$ .

**[0050]** **FIG. 15** shows the relative quantity of a panel of pro- and anti-inflammatory cytokines in clarified middle ear fluids in chinchillas administered either naïve rabbit IgG Fab, rabbit anti-mIhfB2<sub>NTHI</sub> IgG Fab, or rabbit anti-mIhfB4<sub>NTHI</sub> IgG Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. The pro-inflammatory cytokines measured included: IL-1 $\beta$ , IL-6, IL-8, IL-12p70, IL-17A, TNF, and IFN $\gamma$ . The anti-inflammatory cytokines measured included: IL-4, IL-10, and IL-13.

## DETAILED DESCRIPTION

**[0051]** Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, particular, non-limiting exemplary methods, devices, and materials are now described. All technical and patent publications cited herein are incorporated herein by reference in their entirety. Nothing herein is to be construed as an admission that the disclosure is not entitled to antedate such disclosure by virtue of prior invention.

**[0052]** The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell

biology and recombinant DNA, which are within the skill of the art. See, e.g., Green and Sambrook eds. (2012) *Molecular Cloning: A Laboratory Manual*, 4th edition; the series Ausubel et al. eds. (2015) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson et al. (2015) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson et al. (1995) *PCR 2: A Practical Approach*; McPherson et al. (2006) *PCR: The Basics* (Garland Science); Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Greenfield ed. (2014) *Antibodies, A Laboratory Manual*; Freshney (2010) *Culture of Animal Cells: A Manual of Basic Technique*, 6th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Pat. No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Herdewijn ed. (2005) *Oligonucleotide Synthesis: Methods and Applications*; Hames and Higgins eds. (1984) *Transcription and Translation*; Buzdin and Lukyanov ed. (2007) *Nucleic Acids Hybridization: Modern Applications; Immobilized Cells and Enzymes* (IRL Press (1986)); Grandi ed. (2007) *In Vitro Transcription and Translation Protocols*, 2nd edition; Guisan ed. (2006) *Immobilization of Enzymes and Cells*; Perbal (1988) *A Practical Guide to Molecular Cloning*, 2nd edition; Miller and Calos eds, (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Lundblad and Macdonald eds. (2010) *Handbook of Biochemistry and Molecular Biology*, 4th edition; and Herzenberg et al. eds (1996) *Weir's Handbook of Experimental Immunology*, 5th edition.

**[0053]** All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate or alternatively by a variation of +/- 15%, or alternatively 10% or alternatively 5% or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

**[0054]** As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a polypeptide" includes a plurality of polypeptides, including mixtures thereof.

**[0055]** As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but do not exclude others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the intended use. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives (e.g., sodium benzoate, potassium sorbate, and methyl hydroxybenzoate), and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions disclosed herein. Embodiments defined by each of these transition terms are within the scope of this disclosure.

**[0056]** A “biofilm” intends an organized community of microorganisms that at times adhere to the surface of a structure, that may be organic or inorganic, together with the polymers such as DNA that they secrete and/or release. The biofilms are very resistant to microbiotics and antimicrobial agents. They live on gingival tissues, teeth and restorations, causing caries and periodontal disease, also known as periodontal plaque disease. They also cause middle ear infections. Biofilms can also form on the surface of dental implants, stents, catheter lines and contact lenses. They grow on pacemakers, heart valve replacements, artificial joints and other surgical implants. The Centers for Disease Control) estimate that over 65% of nosocomial (hospital-acquired) infections are caused by biofilms. They cause vaginal infections and lead to life-threatening systemic infections in people with hobbled immune systems. Biofilms also are involved in numerous diseases, including but not limited to those caused by *Aggregatibacter actinomycetemcomitans*, *Borrelia burgdorferi* (e.g., B31), *Bordetella pertussis* (e.g., Tohama I), *Burkholderia pseudomallei* (e.g., 668), *Burkholderia cenocepacia* (e.g., HI2424), *Escherichia coli* (e.g., K12 MG1655), *Enterococcus faecalis* (e.g., V583), *Haemophilus influenzae* (e.g., Rd KW20), *Helicobacter pylori* (e.g., 26695), *Klebsiella pneumoniae*, *Moraxella catarrhalis* (e.g., RH4), *Mycobacterium smegmatis* (e.g., MC2), *Mycobacterium tuberculosis* (e.g., CDC1551), *Neisseria gonorrhoeae* (e.g., FA1090), *Neisseria meningitidis* (e.g., MC58), *Pseudomonas aeruginosa*, *Porphyromonas gingivalis* (e.g., W83), *Prevotella intermedia* (e.g., 17), *Prevotella melaninogenica* (e.g., ATCC ® 25845), *Staphylococcus aureus* (e.g., MW2), *Staphylococcus epidermidis* (e.g., RP62A), *Streptococcus agalactiae* (e.g., 2603V/R), *Streptococcus bovis*, *Streptococcus gallolyticus* (e.g., UCN34), *Streptococcus gordonii* (e.g., NCTC 7868

(Challis)), *Streptococcus mutans* (e.g., UA159), *Streptococcus pneumoniae* (e.g., R6), *Streptococcus pyogenes* (e.g., MGAS10270), *Streptococcus sobrinus* (e.g., 6715), *Salmonella enterica* (e.g., *typhi*, CT18), *Treponema denticola* (e.g., ATCC ® 35405), *Treponema palladum* (e.g., Nichols), *Vibrio cholera* (e.g., El Tor, N16961). Additional organisms known to associate with and/or form biofilms include but are not limited to *Campylobacter* spp., *Candida* spp., *Legionella pneumophila*, and *Listeria monocytogenes*. For instance, cystic fibrosis patients have *Pseudomonas* infections that often result in antibiotic resistant biofilms. Other diseases associated with biofilms include, but are not limited to, lung infections of cystic fibrosis patients, otitis media, post-tympanostomy tube otitis media, chronic suppurative otitis media, native valve infectious endocarditis, osteomyelitis, rhinosinusitis, prostatitis, urinary tract infection, wounds, dental caries and periodontitis. Foodborne pathogens, such as but not limited to some of the above listed organisms (e.g., *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*) may also form biofilms on the food which they contaminate. Disease causing biofilms in animals (e.g., *Escherichia coli*, *Salmonella*, and *Shigella* species) may also cause downstream food contamination and/or disease in human hosts. Further, biofilms need not be of one homogeneous microbial population and may incorporate other pathogens and even host cells. In addition to being associated with disease – both nosocomial and otherwise – and food contamination, biofilms are often causes of industrial contamination, most notably in relation to process waters and surfaces in contact therewith. Complications involving organisms that form biofilm as industrial contaminants include but are not limited to biocorrosion, biofouling, and equipment damage as a result of biofilm formation. Non-limiting exemplary organisms associated with biofilms in industrial settings include those disclosed in Ferrera et al. (2015) Biofouling 31(2):173-180 and *Desulfovibrio* species. Additional details regarding biofilms may be found in, for example, Donlan (2002) Emerging Infectious Diseases 8(9):881-890.

**[0057]** The terms “to prevent formation of” or “to disrupt a biofilm” intends a prevention, reduction, or disruption in the formation of, or structure of, the DNA/protein matrix that is a component of a microbial biofilm.

**[0058]** The term “nucleoid associated protein” or “NAP” as used herein refers to a class of proteins that affect the dynamic spatial organization of nucleic acids in the nucleoid of prokaryotic cells. These proteins organize the genome through effecting DNA bending, binding and aggregation. Certain NAPs are DNA binding proteins and may be associated with the biofilm including, DNABII proteins, DPS (Genbank Accession No.: CAA49169), H-

NS (Genbank Accession No.: CAA47740), Hfq (Genbank Accession No.: ACE63256), CbpA (Genbank Accession No.: BAA03950) and CbpB (Genbank Accession No.: NP\_418813).

Of the NAPs, DNABII proteins are distinct and generally have strong sequence identity with alpha helical dimerization domains and may comprise anti-parallel beta ribbons, which often have **NPXT** (wherein X is any amino acid or may be selected from amino acids Q, R, K, S, or T) comprising tips that bind and intercalate into the minor groove of DNA and kink it.

The functional protomer is a dimer of identical or homologous subunits.

**[0059]** A “DNABII polypeptide or protein” intends a DNA binding protein or polypeptide that is composed of DNA-binding domains and thus have a specific or general affinity for microbial DNA. In one aspect, they bind DNA in the minor groove. Non-limiting examples of DNABII proteins are an integration host factor (IHF) protein and a histone-like protein (HU).

**[0060]** An “integration host factor” or “IHF” protein is a bacterial protein that is used by bacteriophages to incorporate their DNA into the host bacteria. They also bind extracellular microbial DNA. The genes that encode the IHF protein subunits in *E. coli* are himA (Genbank Accession No.: POA6X7.1) and himD (POA6Y1.1) genes. Homologs for these genes are found in other organisms, and peptides corresponding to these genes from other organisms can be found in Table 10 of U.S. Pat. No. 8,999,291, incorporated herein by reference.

**[0061]** “HMGB1” is a high mobility group box (HMGB) 1 protein that is reported to bind to and distort the minor groove of DNA and is an example of an agent. Recombinant or isolated protein and polypeptide are commercially available from Atgenglobal, ProSpecBio, Protein1 and Abnova.

**[0062]** “HU” or “histone-like protein” refers to a class of heterodimeric proteins typically associate with *E. coli*. HU proteins are known to bind DNA junctions. Related proteins have been isolated from other microorganisms. The complete amino acid sequence of *E. coli* HU was reported by Laine et al. (1980) Eur. J. Biochem 103(3):447-481. The genes that encode the HU protein subunits in *E. coli* are hupA and hupB corresponding to SEQ ID NOs. 8 and 9, respectively. A *Haemophilus influenzae* homolog derived from non-typeable *Haemophilus influenzae* (NTHI) corresponds to SEQ ID NO. 3. Homologs for these genes are found in other organisms, and peptides corresponding to these genes from other organisms can be found in Table 10 of U.S. Pat. No. 8,999,291, incorporated herein by reference.

**[0063]** The term “surface antigens” or “surface proteins” refers to proteins or peptides on the surface of cells such as bacterial cells that are at least in part exposed to the extracellular environment. Examples of surface antigens are outer membrane proteins such as OMP P5 (Genbank Accession No.: YP\_004139079.1), OMP P2 (Genbank Accession No.: ZZX87199.1), OMP P26 (Genbank Accession No.: YP\_665091.1), rsPilA or recombinant soluble PilA (Genbank Accession No.: EFU96734.1) and Type IV Pilin (Genbank Accession No.: Yp\_003864351.1).

**[0064]** The term “*Haemophilus influenzae*” refers to pathogenic bacteria that can cause many different infections such as, for example, ear infections, eye infections, and sinusitis. Many different strains of *Haemophilus influenzae* have been isolated and have an IhfA gene or protein. Some non-limiting examples of different strains of *Haemophilus influenzae* include Rd KW20, 86-028NP, R2866, PittGG, PittEE, R2846, and 2019.

**[0065]** “Microbial DNA” intends single or double stranded DNA from a microorganism that produces a biofilm.

**[0066]** “A conformational tip domain” of a polypeptide refers to a polypeptide that comprises a primary amino acid sequence wherein the structure has a anti-parallel beta ribbon with a sharp turn that is typically mediated by a proline residue.

**[0067]** “Inhibiting or preventing a biofilm” intends the therapeutic reduction or prevention in the structure of a biofilm.

**[0068]** “Treating an infection” intends a reduction in the number of microbes, e.g., bacteria, associated with the formation of a biofilm. Methods to determine if the number of microbes has been reduced are known in the art and include *in vivo* and *ex vivo* assays, as well as a reduction in the clinical symptoms of an infection. Because bacteria are protected by the biofilms, the bacteria become resistance to the use of antibacterials. By breaking down the biofilm one can reduce or inhibit bacterial resistance to antibacterial and other agents as well as treat the bacterial infection.

**[0069]** A “bent polynucleotide” intends a double strand polynucleotide that contains a small loop on one strand which does not pair with the other strand. In some embodiments, the loop is from 1 base to about 20 bases long, or alternatively from 2 bases to about 15 bases long, or alternatively from about 3 bases to about 12 bases long, or alternatively from about 4 bases to about 10 bases long, or alternatively has about 4, 5, or 6, or 7, or 8, or 9, or 10 bases.

**[0070]** “Polypeptides that compete with DNABII proteins in DNA binding” intend proteins or peptides that compete with IHF or HU in binding bent or distorted DNA structures but do not form a biofilm with the DNA. Examples, without limitation, include fragments of IHF that include one or more DNA binding domains of the IHF, or the biological equivalents thereof.

**[0071]** As used herein, the term “specifically recognize or bind” intends that the binding agent, e.g., Fab (fragment antigen binding) or monoclonal antibody, is more likely than not to bind to its intended target or binding partner.

**[0072]** A “subject” of diagnosis or treatment is a cell or an animal such as a mammal, or a human. Non-human animals subject to diagnosis or treatment and are those subject to infections or animal models, for example, simians, murines, such as, rats, mice, chinchilla, canine, such as dogs, leporids, such as rabbits, livestock, sport animals, and pets.

**[0073]** The term “protein”, “peptide” and “polypeptide” are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein's or peptide's sequence. As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

**[0074]** The terms “polynucleotide” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, RNAi, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can

be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment disclosed herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

**[0075]** A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

**[0076]** The term “isolated” or “recombinant” as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively that are present in the natural source of the macromolecule as well as polypeptides. The term “isolated or recombinant nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polynucleotides, polypeptides and proteins that are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. In other embodiments, the term “isolated or recombinant” means separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature. For example, an isolated cell is a cell that is separated from tissue or cells of dissimilar phenotype or genotype. An isolated polynucleotide is separated from the 3' and 5' contiguous nucleotides with which it is normally associated in its native or natural environment, e.g., on the chromosome. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart.

**[0077]** It is to be inferred without explicit recitation and unless otherwise intended, that when the present disclosure relates to a polypeptide, protein, polynucleotide or antibody, an equivalent or a biologically equivalent of such is intended within the scope of this disclosure. As used herein, the term “biological equivalent thereof” is intended to be synonymous with “equivalent thereof” when referring to a reference protein, antibody, fragment, polypeptide or

nucleic acid, intends those having minimal homology while still maintaining desired structure or functionality. Unless specifically recited herein, it is contemplated that any polynucleotide, polypeptide or protein mentioned herein also includes equivalents thereof. In one aspect, an equivalent polynucleotide is one that hybridizes under stringent conditions to the polynucleotide or complement of the polynucleotide as described herein for use in the described methods. In another aspect, an equivalent antibody or antigen binding polypeptide or Fab (fragment antigen binding) intends one that binds with at least 70%, or alternatively at least 75%, or alternatively at least 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% affinity or higher affinity to a reference antibody or antigen binding fragment. In another aspect, the equivalent thereof competes with the binding of the antibody or antigen binding fragment to its antigen under a competitive ELISA assay. In another aspect, an equivalent intends at least about 80% homology or identity and alternatively, at least about 85%, or alternatively at least about 90%, or alternatively at least about 95%, or alternatively 98% percent homology or identity and exhibits substantially equivalent biological activity to the reference protein, polypeptide or nucleic acid. Examples of biologically equivalent polypeptides are provided in Table 9 of U.S. Pat. No. 8,999,291, incorporated herein by reference, which identify conservative amino acid substitutions to the disclosed amino acid sequences.

**[0078]** A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) having a certain percentage (for example, 80%, 85%, 90%, or 95%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. The alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology (Ausubel et al., eds. 1987) Supplement 30, section 7.7.18, Table 7.7.1. In certain embodiments, default parameters are used for alignment. A non-limiting exemplary alignment program is BLAST, using default parameters. In particular, exemplary programs include BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address: [ncbi.nlm.nih.gov/cgi-bin/BLAST](http://ncbi.nlm.nih.gov/cgi-bin/BLAST). Sequence identity and percent

identity were determined by incorporating them into clustalW (available at the web address:align.genome.jp, last accessed on Mar. 7, 2011).

**[0079]** “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present disclosure.

**[0080]** “Homology” or “identity” or “similarity” can also refer to two nucleic acid molecules that hybridize under stringent conditions.

**[0081]** “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

**[0082]** Examples of stringent hybridization conditions include: incubation temperatures of about 25° C. to about 37° C.; hybridization buffer concentrations of about 6×SSC to about 10×SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4×SSC to about 8×SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40° C. to about 50° C.; buffer concentrations of about 9×SSC to about 2×SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5×SSC to about 2×SSC. Examples of high stringency conditions include: incubation temperatures of about 55° C. to about 68° C.; buffer concentrations of about 1×SSC to about 0.1×SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1×SSC, 0.1×SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation

times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

**[0083]** As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in an eukaryotic cell.

**[0084]** The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

**[0085]** As used herein, the terms “treating,” “treatment” and the like are used herein to mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disorder or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse effect attributable to the disorder. Methods to determine if treatment has occurred are known in the art and briefly described herein.

**[0086]** To prevent intends to prevent a disorder or effect *in vitro* or *in vivo* in a system or subject that is predisposed to the disorder or effect. An example of such is preventing the formation of a biofilm in a system that is infected with a microorganism known to produce one.

**[0087]** A “composition” is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

**[0088]** A “pharmaceutical composition” is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

**[0089]** “Pharmaceutically acceptable carriers” refers to any diluents, excipients, or carriers that may be used in the compositions disclosed herein. Pharmaceutically acceptable carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or

electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, microspheres, microparticles, or nanoparticles (comprising e.g., biodegradable polymers such as Poly(Lactic Acid-co-Glycolic Acid)), and wool fat. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field. They may be selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

**[0090]** A "biologically active agent" or an active agent disclosed herein intends one or more of an isolated or recombinant polypeptide, an isolated or recombinant polynucleotide, a vector, an isolated host cell, or an antibody, as well as compositions comprising one or more of same.

**[0091]** "Administration" or "delivery" of a therapeutic or other agent can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated, and target cell or tissue. Non-limiting examples of route of administration include oral administration, nasal administration, inhalation, injection, and topical application. Administration can be for use in industrial as well as therapeutic applications.

**[0092]** An agent (antibody or fragment thereof, polypeptide or polynucleotide, or cell) of the present disclosure can be administered for therapy by any suitable route of administration. It will also be appreciated that the optimal route will vary with the condition and age of the recipient, and the disease being treated. The agent may be used in industrial settings and for

the treatment of animals. When used in industrial settings, the biofilm is contacted with the agent, e.g., Fab (fragment antigen binding) or antibody.

**[0093]** The term “effective amount” refers to a quantity sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, species, and tolerance to pharmaceutical compositions. In the context of this disclosure, in some embodiments the effective amount is the amount sufficient to result a reduction in biofilm mass or in breaking down a biofilm. In other aspects, the amount is effective to treat a bacterial infection associated with a biofilm in a subject. In other embodiments, in the context of a Fab (fragment antigen binding) or antibody, the effective amount is the amount sufficient to result in breaking down, diminishing or disrupting a biofilm. In other embodiments, the effective amount of an agent or an immunogenic composition is the amount sufficient to result in antibody generation against the antigen. In some embodiments, the effective amount is the amount required to confer passive immunity on a subject in need thereof. With respect to compositions, in some embodiments the effective amount will depend on the intended use, the health/responsiveness of the subject's immune system, in addition to the factors described above. The skilled artisan will be able to determine appropriate amounts depending on these and other factors.

**[0094]** In the case of an *in vitro* application, in some embodiments the effective amount will depend on the size and nature of the application in question. It will also depend on the nature and sensitivity of the *in vitro* target and the methods in use. The skilled artisan will be able to determine the effective amount based on these and other considerations. The effective amount may comprise one or more administrations of a composition depending on the embodiment.

**[0095]** The term “potency” as it relates to the potency of a drug, such as an antibiotic refers to a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. A highly potent drug evokes a given response at low concentrations, while a drug of lower potency evokes the same response only at higher concentrations. The potency depends on both the affinity and efficacy.

**[0096]** The term “conjugated moiety” refers to a moiety that can be added to an isolated chimeric polypeptide by forming a covalent bond with a residue of chimeric polypeptide. The

moiety may bond directly to a residue of the chimeric polypeptide or may form a covalent bond with a linker which in turn forms a covalent bond with a residue of the chimeric polypeptide. A “peptide conjugate” refers to the association by covalent or non-covalent bonding of one or more polypeptides and another chemical or biological compound. In a non-limiting example, the “conjugation” of a polypeptide with a chemical compound results in improved stability or efficacy of the polypeptide for its intended purpose. In one embodiment, the polypeptide of this disclosure is conjugated to a carrier, wherein the carrier is a liposome, a micelle, or a pharmaceutically acceptable polymer.

**[0097]** “Liposomes” are microscopic vesicles consisting of concentric lipid bilayers. A liposome is an example of a carrier, e.g., a pharmaceutically acceptable carrier. Structurally, liposomes range in size and shape from long tubes to spheres, with dimensions from a few hundred Angstroms to fractions of a millimeter. Vesicle-forming lipids are selected to achieve a specified degree of fluidity or rigidity of the final complex providing the lipid composition of the outer layer. These are neutral (cholesterol) or bipolar and include phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and sphingomyelin (SM) and other types of bipolar lipids including but not limited to dioleoylphosphatidylethanolamine (DOPE), with a hydrocarbon chain length in the range of 14-22, and saturated or with one or more double C=C bonds. Examples of lipids capable of producing a stable liposome, alone, or in combination with other lipid components are phospholipids, such as hydrogenated soy phosphatidylcholine (HSPC), lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanol-amine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic acid, cerebrosides, distearoylphosphatidylethanol-amine (DSPE), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE) and dioleoylphosphatidylethanolamine 4-(N-maleimido-triethyl)cyclohexane-1-carboxylate (DOPE-mal). Additional non-phosphorous containing lipids that can become incorporated into liposomes include stearylamine, dodecylamine, hexadecylamine, isopropyl myristate, triethanolamine-lauryl sulfate, alkyl-aryl sulfate, acetyl palmitate, glycerol ricinoleate, hexadecyl stearate, amphoteric acrylic polymers, polyethoxylated fatty acid amides, and the cationic lipids mentioned above (DDAB, DODAC, DMRIE, DMTAP, DOGS, DOTAP (DOTMA), DOSPA, DPTAP, DSTAP, DC-Chol). Negatively charged lipids include phosphatidic acid (PA), dipalmitoylphosphatidylglycerol (DPPG),

diotelylphosphatidylglycerol and (DOPG), dicetylphosphate that are able to form vesicles. Typically, liposomes can be divided into three categories based on their overall size and the nature of the lamellar structure. The three classifications, as developed by the New York Academy Sciences Meeting, “Liposomes and Their Use in Biology and Medicine,” December 1977, are multi-lamellar vesicles (MLVs), small uni-lamellar vesicles (SUVs) and large uni-lamellar vesicles (LUVs). The biological active agents can be encapsulated in such for administration in accordance with the methods described herein.

**[0098]** A “micelle” is an aggregate of surfactant molecules dispersed in a liquid colloid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic “head” regions in contact with surrounding solvent, sequestering the hydrophobic tail regions in the micelle center. This type of micelle is known as a normal phase micelle (oil-in-water micelle). Inverse micelles have the head groups at the center with the tails extending out (water-in-oil micelle). Micelles can be used to attach a polynucleotide, polypeptide, antibody or composition described herein to facilitate efficient delivery to the target cell or tissue.

**[0099]** The phrase “pharmaceutically acceptable polymer” refers to the group of compounds which can be conjugated to one or more polypeptides or antibodies described here. It is contemplated that the conjugation of a polymer to the polypeptide or antibody is capable of extending the half-life of the polypeptide *in vivo* and *in vitro*. Non-limiting examples include polyethylene glycols, polyvinylpyrrolidones, polyvinylalcohols, cellulose derivatives, polyacrylates, polymethacrylates, sugars, polyols and mixtures thereof. The biological active agents can be conjugated to a pharmaceutically acceptable polymer for administration in accordance with the methods described herein.

**[0100]** A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, micelles biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

**[0101]** A polynucleotide disclosed herein can be delivered to a cell or tissue using a gene delivery vehicle. “Gene delivery,” “gene transfer,” “transducing,” and the like as used herein,

are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a “transgene”) into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of “naked” polynucleotides (such as electroporation, “gene gun” delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

**[0102]** As used herein the term “eDNA” refers to extracellular DNA found as a component to pathogenic biofilms.

**[0103]** A “plasmid” is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances.

**[0104]** “Plasmids” used in genetic engineering are called “plasmid vectors”. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for.

**[0105]** A “yeast artificial chromosome” or “YAC” refers to a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the telomeric, centromeric, and replication origin sequences needed for replication and preservation in yeast cells. Built using an initial circular plasmid, they are linearized by using restriction enzymes, and then DNA ligase can add a sequence or gene of interest within the linear molecule by the use of cohesive ends. Yeast expression vectors, such as YACs, YIps (yeast integrating plasmid), and YEps (yeast episomal plasmid), are extremely useful as one can get eukaryotic protein products with posttranslational modifications as yeasts are themselves eukaryotic cells, however YACs have been found to be more unstable than BACs, producing chimeric effects.

**[0106]** A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Infectious tobacco mosaic virus (TMV)-based vectors can be used to manufacturer proteins and have been reported to express Griffithsin in tobacco leaves (O'Keefe et al. (2009) Proc. Nat. Acad. Sci. USA 106(15):6099-6104). Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger & Dubensky (1999) Curr. Opin. Biotechnol. 5:434-439 and Ying et al. (1999) Nat. Med. 5(7):823-827. In aspects where gene transfer is mediated by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene. Further details as to modern methods of vectors for use in gene transfer may be found in, for example, Kotterman et al. (2015) Viral Vectors for Gene Therapy: Translational and Clinical Outlook Annual Review of Biomedical Engineering 17.

**[0107]** As used herein, “retroviral mediated gene transfer” or “retroviral transduction” carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

**[0108]** Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus.

**[0109]** In aspects where gene transfer is mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a transgene. Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. See, e.g., PCT International Pat. Application Publication No. WO 95/27071. Ads do not require integration into the host cell genome. Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. See, PCT International Pat. Application Publication Nos. WO 95/00655 and WO 95/11984, Wild-type AAV has high infectivity and specificity integrating into the host cell's genome. See, Hermonat & Muzyczka (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470 and Lebkowski et al. (1988) Mol. Cell. Biol. 8:3988-3996.

**[0110]** Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression.

**[0111]** Gene delivery vehicles also include DNA/liposome complexes, micelles and targeted viral protein-DNA complexes. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods disclosed herein. In addition to the delivery of polynucleotides to a cell or cell population, direct introduction of the proteins described herein to the cell or cell population can be done by the non-limiting technique of protein transfection, alternatively culturing conditions that can enhance the expression and/or promote the activity of the proteins disclosed herein are other non-limiting techniques.

**[0112]** As used herein, the terms “antibody,” “antibodies” and “immunoglobulin” includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term

“antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. The terms “antibody,” “antibodies” and “immunoglobulin” also include immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fab', F(ab)<sub>2</sub>, Fv, scFv, dsFv, Fd fragments, dAb, VH, VL, VhH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies and kappa bodies; multispecific antibody fragments formed from antibody fragments and one or more isolated. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, at least one portion of a binding protein, chimeric antibodies, humanized antibodies, species-sized antibodies, single-chain antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein. The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues. The term “anti-” when used before a protein name, anti-IHF, anti-HU, anti-OMP P5, for example, refers to a monoclonal or polyclonal antibody that binds and/or has an affinity to a particular protein. For example, “anti-IHF” refers to an antibody that binds to the IHF protein. The specific antibody may have affinity or bind to proteins other than the protein it was raised against. For example, anti-IHF, while specifically raised against the IHF protein, may also bind other proteins that are related either through sequence homology or through structure homology.

**[0113]** The antibodies can be polyclonal, monoclonal, multispecific (e.g., bispecific antibodies), a diabody, and antibody fragments, so long as they exhibit the desired biological activity. Antibodies can be isolated from any suitable biological source, e.g., a human, a murine, rat, sheep and canine.

**[0114]** As used herein, “monoclonal antibody” refers to an antibody obtained from a substantially homogeneous antibody population. Monoclonal antibodies are highly specific, as each monoclonal antibody is directed against a single determinant on the antigen. The antibodies may be detectably labeled, e.g., with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member

of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like.

**[0115]** Monoclonal antibodies may be generated using hybridoma techniques or recombinant DNA methods known in the art. A hybridoma is a cell that is produced in the laboratory from the fusion of an antibody-producing lymphocyte and a non-antibody producing cancer cell, usually a myeloma or lymphoma. A hybridoma proliferates and produces a continuous sample of a specific monoclonal antibody. Alternative techniques for generating or selecting antibodies include *in vitro* exposure of lymphocytes to antigens of interest, and screening of antibody display libraries in cells, phage, or similar systems.

**[0116]** The term “human antibody” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies disclosed herein may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human antibody” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C<sub>L</sub>, C<sub>H</sub> domains (e.g., C<sub>H1</sub>, C<sub>H2</sub>, C<sub>H3</sub>), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

**[0117]** As used herein, a human antibody is “derived from” a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, e.g., by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is “derived from” a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

**[0118]** A “human monoclonal antibody” refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. The term also intends recombinant human antibodies. Methods to making these antibodies are described herein.

**[0119]** The term “recombinant human antibody”, as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is

used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. Methods to making these antibodies are described herein.

**[0120]** As used herein, chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species.

**[0121]** As used herein, the term “humanized antibody” or “humanized immunoglobulin” refers to a human/non-human chimeric antibody that contains a minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a variable region of the recipient are replaced by residues from a variable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or non-human primate having the desired specificity, affinity and capacity. Humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. The humanized antibody can optionally also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin, a non-human antibody containing one or more amino acids in a framework region, a constant region or a CDR, that have been substituted with a correspondingly positioned amino acid from a human antibody. In general, humanized antibodies are expected to produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody. The humanized antibodies may have conservative amino acid substitutions which have substantially no effect on antigen binding or other antibody functions. Conservative substitutions groupings include: glycine-alanine, valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, serine-threonine and asparagine-glutamine. The term “species-ized” refers to antibodies that have been modified in the same or a similar manner for a non-human species.

**[0122]** The terms “polyclonal antibody” or “polyclonal antibody composition” as used herein refer to a preparation of antibodies that are derived from different B-cell lines. They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope. In some embodiments, the antibody or antigen binding fragment is not a polyclonal antibody.

**[0123]** As used herein, the term “antibody derivative”, comprises a full-length antibody or a fragment of an antibody, wherein one or more of the amino acids are chemically modified by alkylation, pegylation, acylation, ester formation or amide formation or the like, e.g., for linking the antibody to a second molecule. This includes, but is not limited to, pegylated antibodies, cysteine-pegylated antibodies, and variants thereof. This disclosure also provided antibody derivatives of the antibody fragments, e.g., the polypeptides conjugated to another molecule, e.g., PEG or further modified by acylation.

**[0124]** As used herein, the term “immunoconjugate” comprises an antibody fragment or a antibody derivative associated with or linked to a second agent, such as a cytotoxic agent, a detectable agent, a radioactive agent, a targeting agent, a human antibody, a humanized antibody, a chimeric antibody, a synthetic antibody, a semisynthetic antibody, or a multispecific antibody. This disclosure provides immunoconjugates comprising as one component, an antibody polypeptide as described herein.

**[0125]** As used herein, the term “label” intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., N-terminal histidine tags (N-His), magnetically active isotopes, e.g., <sup>115</sup>Sn, <sup>117</sup>Sn and <sup>119</sup>Sn, a non-radioactive isotopes such as <sup>13</sup>C and <sup>15</sup>N, polynucleotide or protein such as an antibody so as to generate a “labeled” composition. The term also includes sequences conjugated to a polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The term also includes purification tags or labels that aid in the isolation of biological materials from mixed populations. While the term “label” generally intends compositions covalently attached to the composition to be detected, in one aspect it specifically excludes naturally occurring nucleosides and amino acids that are known to fluoresce under certain conditions (e.g., temperature, pH, etc.) when positioned within the polynucleotide or protein in its native environment and generally any natural fluorescence that may be present in the composition to be detected. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to magnetically active isotopes, non-radioactive isotopes, radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally

comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component. Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) *Handbook of Fluorescent Probes and Research Chemicals* (6<sup>th</sup> ed). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

**[0126]** Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, CASCADE BLUE™, and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) *Handbook of Fluorescent Probes and Research Chemicals* (6th ed.).

**[0127]** In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

**[0128]** “Eukaryotic cells” comprise all of the life kingdoms except monera. They can be easily distinguished through a membrane-bound nucleus. Animals, plants, fungi, and protists are eukaryotes or organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane-bound structure is the nucleus. Unless specifically recited, the term “host” includes a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Non-limiting examples of eukaryotic cells or hosts include simian, bovine, porcine, murine, rat, avian, reptilian and human.

**[0129]** “Prokaryotic cells” that usually lack a nucleus or any other membrane-bound organelles and are divided into two domains, bacteria and archaea. In addition to chromosomal DNA, these cells can also contain genetic information in a circular loop called an episome. Bacterial cells are very small, roughly the size of an animal mitochondrion (about 1-2  $\mu\text{m}$  in diameter and 10  $\mu\text{m}$  long). Prokaryotic cells feature three major shapes: rod shaped, spherical, and spiral. Instead of going through elaborate replication processes like eukaryotes, bacterial cells divide by binary fission. Examples include but are not limited to *Bacillus* bacteria, *E. coli* bacterium, and *Salmonella* bacterium.

**[0130]** A “native” or “natural” antigen is a polypeptide, protein or a fragment which contains an epitope, which has been isolated from a natural biological source, and which can specifically bind to an antigen receptor, in particular a T cell antigen receptor (TCR), in a subject.

**[0131]** The terms “antigen” and “antigenic” refer to molecules with the capacity to be recognized by an antibody or otherwise act as a member of an antibody-ligand pair. “Specific binding” refers to the interaction of an antigen with the variable regions of immunoglobulin heavy and light chains. Antibody-antigen binding may occur *in vivo* or *in vitro*. The skilled artisan will understand that macromolecules, including proteins, nucleic acids, fatty acids, lipids, lipopolysaccharides and polysaccharides have the potential to act as an antigen. The skilled artisan will further understand that nucleic acids encoding a protein with the potential to act as an antibody ligand necessarily encode an antigen. The artisan will further understand that antigens are not limited to full-length molecules, but can also include partial molecules. The term “antigenic” is an adjectival reference to molecules having the properties of an antigen. The term encompasses substances that are immunogenic, i.e., immunogens, as well as substances which induce immunological unresponsiveness, or anergy, i.e., anergens.

**[0132]** An “altered antigen” is one having a primary sequence that is different from that of the corresponding wild-type antigen. Polypeptide mB4 (SEQ ID NO.: 17) is an example of an altered antigen (SEQ ID NO.: 16). Altered antigens can be made by synthetic or recombinant methods and include, but are not limited to, antigenic peptides that are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, cross-linking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane molecule or other ligand (Ferguson et al. (1988) Ann. Rev. Biochem. 57:285-320). A synthetic or altered antigen disclosed herein is intended to bind to the same TCR as the natural epitope.

**[0133]** A “self-antigen” also referred to herein as a native or wild-type antigen is an antigenic peptide that induces little or no immune response in the subject due to self-tolerance to the antigen. An example of a self-antigen is the melanoma specific antigen gp100.

**[0134]** “Immune response” broadly refers to the antigen-specific responses of lymphocytes to foreign substances. The terms “immunogen” and “immunogenic” refer to molecules with the capacity to elicit an immune response. All immunogens are antigens; however, not all antigens are immunogenic. An immune response disclosed herein can be humoral (via antibody activity) or cell-mediated (via T cell activation). The response may occur *in vivo* or *in vitro*. The skilled artisan will understand that a variety of macromolecules, including proteins, nucleic acids, fatty acids, lipids, lipopolysaccharides and polysaccharides have the potential to be immunogenic. The skilled artisan will further understand that nucleic acids encoding a molecule capable of eliciting an immune response necessarily encode an immunogen. The artisan will further understand that immunogens are not limited to full-length molecules, but may include partial molecules.

**[0135]** The term “passive immunity” refers to the transfer of immunity from one subject to another through the transfer of antibodies. Passive immunity may occur naturally, as when maternal antibodies are transferred to a fetus. Passive immunity may also occur artificially as when antibody compositions are administered to non-immune subjects. Antibody donors and recipients may be human or non-human subjects. Antibodies may be polyclonal or monoclonal, may be generated *in vitro* or *in vivo*, and may be purified, partially purified, or unpurified depending on the embodiment. In some embodiments described herein, passive immunity is conferred on a subject in need thereof through the administration of antibodies or antigen binding fragments that specifically recognize or bind to a particular antigen. In some embodiments, passive immunity is conferred through the administration of an isolated or recombinant polynucleotide encoding an antibody or antigen binding fragment that specifically recognizes or binds to a particular antigen.

**[0136]** As used herein, the term “inducing an immune response in a subject” is a term well understood in the art and intends that an increase of at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 100-fold, at least about 500-fold, or at least about 1000-fold or more in an immune response to an antigen (or epitope) can be detected or measured, after introducing the antigen (or epitope) into the subject, relative to the immune response (if any) before introduction of the antigen (or epitope) into the subject. An immune response to an antigen (or epitope), includes, but is not limited to, production of an antigen-specific (or

epitope-specific) antibody, and production of an immune cell expressing on its surface a molecule which specifically binds to an antigen (or epitope). Methods of determining whether an immune response to a given antigen (or epitope) has been induced are well known in the art. For example, antigen-specific antibody can be detected using any of a variety of immunoassays known in the art, including, but not limited to, ELISA, wherein, for example, binding of an antibody in a sample to an immobilized antigen (or epitope) is detected with a detectably-labeled second antibody (e.g., enzyme-labeled mouse anti-human Ig antibody).

**[0137]** As used herein, the term “anti-inflammatory cytokines” includes immunoregulatory molecules that control the proinflammatory cytokine response. Cytokines act together with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13. Specific cytokine receptors for IL-1, tumor necrosis factor-alpha, and IL-18 also function as proinflammatory cytokine inhibitors. Methods of measuring cytokine, including anti-inflammatory cytokine, levels are well known in the art. For example, serum cytokine levels can be measured using commercially available enzyme-linked immuno-sorbent assay (ELISA) kits.

**[0138]** The term “effective amount” refers to a quantity sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, and tolerance to pharmaceutical compositions. In the context of an immunogenic composition, in some embodiments the effective amount is the amount sufficient to result in a protective response against a pathogen. In other embodiments, the effective amount of an immunogenic composition is the amount sufficient to result in antibody generation against the antigen. In some embodiments, the effective amount is the amount required to confer passive immunity on a subject in need thereof. With respect to immunogenic compositions, in some embodiments the effective amount will depend on the intended use, the degree of immunogenicity of a particular antigenic compound, and the health/responsiveness of the subject's immune system, in addition to the factors described above. The skilled artisan will be able to determine appropriate amounts depending on these and other factors.

**[0139]** In the case of an *in vitro* application, in some embodiments the effective amount will depend on the size and nature of the application in question. It will also depend on the nature and sensitivity of the *in vitro* target and the methods in use. The skilled artisan will be

able to determine the effective amount based on these and other considerations. The effective amount may comprise one or more administrations of a composition depending on the embodiment.

**[0140]** As used herein, “solid phase support” or “solid support”, used interchangeably, is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, alumina gels. As used herein, “solid support” also includes synthetic antigen-presenting matrices, cells, and liposomes. A suitable solid phase support may be selected on the basis of desired end use and suitability for various protocols. For example, for peptide synthesis, solid phase support may refer to resins such as polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), POLYHIPE® resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGel®, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Bioscience, Calif.).

**[0141]** An example of a solid phase support include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to a polynucleotide, polypeptide or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. or alternatively polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

### **Modes for Carrying Out the Disclosure**

#### **Antibody Fragments and Derivatives Thereof**

**[0142]** This disclosure provides an antibody fragment (e.g., a Fab fragment or an antigen binding fragment). This disclosure also provides an isolated polypeptide that comprises, or alternatively consists essentially of, or yet further consists of the amino acid sequence of the Fab fragment or antigen binding fragment (e.g., a Fab fragment) wherein the fragment or the

polypeptide binds and/or specifically recognizes a DNABII polypeptide and/or biofilm component comprising a DNABII protein or polypeptide.

**[0143]** The antibody fragments or isolated polypeptides comprising, consisting essentially of, or yet further consisting of the antibody fragments, can be any of the Fab fragments from a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a human antibody, a veneered antibody, a diabody, a humanized antibody, an antibody derivative, or a recombinant humanized antibody. The antibody can be from any appropriate species, e.g., a mammalian species, e.g., a murine, a feline, a canine, a rabbit, or a human. Methods to prepare Fab antibody fragments are known in the art and briefly described herein. One exemplary method is outlined in **FIG. 1**.

**[0144]** The antibody fragments are derived from or produced from antibodies that specifically recognize and bind a DNABII protein, non-limiting examples of such include IHFalpha, IHFbeta, and HU. Non-limiting examples of such polypeptides and antibodies that bind such polypeptides are provided in US Patent No. 8,999,291, incorporated herein by reference. In one aspect, the antibody specifically binds the “tip domain” of the DNABII polypeptide that contains a conformational epitope. In one aspect, the DNABII polypeptide comprises, or consists essentially of, or yet further consists of one of SEQ ID NOS.: 1 to 33. Additional non-limiting examples of exemplary DNABII polypeptides include, for example, the conformational epitope within the tip domain of IHFalpha, IHFbeta or HU, e.g., provided in SEQ ID NOS.: 13, 14, 17, 26-32. In one aspect, the SEQ ID NOS. 13, 14, 17 and 26-32 are contained within a larger polypeptide, that in one aspect, is not the wild-type native polypeptide, e.g., SEQ ID NOS.: 1 to 11. The larger polypeptide can contain additional amino acids at the amine and/or the carboxy terminus, e.g., at least 3, or 4, or 5, or 10, or 15, or 20 or more amino acids at one or both termini.

**[0145]** In addition, antibodies can be generated against the “tip” region of an DNABII protein, that in one aspect contains or is altered to contain turn of the antiparallel beta ribbon and/or the sequence, **NPXT** wherein “X” refers to any amino acid, or alternatively X is selected from the amino acids Q, R, K, S, or T. Such antibodies may be generated using a fragment of the DNABII protein comprising the **NPXT** sequence, optionally flanked by between about 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids on one or both sides of the sequence. Non-limiting examples of such are disclosed herein, i.e., those with the consensus amino acid sequence **NPXT**. In another aspect, antibodies are generated against the tip region of a DNABII protein providing the consensus sequence **NPXT**, wherein

“X” is any amino acid or alternatively X is selected from the amino acids Q, R, K, S, or T. A skilled artisan will appreciate that in any one of these sequences, the residue in the X position may be substituted with any amino acid – e.g., Q, R, K, S, or T. Examples include:

SEQ ID NO. 13: *Haemophilus influenzae* IhfA, A5 fragment:

RPGRNPKTGDVVPVSARRVV.

SEQ ID NO. 14: *Haemophilus influenzae* HU, A5 fragment:

RTGRNPQTGAEIQIAASKVP.

SEQ ID NO. 17: *Haemophilus influenzae* IhfB, modified B4 (mB4) fragment:

FSLHHRQPRLGRNPKTGDSV.

SEQ ID NO. 26: *Haemophilus influenzae* IhfA, A tip fragment:

NFELRDKSSRPGRNPKTGDVV.

SEQ ID NO. 27: *Haemophilus influenzae* IhfB, B tip fragment:

SLHHRQPRLGRNPKTGDSVNL.

SEQ ID NO. 31 *Haemophilus influenzae* HU, fragment:

VNERAARTGRNPQTGAEIQIAA.

**[0146]** In some embodiments, the polypeptide comprising **NPXT** is at least about 20 amino acids long and the **NPXT** is centrally in the sequence. Non-limiting examples of such sequences include SEQ ID NOs. 13, 14, and 31. Alternatively, the polypeptides having the **NPXT** motif (e.g., as noted above) can be modified to remove the **NPXT** motif either by substitution or deletion of one or more amino acids and monoclonal antibodies can be raised against these polypeptides. Applicants have determined that Fab fragments of antibodies raised against DNABII polypeptides lacking the **NPXT** motif are useful in diagnostic methods to image and monitor biofilm formation and/or disruption. In one aspect, a kit is provided comprising a Fab fragment of an antibody raised against or that binds a DNABII having the **NPXT** motif and a Fab fragment of an antibody raised against or binds a DNABII (e.g. a modified or naturally occurring polypeptide) that lacks the **NPXT** motif. For example a kit can comprise an antibody that recognizes and binds the polypeptide A5, B4, or mB4 (therapeutic) can be combined in a kit with an Fab fragment of an antibody that recognizes and binds the polypeptide A3 or B2 (diagnostic). The kit is useful for diagnosis, treatment and monitoring biofilm treatment.

**[0147]** In one aspect, the isolated polypeptide comprises, or alternatively consists essentially of, or yet further consists of a Fab fragment of an mIhfB4<sub>NTHI</sub> antibody or an equivalent of the mIhfB4<sub>NTHI</sub> antibody, examples of such are provided herein.

**[0148]** In another aspect, the Fab fragment is prepared from an antibody that is produced by a hybridoma cell line, e.g. the hybridomas listed in **Table 1**.

**Table 1**

Specificity	SEQ ID NO.	ATCC Accession No.	Hybridoma Cell Line
IhfA frag. A5	SEQ ID NO. 13	PTA-122334	IhfA5 NTHI 14G8.F5.G6
IhfB frag. B4	SEQ ID NO. 16	PTA-122336	IhfB4 NTHI 4E11.E5.G2
IhfB frag. mB4	SEQ ID NO. 17	PTA-122335	mIhfB4 NTHI 12E6.F8.D12.D5
IhfA frag. A3	SEQ ID NO. 12	PTA-123393	IhfA3 NTHI 9B10.F2.H3
IhfB frag. B2	SEQ ID NO. 15	PTA-123392	IhfB2 NTHI 7A4.E4.G4
		PTA-123394	IhfB2 NTHI 7A4.E4.G11

**[0149]** As noted above, this disclosure also provides an antibody fragment, antigen binding fragment or polypeptide that comprises, or consists essentially of, or yet consists of, a Fab fragment of an antibody disclosed in Table 1, e.g., an IhfmB4<sub>NTHI</sub> antibody or an equivalent of the antibody. In another aspect, the isolated polypeptide comprises, or alternatively consists essentially of, or yet further consists of a Fab fragment of an IhfA5<sub>NTHI</sub> antibody or an equivalent of the antibody.

**[0150]** In a further aspect, the present disclosure provides an isolated antibody disclosed in Table 1 or antigen binding fragment of an antibody disclosed in Table 1 that is at least 85% identical, or alternatively at least 90%, or alternatively at least 95 %, or alternatively at least 100% identical to an antibody fragment from an antibody disclosed in Table 1, or selected from the group consisting of (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5.

**[0151]** In one aspect, the present disclosure provides an isolated antibody fragment, antigen binding fragment or polypeptide comprising the CDRs of (i) the antibody produced by hybridoma cell line disclosed in Table 1, e.g., IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, or (iii) the antibody produced by

hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5. In one aspect, the present disclosure provides an isolated antibody fragment, antigen binding fragment or polypeptide from an antibody that has a CDR that is at least 85% or alternatively at least 90%, or alternatively at least 95 %, or alternatively at least 100% identical to (i) the antibody produced by hybridoma disclosed in Table 1, e.g., the cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, or (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5.

**[0152]** In some aspects of the antibody fragments, antigen binding fragments or polypeptides provided herein, the HC variable domain sequence of the antibody that provides such, comprises the HC variable domain sequence of (i) the antibody produced by a hybridoma cell line disclosed in Table 1, e.g., IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, or (iii) the antibody produced by hybridoma cell line MIhfB4 NTHI 12E6.F8.D12.D5; and/or the LC variable domain sequence comprises the LC variable domain sequence of antibody disclosed in Table 1, e.g., (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, or (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5.

**[0153]** In some aspects of the antibody fragment, antigen binding fragment or polypeptide provided herein, the HC variable domain sequence of the antibody that provides such sequence comprises a HC variable domain sequence at least 85% identical, or alternatively at least 90%, or alternatively at least 95 %, or alternatively at least 100% identical to a HC variable domain sequence of an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5; and/or the LC variable domain sequence comprises a LC variable domain sequence at least 85% identical or alternatively at least 90%, or alternatively at least 95 %, or alternatively at least 100% identical to the of LC variable domain sequence an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5.

**[0154]** In one aspect, the present disclosure provides Fab fragments comprising a heavy chain (HC) variable domain sequence and a light chain (LC) variable domain sequence,

wherein the heavy chain and light chain immunoglobulin variable domain sequences form an antigen binding site that binds to an epitope of a DNABII protein.

**[0155]** In some embodiments, the heavy chain variable region comprises a CDRH1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising the CDRH1 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0156]** In some embodiments, the heavy chain variable region comprises a CDRH2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising the CDRH2 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0157]** In some embodiments, the heavy chain variable region comprises a CDRH3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising the CDRH3 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0158]** In some embodiments, the heavy chain variable region comprises, or alternatively consists essentially of, or yet further consists of, the amino acid sequence comprising the heavy chain variable region sequence of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0159]** In some embodiments, the light chain variable region comprises a CDRL1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino

acid sequence comprising the CDRL1 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0160]** In some embodiments, the light chain variable region comprises a CDRL2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising the CDRL2 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0161]** In some embodiments, the light chain variable region comprises a CDRL3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising the CDRL3 of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0162]** In some embodiments, the light chain variable region comprises, or alternatively consists essentially of, or yet further consists of, the polypeptide encoded by the polynucleotide sequence comprising the light chain variable region sequence of any one of the following antibodies: an antibody disclosed in Table 1, e.g. (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0163]** In some embodiments, the heavy chain variable region of the antibody or the fragment thereof comprises a CDRH1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising FSLTSYS (SEQ ID NO. 58), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of FSLTSYSV (SEQ ID NO. 59), FSLTSYSVH (SEQ ID NO. 60), GFSLTSYS (SEQ ID NO. 61), or an equivalent each thereof.

**[0164]** In some embodiments, the heavy chain variable region of the antibody or fragment thereof comprises a CDRH1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising FNIKDYY (SEQ ID NO. 110), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of FNIKDYYM (SEQ ID NO. 111), FNIKDYYMH (SEQ ID NO. 112), GFNIKDYY (SEQ ID NO. 113), or an equivalent each thereof.

**[0165]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises a CDRH2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising IWAGGST (SEQ ID NO. 62), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of VIWAGGST (SEQ ID NO. 63), GVIWAGGST (SEQ ID NO. 64), LGVIWAGGST (SEQ ID NO. 65), WLGVVIWAGGST (SEQ ID NO. 66), IWAGGSTN (SEQ ID NO. 67), VIWAGGSTN (SEQ ID NO. 68), GVIWAGGSTN (SEQ ID NO. 69), LGVIWAGGSTN (SEQ ID NO. 70), WLGVVIWAGGSTN (SEQ ID NO. 71), IWAGGSTNY (SEQ ID NO. 72), VIWAGGSTNY (SEQ ID NO. 73), GVIWAGGSTNY (SEQ ID NO. 74), LGVIWAGGSTNY (SEQ ID NO. 75), WLGVVIWAGGSTNY (SEQ ID NO. 76), or an equivalent each thereof.

**[0166]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises a CDRH2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising IDPENDDT (SEQ ID NO. 114), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of WIDPENDDT (SEQ ID NO. 115), GWIDPENDDT (SEQ ID NO. 116), IGWIDPENDDT (SEQ ID NO. 117), WIGWIDPENDDT (SEQ ID NO. 118), IDPENDDTE (SEQ ID NO. 119), WIDPENDDTE (SEQ ID NO. 120), GWIDPENDDTE (SEQ ID NO. 121), IGWIDPENDDTE (SEQ ID NO. 122), WIGWIDPENDDTE (SEQ ID NO. 123), IDPENDDTEY (SEQ ID NO. 124), WIDPENDDTEY (SEQ ID NO. 125), GWIDPENDDTEY (SEQ ID NO. 126), IGWIDPENDDTEY (SEQ ID NO. 127), WIGWIDPENDDTEY (SEQ ID NO. 128), or an equivalent each thereof.

**[0167]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises a CDRH3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising REDS (SEQ ID NO. 77), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of AREDS (SEQ ID NO. 78) or an equivalent thereof.

**[0168]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises a CDRH3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising TELGAY (SEQ ID NO. 129) or an equivalent thereof.

**[0169]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the polypeptide encoded by the below noted polynucleotide sequences:

GAGGTGCAGCTGCAGGAGTCTGGACCTGGCCTGGTGACGCCCTCACAGAGCCTG  
TCCATGACTTGCACTGTCTCTGGGTTTCATTAACCAGCTATAAGTGTACACTGGGT  
TCGCCAGCCTCCAGGAAAGAGTCTGGAGTGGCTGGGAGTAATATGGGCTGGTGG  
AAGCACAAATTATAATTGGCTCTCATGTCCAGACTGAGCATCAGCAAAGACAA  
CTCCAAGAGCCAAGTTTCTAAAAATGGACAGTCTGCAAACACTGATGACACAGCC  
ATATACTACTGTGCCAGAGAGGACTCCTGGGTCAAGGAACCTCAGTCACCGTCT  
CCTCA (SEQ ID NO. 50) or an equivalent thereof.

**[0170]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the amino acid sequence:

EVQLQESGPLVTPSQSLSMTCVSGFSLTSYSVHWVRQPPGKSLEWLGVIWAGGST  
NYNSALMSRLSISKDNSKSQVFLKMDSLQTDDTAIYYCAREDSWGQGTSVTVSS  
(SEQ ID NO. 51) or an equivalent thereof.

**[0171]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the polypeptide encoded by the below noted polynucleotide sequences:

GAGGTGCAGCTGCAGGAGTCTGGGCAGAGCTTGTGAGGTAGGGCCTCAGTC  
AAGTTGCCTGCACAGCTTCTGGCTAACATTAAAGACTACTATATGCACTGGG  
TGAAGCAGAGGCCTGAACAGGGCCTGGAGTGGATTGGATGGATTGATCCTGAAA  
ATGATGATACTGAATATGTCCGAAGTCCAGGGCAAGGCCAGTATGACTGCAG  
ACACATCCTCCAACACACAGCCTACCTGCAGCTCAGCAGCCTGACATCTGAGGACA  
CTGCCGTCTATTACTGTACAGAGCTGGAGCTTACTGGGCCAGGGACTCTGGT  
C (SEQ ID NO. 52) or an equivalent thereof.

**[0172]** In some embodiments, the heavy chain variable region of the antibody or a fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the amino

acid sequence:

EVQLQESGAELVRSGASVKLSCTASGFNIKDYYMHWVKQRPEQGLEWIGWIDPEND  
DTEYVPKFQGKASMTADTSSNTAYLQLSSLTSEDTAVYYCTELGAYWGQQGTLV  
(SEQ ID NO. 53) or an equivalent thereof.

**[0173]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising QNVGTN (SEQ ID NO. 79), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of QNVGTNV (SEQ ID NO. 80), QNVGTNVA (SEQ ID NO. 81), or an equivalent each thereof.

**[0174]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL1 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising QSLLDSNGKTY (SEQ ID NO. 130), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of QSLLDSNGKTYL (SEQ ID NO. 131), QSLLDSNGKTYLN (SEQ ID NO. 132), or an equivalent each thereof.

**[0175]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising SAS (SEQ ID NO. 82), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of YSAS (SEQ ID NO. 83), IYSAS (SEQ ID NO. 84), LIYSAS (SEQ ID NO. 85), ALIYSAS (SEQ ID NO. 86), SASY (SEQ ID NO. 87), YSASY (SEQ ID NO. 88), IYSASY (SEQ ID NO. 89), LIYSASY (SEQ ID NO. 90), ALIYSASY (SEQ ID NO. 91), SASYR (SEQ ID NO. 92), YSASYR (SEQ ID NO. 93), IYSASYR (SEQ ID NO. 94), LIYSASYR (SEQ ID NO. 95), ALIYSASYR (SEQ ID NO. 96), SASYRY (SEQ ID NO. 97), YSASYRY (SEQ ID NO. 98), IYSASYRY (SEQ ID NO. 99), LIYSASYRY (SEQ ID NO. 100), ALIYSASYRY (SEQ ID NO. 101), SASYRYS (SEQ ID NO. 102), YSASYRYS (SEQ ID NO. 103), IYSASYRYS (SEQ ID NO. 104), LIYSASYRYS (SEQ ID NO. 105), ALIYSASYRYS (SEQ ID NO. 106), or an equivalent each thereof.

**[0176]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL2 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising LVS (SEQ ID NO. 133),

such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of YLVS (SEQ ID NO. 134), IYLV (SEQ ID NO. 135), LIYLV (SEQ ID NO. 136), RLIYLV (SEQ ID NO. 137), LVS (SEQ ID NO. 138), YLVS (SEQ ID NO. 139), IYLV (SEQ ID NO. 140), LIYLV (SEQ ID NO. 141), RLIYLV (SEQ ID NO. 142), LVS (SEQ ID NO. 143), YLVS (SEQ ID NO. 144), IYLV (SEQ ID NO. 145), LIYLV (SEQ ID NO. 146), RLIYLV (SEQ ID NO. 147), LVS (SEQ ID NO. 148), YLVS (SEQ ID NO. 149), IYLV (SEQ ID NO. 150), LIYLV (SEQ ID NO. 151), RLIYLV (SEQ ID NO. 152), LVS (SEQ ID NO. 153), YLVS (SEQ ID NO. 154), IYLV (SEQ ID NO. 155), LIYLV (SEQ ID NO. 156), RLIYLV (SEQ ID NO. 157), or an equivalent each thereof.

**[0177]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising QQYNSYP (SEQ ID NO. 108), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of QQYNSYPT (SEQ ID NO. 109), or an equivalent thereof.

**[0178]** In some embodiments, the light chain variable region of the antibody or a fragment thereof comprises a CDRL3 sequence comprising, or alternatively consisting essentially of, or yet further consisting of, an amino acid sequence comprising WQSTHFPH (SEQ ID NO. 158), such as but not limited to an amino acid sequence beginning with, ending with, or consisting essentially of WQSTHFPH (SEQ ID NO. 159) or an equivalent thereof.

**[0179]** In some embodiments, the light chain variable region of the antibody or fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the polypeptide encoded by the polynucleotide sequence:

GACATTGTGATGACCCAGTCTAAAAATTATGTCCACATCAGTAGGAGACAGG  
GTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGTACTAATGTAGCCTGGTATC  
AACAGAAACCAGGGCAATCTCCTAAAGCACTGATTACTCGGCATCCTACCGGTA  
CAGTGGAGTCCCTGATCGCTCACAGGCAGTGGATCTGGACAGATTCACTCTC  
ACCATCAGCAATGTGCAGTCTGAAGACTTGGCAGAGTATTCTGTCAGCAATATA  
ACAGCTATCCCACGTTGGAGGGGGACCAAGTTGGAAATAAAA (SEQ ID NO. 54) or an equivalent thereof.

**[0180]** In some embodiments, the light chain variable region of the antibody or fragment thereof comprises, or alternatively consists essentially of, or yet further consists of, the amino

acid sequence:

DIVMTQSQKFMSTSVGDRSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRYS  
GVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYNSYPTFGGGTKLEIK (SEQ ID  
NO. 55) or an equivalent thereof.

**[0181]** In some embodiments, the light chain variable region comprises, or alternatively consists essentially of, or yet further consists of, the polypeptide encoded by the polynucleotide sequence:

GATGTTGTGATGACCCAGATTCCACTCACTTGTGGTTACCATTGGACAACCAG  
CCTCCATCTCTTGCAAGTCAGTCAGAGCCTCTAGATAGTAATGGAAAGACATA  
TTTGAATTGGTTGTTTCAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTG  
GTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGA  
CAGATTCACACTGAAAATCAGCAGAGTTGAGGCTGAGGATTGGGAATTATTAA  
TTGCTGGCAAAGTACACATTTCCACACGTTGGAGGGGGACCAAGTTGGAA  
ATCAAA (SEQ ID NO. 56) or an equivalent thereof.

**[0182]** In some embodiments, the light chain variable region comprises, or alternatively consists essentially of, or yet further consists of, the amino acid sequence:

DVVMTQIPLTLSVTIGQPASISCKSSQSLDSNGKTYLNWLFQRPGQSPKRLIYLVSKL  
DSGVPDRFTGSGSGTDFTLKISRVEAEDLGIYYCWQSTHFPHTFGGGTKLEIK (SEQ  
ID NO. 57) or an equivalent thereof.

**[0183]** Exemplary antibodies comprising the disclosed CDR sequences and heavy and light chain variable sequences from which the antibody fragments, antigen binding fragments or polypeptides are derived are disclosed in **Table 2** and **Table 3**, respectively. Alternate CDR predictions may be made based on the heavy and/or light chain sequences – e.g., based on the Kabat, Clothia, AbM, or contact definitions of CDR specificity; details of these CDR prediction methods are known in the art (see, e.g., [bioinf.org.uk/abs/#cdrid](http://bioinf.org.uk/abs/#cdrid)) and/or commercially available. Those disclosed in **Table 2** are the result of utilizing the CDR prediction algorithms provided by the Oftran Lab (Paratome available at [ofranservices.biu.ac.il/site/services/paratome/index.html](http://ofranservices.biu.ac.il/site/services/paratome/index.html)) and Green Mountain Antibodies' CDR prediction program.

**Table 2**

ANTIBODY	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
Ihf A5	SEQ ID					

	NO. 60 or SEQ ID NO. 61	NO. 62 or SEQ ID NO. 76	NO. 77 or SEQ ID NO. 78	NO. 79 or SEQ ID NO. 81	NO. 82 or SEQ ID NO. 106	NO. 108 or SEQ ID NO. 109
Ihf mB4	SEQ ID NO. 112 or SEQ ID NO. 113	SEQ ID NO. 114 or SEQ ID NO. 128	SEQ ID NO. 129	SEQ ID NO. 131 or SEQ ID NO. 132	SEQ ID NO. 133 or SEQ ID NO. 157	SEQ ID NO. 158 or SEQ ID NO. 159

**Table 3**

ANTIBODY	Heavy Chain Variable Region	Light Chain Variable Region
Ihf A5	SEQ ID NO. 51	SEQ ID NO. 55
Ihf mB4	SEQ ID NO. 53	SEQ ID NO. 57

**[0184]** In one aspect, the present disclosure provides antibody fragments (e.g., a Fab fragment) of an isolated antibody that is at least 85%, or alternatively at least 90%, or alternatively at least 95%, or 100% identical to an antibody selected from the group consisting of an antibody disclosed in Table 1, e.g. Ihf A5, Ihf mB4, or a biological equivalent of each thereof.

**[0185]** In one aspect, the present disclosure provides isolated antibody fragments comprising the CDRs of Ihf A5. In one aspect, the present disclosure provides isolated antibody fragments of an antibody that is at least 85%, or alternatively at least 90%, or alternatively at least 95 %, or 100% identical to Ihf A5 or a biological equivalent thereof.

**[0186]** In one aspect, the present disclosure provides isolated antibody fragments comprising the CDRs of Ihf mB4. In one aspect, the present disclosure provides isolated antibody fragments of an isolated antibody that is at least 85%, or alternatively at least 90%, or alternatively at least 95%, or 100% identical to Ihf mB4 or a biological equivalent thereof.

**[0187]** In some aspects of the antibody fragments provided herein, the HC variable domain sequence comprises a variable domain sequence of Ihf A5 and the LC variable domain sequence comprises a variable domain sequence of Ihf A5.

**[0188]** In some aspects of the antibody fragments provided herein, the HC variable domain sequence comprises a variable domain sequence of Ihf mB4 and the LC variable domain sequence comprises a variable domain sequence of Ihf mB4.

**[0189]** In another aspect of the present technology, the antibody fragments include one or more of the following characteristics:

(a) the light chain immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85%, or alternatively at least 90%, or alternatively at least 95 % or 100% identical to a CDR of a light chain variable domain of any of the disclosed light chain sequences;

(b) the heavy chain immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85% , or alternatively at least 90%, or alternatively at least 95 %, or 100% identical to a CDR of a heavy chain variable domain of any of the disclosed heavy chain sequences;

(c) the light chain immunoglobulin variable domain sequence is at least 85% , or alternatively at least 90%, or alternatively at least 95 %, or 100% identical to a light chain variable domain of any of the disclosed light chain sequences;

(d) the HC immunoglobulin variable domain sequence is at least 85% , or alternatively at least 90%, or alternatively at least 95 %, or 100% identical to a heavy chain variable domain of any of the disclosed light chain sequences; and

(e) the antibody binds an epitope that overlaps with an epitope bound by any of the disclosed sequences.

**[0190]** In some of the aspects of the antibodies provided herein, the antibody fragment, antigen binding fragment or polypeptides binds a DNABII protein with a dissociation constant (KD) of less than  $10^{-4}$  M,  $10^{-5}$  M,  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M, or  $10^{-12}$  M. In some of the aspects of the antibody fragments or polypeptides provided herein, the antigen binding site specifically binds to a DNABII protein. In another aspect, the affinity of the antibody or antigen binding fragment is less than or about 1000 picoMole (pM), 900 pM, 800 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM, about 100 pM, 50 pM, 40 pM , 30 pM, 20 pM, 10 pM, 9 pM, and 8 pM.

**[0191]** In some of the aspects of the antibodies provided herein, the antibody fragment or antigen binding fragment is soluble Fab. In another aspect, the polypeptide comprises, or alternatively consists essentially of, or yet further consists of, a soluble Fab of antibody disclosed herein, or an equivalent of the soluble Fab.

**[0192]** In some of the aspects of the antibodies provided herein, the antibody from which the antibody fragment, antigen binding fragment or polypeptide is a rabbit antibody. In some of the aspects of the antibodies provided herein, the antibody from which the antibody fragment, antigen binding fragment or polypeptide is derived is a human or humanized antibody or is non-immunogenic in a human.

**[0193]** In some of the aspects of the antibodies provided herein, the antibody from which the antibody fragment, antigen binding fragment or polypeptide is derived comprises a human antibody framework region (e.g., a humanized antibody). In some aspects of the antibodies from which the antibody fragment, antigen binding fragment or polypeptide provided herein is derived, the antibody comprises an antibody framework region from a non-human species such as a simian, equine, feline, canine, bovine, porcine, caprine, or ovine. In some aspects of the antibodies from which the antibody fragment, antigen binding fragment or polypeptide is derived, the antibody is a chimeric antibody. In any one or more of these aspects, the antibody may further comprise one or more of the CDR regions and/or the heavy and/or light chains disclosed herein.

**[0194]** In other aspects, one or more amino acid residues in a CDR of the antibodies provided herein are substituted with another amino acid. The substitution may be “conservative” in the sense of being a substitution within the same family of amino acids. The naturally occurring amino acids may be divided into the following four families and conservative substitutions will take place within those families.

- 1) Amino acids with basic side chains: lysine, arginine, histidine.
- 2) Amino acids with acidic side chains: aspartic acid, glutamic acid
- 3) Amino acids with uncharged polar side chains: asparagine, glutamine, serine, threonine, tyrosine.
- 4) Amino acids with nonpolar side chains: glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, cysteine.

**[0195]** In another aspect, one or more amino acid residues are added to or deleted from one or more CDRs of an antibody. Such additions or deletions occur at the N or C termini of the CDR or at a position within the CDR.

**[0196]** By varying the amino acid sequence of the CDRs of an antibody by addition, deletion or substitution of amino acids, various effects such as increased binding affinity for the target antigen may be obtained.

**[0197]** It is to be appreciated that antibodies of the present disclosure comprising such varied CDR sequences still bind a DNABII protein with similar specificity and sensitivity profiles as the disclosed antibodies. This may be tested by way of the binding assays.

**[0198]** The constant regions of antibodies may also be varied. For example, antibodies may be provided with Fc regions of any isotype: IgA (IgA1, IgA2), IgD, IgE, IgG (IgG1, IgG2, IgG3, IgG4) or IgM. The constant regions of antibodies may further be adapted for specific therapeutic indications, e.g., according to the teachings of Irani et al. (2015) Molecular Immunol. 67:171-182.

**[0199]** As noted in Irani et al. (2015), Fc engineering is important in developing therapeutic antibodies with potent and specific activity, therefore reducing both dosage and potential side-effects. An understanding of the differences across the IgG subclasses has been used in Fc engineering and studies have shown that introducing specific residues from one subclass to another can transform certain effector functions, while retaining others (Armour et al. (1999) Eur. J. Immunol. 29:2613-2624; Armour et al. (2003) Mol. Immunol. 40:585-593; Hessel et al. (2007) Nature 449:101-104; Redpath et al. (1998) Hum. Immunol. 59:720-727; Vafa et al. (2014) Methods (San Diego, CA) 65:114-126). These Fc engineering approaches are pertinent in the context of infectious diseases, as specific antibody effector function is often critical in efficient pathogen clearance.

**[0200]** The structural and functional properties of the IgG subclasses vary, as do their response profiles to different infectious diseases, and these differences can be utilized in the development of effective therapeutic antibodies (Carter (2006) Nat. Rev. Immunol. 6:343-357; Jefferis (2012) Arch. Biochem. Biophys. 526:159-166). Although the heavy chains share greater than 90% sequence identity across IgG sub classes (Rispens and Vidarsson (2014) Nimmerjahn, M.E.A. (Ed.), Chapter 9 – Human IgG Subclasses. Academic Press. Boston. pp.159-177.), there are differences in surface exposed residues on the constant (CH1, CH2 and CH3) domains, as well as substantial variation within the hinge region. It is the hinge structure that confers many of the unique properties to each IgG subclass such as stability, flexibility and distances spanned by the two Fabs and the attendant Fc (Liu and May (2012) mAbs 4:17-23; Roux et al. (1997) J. Immunol. (Baltimore, MD: 1950) 159:3372-3382; Tian et al. (2014) Pharm. Sci. 103:1701-1710). Importantly, some areas of the Fc and the hinge that differ between IgG subclasses clearly overlap with residues known to be involved with binding to both activating and inhibitory Fc $\gamma$  receptors (Fc $\gamma$ R), the neonatal receptor for IgG (FcRn) and complement component C1q. The occurrence of key amino acid differences

within the binding sites of these effector molecules helps explain the observed differences in the effector properties of the IgG subclasses. This structural and molecular information is important when choosing a subclass backbone for a therapeutic antibody or introducing changes in key amino acids to tailor antibodies for a specific purpose.

**[0201]** Non-limiting examples of constant region sequences include:

Human IgD constant region, Uniprot: P01880 SEQ ID NO. 34:

APTKAPDVFPPISGCRHPKDNSPVVLACLITGYHPTSVTVWYMGTSQSPQRTFPEIQ  
RRDSYYMTSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPEPKAQASSVPTA  
QPQAEGLAKATTAPATTRNTGRGGEEKKEKEKEEQQEERETKTPECPSPHTQPLGVY  
LLTPAVQDLWLRDKATFTCFVVGSPLKDAHLTWEVAGKVPTGGVEEGLLERHSNG  
SQSQHSRLLTLPRSLWNAGTSVTCTLNHPQLPQRLMALREPAAQAPVKLSLNLLASS  
DPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVL  
RVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDHGP MK.

Human IgG1 constant region, Uniprot: P01857 SEQ ID NO. 35:

ASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHCPPCPAP  
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVFKFNWYVDGVEVHNAK  
TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE  
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDG  
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

Human IgG2 constant region, Uniprot: P01859 SEQ ID NO. 36:

ASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSNFGTQTYTCNVNDHKPSNTKVDKTVERKCCVECPCPAPPVA  
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR  
EEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKKGQPREPQVY  
TLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTPVLDSDGSFLL  
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

Human IgG3 constant region, Uniprot: P01860 SEQ ID NO. 37:

ASTKGPSVFP LAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
QSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKVELKPLGDTTHCPRC  
PEPKSCDTPPPCPRCPEPKSCDTPPPCCPRCPEPKSCDTPPPCCPRCPAPELLGGPSVFLFPP  
KPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFR

VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKGQPREPVYTLPPSREEM  
TKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLSDGSFFLYSKLTVDKS  
RWQQGNIFSCSVMHEALHNRTQKSLSLSPGK.

Human IgM constant region, Uniprot: P01871 SEQ ID NO. 38:

GSASAPTLFPLVSCENSPSDTSSAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPSV  
LRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVS  
FVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESG  
PTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPS  
FASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKHTHNISESHPNATFSAVGEAS  
ICEDDWNSGERFTCTVHTDLPPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESA  
TITCLVTGFSPADVFWQMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEE  
WNTGETYTCVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY.

Human IgG4 constant region, Uniprot: P01861 SEQ ID NO. 39:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTKYTCNCVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLG  
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR  
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPVY  
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFL  
YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK.

Human IgA1 constant region, Uniprot: P01876 SEQ ID NO. 40:

ASPTSPKVFPPLSLCSTQPDGNVVIACLVQGFPQEPLSVTWSESGQGVTARNFPPSQD  
ASGDLYTTSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCTPVPSTPPTPSPSTPP  
TPSPSCCHPRLSLHRALEDELLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGP  
PERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKPLTATLSKSGNTFRPEVH  
LLPPPSEELALNELVLTCLARGFSPKDVLVRWLQGSQELPREKYLTwASRQEPSQG  
TTTFAVTSILRVAEAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVV  
MAEVDGTCY.

Human IgA2 constant region, Uniprot: P01877 SEQ ID NO. 41:

ASPTSPKVFPPLSLDSTPQDGNNVVACLVQGFPQEPLSVTWSESGQNVTARNFPPSQD  
ASGDLYTTSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPCTPVPFFFFCCHPRLSL  
HRALEDELLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGP PERDLCGCYSVS  
SVLPGCAQPWNHGETFTCTAAHPELKPLTANITKSGNTFRPEVHLLPPPSEELALNE

LVTLTCLARGFSPKDVLVRWLQGSQELPREKYL TWASRQE PSQGTTFAVTSILRVA  
AEDWKKGDTFSCMV GHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEV DGT CY.

Human Ig kappa constant region, Uniprot: P01834 SEQ ID NO. 42:

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
QDSKDSTYSLSTTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

**[0202]** In some embodiments, the antibody fragments, antigen binding fragments or polypeptides are derived from antibodies specific to the IhfA fragment A5 (SEQ ID NO. 13) and comprise constant regions derived from IgM or IgG, such as but not limited to IgG2 (in certain aspects IgG2a). In instances where the parental antibody is a monoclonal antibody, exemplary isotypes include, but are not limited to, IgG such as IgG1, IgG2, IgG3, and IgG4, IgM, IgA such as IgA1 and IgA2, IgD, and IgE and can preferably include IgG and IgM. The isotype and subclass of the monoclonal antibody can be determined by, for example, an Ouchterlony test, ELISA, or radio immunoassay (hereinafter, referred to as "RIA"). A commercially available kit for identification (e.g., Mouse Typer Kit; Bio-Rad Laboratories, Inc., and RAT MONOCLONAL ANTIBODY ISOTYPING TEST KIT: AbD Serotec) may be used.

**[0203]** In some embodiments, the antibody fragments, antigen binding fragments or polypeptides are derived from antibodies specific to the IhfB fragment mB4 (SEQ ID NO. 17) or an equivalent thereof, and comprise constant regions derived from IgG, such as but not limited to IgG1.

**[0204]** In some aspects, the antibody fragments, antigen binding fragments or polypeptides are derived from an antibodies that comprise a heavy chain constant region that is at least 80% identical, or alternatively as least 85%, or alternatively at least 90%, or alternatively at least 95 % or alternatively at least 100% identical, to the heavy chain constant region sequence of any one of the following antibodies: (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5 or an equivalent of each thereof.

**[0205]** In some aspects, the antibody fragments, antigen binding fragments or polypeptides are derived from an antibody that comprises a light chain constant region that is at least 80% identical, or alternatively as least 85%, or alternatively at least 90%, or alternatively at least 95 % or alternatively at least 100% identical, to the light chain constant region sequence of

any one of the following parental antibodies: (i) the antibody produced by hybridoma cell line IhfA5 NTHI 14G8.F5.G6, (ii) the antibody produced by hybridoma cell line IhfB4 NTHI 4E11.E5.G2, and (iii) the antibody produced by hybridoma cell line mIhfB4 NTHI 12E6.F8.D12.D5, or an equivalent of each thereof.

**[0206]** In some aspects of the antibodies from which the antibody fragment, antigen binding fragment or polypeptide as provided herein, the parental antibody or Fab (fragment antigen binding) fragments bind to the epitope bound by DNABII antibodies.

**[0207]** In some aspects of the parental antibodies provided herein, the antibody fragment contains structural modifications to facilitate rapid binding and cell uptake and/or slow release..

**[0208]** The antibody fragments, antigen binding fragments and polypeptides and equivalents thereof can be combined with a detectable or purification label and/or a carrier, e.g., a pharmaceutically acceptable carrier or other agents to provide a formulation for use and/or storage.

#### *Methods for antibody production*

**[0209]** The general structure of antibodies is known in the art and will only be briefly summarized here. An immunoglobulin monomer comprises two heavy chains and two light chains connected by disulfide bonds. Each heavy chain is paired with one of the light chains to which it is directly bound *via* a disulfide bond. Each heavy chain comprises a constant region (which varies depending on the isotype of the antibody) and a variable region. The variable region comprises three hypervariable regions (or complementarity determining regions) which are designated CDRH1, CDRH2 and CDRH3 and which are supported within framework regions. Each light chain comprises a constant region and a variable region, with the variable region comprising three hypervariable regions (designated CDRL1, CDRL2 and CDRL3) supported by framework regions in an analogous manner to the variable region of the heavy chain.

**[0210]** The hypervariable regions of each pair of heavy and light chains mutually cooperate to provide an antigen binding site that is capable of binding a target antigen. The binding specificity of a pair of heavy and light chains is defined by the sequence of CDR1, CDR2 and CDR3 of the heavy and light chains. Thus once a set of CDR sequences (i.e., the sequence of CDR1, CDR2 and CDR3 for the heavy and light chains) is determined which gives rise to a particular binding specificity, the set of CDR sequences can, in principle, be inserted into the

appropriate positions within any other antibody framework regions linked with any antibody constant regions in order to provide a different antibody with the same antigen binding specificity.

**[0211]** Antibodies for the production of the Fab fragments can be generated using conventional techniques known in the art and are well-described in the literature. Several methodologies exist for production of polyclonal antibodies. For example, polyclonal antibodies are typically produced by immunization of a suitable mammal such as, but not limited to, chickens, goats, sheep, guinea pigs, hamsters, horses, dogs, mice, rats, and rabbits. An antigen is injected into the mammal, and induces the B-lymphocytes to produce immunoglobulins specific for the antigen. Immunoglobulins may be purified from the mammal's serum. Antibodies specific to an IHF $\alpha$  and/or an IHF $\beta$  subunit can be generated by injection of polypeptides corresponding to different epitopes of IHF $\alpha$  and IHF $\beta$ . For example, antibodies can be generated using the 20 amino acids of each subunit such as SEQ ID NOS. 12 and 13 for IHF $\alpha$  (fragments A3 and A5 of IHF, respectively), SEQ ID NO. 14 for HU (fragment A5 of HU), SEQ ID NOS. 15 to 17 for IHF $\beta$  (fragments B2, B4, and mB4 of IHF).

**[0212]** Variations of this methodology include modification of adjuvants, routes and site of administration, injection volumes per site and the number of sites per animal for optimal production and humane treatment of the animal. For example, adjuvants typically are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site antigen depot, which allows for a slow release of antigen into draining lymph nodes. Other adjuvants include surfactants which promote concentration of protein antigen molecules over a large surface area and immunostimulatory molecules. Non-limiting examples of adjuvants for polyclonal antibody generation include Freund's adjuvants, Ribi adjuvant system, MPL derived adjuvants, those derived from the heat labile enterotoxin of *E. coli* (e.g., dLT = double mutant labile toxin), and Titermax. Polyclonal antibodies can be generated using methods known in the art some of which are described in U.S. Patent Nos. 7,279,559; 7,119,179; 7,060,800; 6,709,659; 6,656,746; 6,322,788; 5,686,073; and 5,670,153.

**[0213]** Monoclonal antibodies can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, P3X63Ag8.653, Sp2 SA3, Sp2 MA1, Sp2

SS1, Sp2 SA5, U397, MIA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 313, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/O) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived there from, or any other suitable cell line as known in the art (see, those at the following web addresses, e.g., atcc.org, lifetech.com, last accessed on Nov. 26, 2007), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. Antibody producing cells can also be obtained from the peripheral blood or, in particular embodiments, the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing-heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present disclosure. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Some embodiments disclosed herein relate to specific hybridomas that produce monoclonal antibodies to IHF fragments; non-limiting examples include IhfA5 NTHI 14G8.F5.G6 (ATCC No. PTA-122334), IhfB4 NTHI 4E11.E5.G2 (ATCC No. PTA-122336), mIhfB4 NTHI 12E6.F8.D12.D5 (ATCC No. PTA-122335).

**[0214]** Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, cDNA, or the like, display library; e.g., as available from various commercial vendors such as MorphoSys (Martinsreid/Planegg, Del.), BioInvent (Lund, Sweden), Affitech (Oslo, Norway) using methods known in the art. Art known methods are described in the patent literature some of which include U.S. Pat. Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al. (1977) *Microbiol. Immunol.* 41:901-907 (1997); Sandhu et al. (1996) *Crit. Rev. Biotechnol.* 16:95-118; Eren et

al. (1998) *Mumma* 93:154-161) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display, e.g., Wanes et al. (1997) *Proc. Natl. Acad. Sci. USA*, 94:4937-4942; Hanes et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:14130-14135); single cell antibody producing technologies (e.g., selected lymphocyte antibody method (“SLAM”) (U.S. Pat. No. 5,627,052, Wen et al. (1987) *J. Immunol* 17:887-892; Babcock et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848); gel microdroplet and flow cytometry (Powell et al. (1990) *Biotechnol.* 8:333-337; One Cell Systems, (Cambridge, Mass.); Gray et al. (1995) *J. Imm. Meth.* 182:155-163; and Kenny et al. (1995) *Bio. Technol.* 13:787-790); B-cell selection (Steenbakkers et al. (1994) *Molec. Biol. Reports* 19:125-134). An additional method relies on the isolation of human antibodies from human serum, using techniques known in the art, see U.S. Patent No. 7,939,344.

**[0215]** Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents (Orlandi et al. (1989) *PNAS* 86: 3833-3837; Winter et al. (1991) *Nature* 349: 293-299).

**[0216]** Alternatively, techniques for the production of single chain antibodies may be used. Single chain antibodies (scF<sub>v</sub>s) comprise a heavy chain variable region and a light chain variable region connected with a linker peptide (typically around 5 to 25 amino acids in length). In the scF<sub>v</sub>, the variable regions of the heavy chain and the light chain may be derived from the same antibody or different antibodies. scF<sub>v</sub>s may be synthesized using recombinant techniques, for example by expression of a vector encoding the scF<sub>v</sub> in a host organism such as *E. coli*. DNA encoding scF<sub>v</sub> can be obtained by performing amplification using a partial DNA encoding the entire or a desired amino acid sequence of a DNA selected from a DNA encoding the heavy chain or the variable region of the heavy chain of the above-mentioned antibody and a DNA encoding the light chain or the variable region of the light chain thereof as a template, by PCR using a primer pair that defines both ends thereof, and further performing amplification combining a DNA encoding a polypeptide linker portion and a primer pair that defines both ends thereof, so as to ligate both ends of the linker to the heavy chain and the light chain, respectively. An expression vector containing the DNA encoding scF<sub>v</sub> and a host transformed by the expression vector can be obtained according to conventional methods known in the art.

**[0217]** Antigen binding fragments, for example the F(ab')<sub>2</sub> fragments, can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Fab fragments, in particular murine Fab fragments produced from murine monoclonal antibodies, can also be produced by digestion of the antibody with the thiol protease ficin in the presence of cysteine. Fab fragments, in particular rabbit Fab fragments produced from rabbit polyclonal antibodies, may also be produced by digestion of the antibody with the protease papain in the presence of cysteine-HCl. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al. (1989) *Science* 256:1275-1281). Upon generation of the fragment, the fragment can be isolated and sequenced using conventional techniques and the amino acid sequence is determined.

**[0218]** Antibody derivatives of the present disclosure can also be prepared by delivering a polynucleotide encoding an antibody fragment, antigen binding fragment or polypeptide as disclosed herein to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are known in the art and are described for example in U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

**[0219]** The term “antibody derivative” includes post-translational modification to linear polypeptide sequence of the antibody or fragment. For example, U.S. Patent No. 6,602,684 B1 describes a method for the generation of modified glycol-forms of antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having enhanced Fe-mediated cellular toxicity, and glycoproteins so generated.

**[0220]** The parental antibodies from which the antibody fragments, antigen binding fragments and polypeptides as disclosed herein, also include derivatives that are modified by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. Antibody derivatives include, but are not limited to, antibodies that have been modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Non-limiting examples of the modified antibodies contemplated herein are agylcosylated whole antibody molecules, antibody fragments, or fusion proteins that include a region

equivalent to the Fc region of an immunoglobulin. Such aglycosyltae forms can be generated, for example, in host cells that lack the ability to modify proteins with N-linked glycans or by mutating N-linked consensus sites on the antibody of interest. Additionally, the derivatives may contain one or more non-classical amino acids.

**[0221]** Antibody derivatives of the parental antibodies also can be prepared by delivering a polynucleotide disclosed herein to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al. (1999) *Curr. Top. Microbiol. Immunol.* 240:95-118 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al. (1999) *Adv. Exp. Med. Biol.* 464:127-147 and references cited therein. Antibody derivatives have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al. (1998) *Plant Mol. Biol.* 38:101-109 and references cited therein. Thus, antibodies can also be produced using transgenic plants, according to known methods.

**[0222]** Antibody derivatives of the parental antibodies also can be produced, for example, by adding exogenous sequences to modify immunogenicity or to reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

**[0223]** Humanization or engineering of antibodies can be performed using any known method such as, but not limited to, those described in U.S. Pat. Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

**[0224]** Further provided are the isolated polypeptides comprising, or alternatively consisting essentially of, or yet further consisting of, the Fab fragment or antigen binding

fragmet, or a polypeptide as disclosed herein tht further comprises a detectable or a purification label, as described herein.

**[0225]** Chimeric, humanized or primatized parental antibodies of the antibody fragments, antigen binding fragments or polypeptides of the present disclosure can be prepared based on the sequence of a reference monoclonal antibody prepared using standard molecular biology techniques. Such antibodies may be used to generate the antibody fragments (e.g., Fab fragment or antigen binding fragment) of the present disclosure.

**[0226]** DNA encoding the heavy and light chain immunoglobulins can be obtained from the hybridoma of interest and engineered to contain non-reference (e.g., human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (U.S. Patent No. 4,816,567). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (U.S. Patent No. 5,225,539 and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762; and 6,180,370). Similarly, to create a primatized antibody the murine CDR regions can be inserted into a primate framework using methods known in the art (PCT International Pat. Application Publication Nos. WO 93/02108 and WO 99/55369).

**[0227]** Techniques for making partially to fully human antibodies are known in the art and any such techniques can be used. According to one embodiment, fully human antibody sequences are made in a transgenic mouse which has been engineered to express human heavy and light chain antibody genes. Multiple strains of such transgenic mice have been made which can produce different classes of antibodies. B cells from transgenic mice which are producing a desirable antibody can be fused to make hybridoma cell lines for continuous production of the desired antibody. (See, for example, Russel et al. (2000) Infection and Immunity April 2000:1820-1826; Gallo et al. (2000) European J. of Immun. 30:534-540; Green (1999) J. of Immun. Methods 231:11-23; Yang et al. (1999A) J. of Leukocyte Biology 66:401-410; Yang (1999B) Cancer Research 59(6):1236-1243; Jakobovits (1998) Advanced Drug Reviews 31:33-42; Green and Jakobovits (1998) J. Exp. Med. 188(3):483-495; Jakobovits (1998) Exp. Opin. Invest. Drugs 7(4):607-614; Tsuda et al. (1997) Genomics 42:413-421; Sherman-Gold (1997) Genetic Engineering News 17(14); Mendez et al. (1997) Nature Genetics 15:146-156; Jakobovits (1996) Weir's Handbook of Experimental Immunology, The Integrated Immune System Vol. IV, 194.1-194.7; Jakobovits (1995) Current Opinion in Biotechnology 6:561-566; Mendez et al. (1995) Genomics 26:294-307;

Jakobovits (1994) Current Biology 4(8):761-763; Arbones et al. (1994) Immunity 1(4):247-260; Jakobovits (1993) Nature 362(6417):255-258; Jakobovits et al. (1993) Proc. Natl. Acad. Sci. USA 90(6):2551-2555; and U.S. Pat. No. 6,075,181.)

**[0228]** The antibodies disclosed herein also can be modified to create chimeric antibodies. Chimeric antibodies are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species. See, e.g., U.S. Pat. No. 4,816,567.

**[0229]** Alternatively, the antibodies disclosed herein can also be modified to create veneered antibodies. Veneered antibodies are those in which the exterior amino acid residues of the antibody of one species are judiciously replaced or "veneered" with those of a second species so that the antibodies of the first species will not be immunogenic in the second species thereby reducing the immunogenicity of the antibody. Since the antigenicity of a protein is primarily dependent on the nature of its surface, the immunogenicity of an antibody could be reduced by replacing the exposed residues which differ from those usually found in another mammalian species antibodies. This judicious replacement of exterior residues should have little, or no, effect on the interior domains, or on the interdomain contacts. Thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues. The process is referred to as "veneering" since only the outer surface or skin of the antibody is altered, the supporting residues remain undisturbed.

**[0230]** The procedure for "veneering" makes use of the available sequence data for human antibody variable domains compiled by Kabat et al. (1987) Sequences of Proteins of Immunological interest, 4th ed., Bethesda, Md., National Institutes of Health, updates to this database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Non-limiting examples of the methods used to generate veneered antibodies include EP 519596; U.S. Pat. No. 6,797,492; and described in Padlan et al. (1991) Mol. Immunol. 28(4-5):489-498.

**[0231]** The antibody fragments, antigen binding fragments and polypeptides as disclosed herein can be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,

hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography (“HPLC”) can also be used for purification.

**[0232]** Antibodies for the preparation the antibody fragments, antigen binding fragments or of the present disclosure include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells, or alternatively from a prokaryotic host as described above. A number of antibody production systems are described in Birch & Radner (2006) *Adv. Drug Delivery Rev.* 58:671-685.

**[0233]** If an antibody fragment being tested binds with protein or polypeptide, then the antibody fragment being tested and the antibody fragment provided by this disclosure are equivalent. It also is possible to determine without undue experimentation, whether an antibody fragment has the same specificity as the antibody fragment disclosed herein by determining whether the antibody being tested prevents an antibody fragment disclosed herein from binding the protein or polypeptide with which the antibody fragment is normally reactive. If the antibody fragment being tested competes with the antibody fragment disclosed herein as shown by a decrease in binding by the antibody fragment disclosed herein, then it is likely that the two antibody fragments bind to the same or a closely related epitope. Alternatively, one can pre-incubate the antibody fragment disclosed herein with a protein with which it is normally reactive, and determine if the antibody fragment being tested is inhibited in its ability to bind the antigen. If the antibody fragment being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the antibody fragment disclosed herein.

**[0234]** The term “antibody” and “antibody fragment” also is intended to include antibodies of all immunoglobulin isotypes and subclasses. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from an initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:8653 or Spira et al. (1984) *J. Immunol. Methods* 74:307. Alternatively, recombinant DNA techniques may be used.

**[0235]** The isolation of other monoclonal antibodies for use in generating antibody fragments, antigen binding fragments and polypeptides, with the specificity of the monoclonal antibodies described herein can also be accomplished by one of ordinary skill in

the art by producing anti-idiotypic antibodies. Herlyn et al. (1986) *Science* 232:100. An anti-idiotypic antibody is an antibody that recognizes unique determinants present on the monoclonal antibody of interest.

**[0236]** In some aspects disclosed herein, it will be useful to detectably or therapeutically label the antibody fragment, antigen binding fragment or polypeptide. Suitable labels are described herein. Methods for conjugating antibodies and polypeptides to these agents are known in the art. For the purpose of illustration only, the antibody fragment, antigen binding fragment or polypeptide can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either *in vivo*, or in an isolated test sample.

**[0237]** The coupling of antibodies, fragments and polypeptides to low molecular weight haptens can increase the sensitivity of the antibody fragment or polypeptide in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See, Harlow and Lane (1988) *supra*.

**[0238]** The variable region of the parental antibodies of the present disclosure can be modified by mutating amino acid residues within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions to improve one or more binding properties (e.g., affinity) of the antibody. Mutations may be introduced by site-directed mutagenesis or PCR-mediated mutagenesis and the effect on antibody binding, or other functional property of interest, can be evaluated in appropriate *in vitro* or *in vivo* assays. In certain embodiments, conservative modifications are introduced and typically no more than one, two, three, four or five residues within a CDR region are altered. The mutations may be amino acid substitutions, additions or deletions.

**[0239]** Framework modifications can be made to the parental antibodies to decrease immunogenicity, for example, by “backmutating” one or more framework residues to the corresponding germline sequence.

**[0240]** In addition, the antibody fragments, antigen binding fragments or polypeptides disclosed herein may be engineered to include modifications within the Fc region to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Such modifications include, but are not limited to, alterations of the number of cysteine residues in the hinge

region to facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody (U.S. Patent No. 5,677,425) and amino acid mutations in the Fc hinge region to decrease die biological half-life of the antibody (U.S. Patent No. 6,165,745).

**[0241]** Additionally, the antibody fragments disclosed herein may be chemically modified. Glycosylation of an antibody can be altered, for example, by modifying one or more sites of glycosylation within the antibody sequence to increase the affinity of the antibody for antigen (U.S. Patent Nos. 5,714,350 and 6,350,861). Alternatively, to increase antibody-dependent cell-mediated cytotoxicity, a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures can be obtained by expressing the antibody in a host cell with altered glycosylation mechanism (Shields et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-180).

**[0242]** The antibodies fragment, antigen binding fragment or polypeptide as disclosed herein can be pegylated to increase biological half-life by reacting the antibody or fragment thereof with polyethylene glycol (PEG) or a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Pegylation may be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water soluble polymer). As used herein, the term “polyethylene glycol” is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. The antibody to be pegylated can be an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies disclosed herein (EP 0154316 and EP 0401384).

**[0243]** Additionally, the antibody binding fragment, antigen binding fragment or polypeptides may be chemically modified by conjugating or fusing the antigen-binding region to serum protein, such as human serum albumin, to increase half-life of the resulting molecule. Such approach is for example described in EP 0322094 and EP 0486525.

**[0244]** The antibody fragment, antigen binding fragment or polypeptide as disclosed herein may be conjugated to a diagnostic agent and used diagnostically, for example, to monitor the development or progression of a disease and determine the efficacy of a given treatment regimen. Examples of diagnostic agents include enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive

paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or fragment thereof, or indirectly, through a linker using techniques known in the art. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. An example of a luminescent material includes luminol. Examples of bioluminescent materials include luciferase, luciferin, and aequorin. Examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-1105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199, and Lead-211. Monoclonal antibodies may be indirectly conjugated with radiometal ions through the use of bifunctional chelating agents that are covalently linked to the antibodies. Chelating agents may be attached through amities (Meares et al. (1984) *Anal. Biochem.* 142:68-78); sulfhydral groups (Koyama 1994 *Chem. Abstr.* 120: 217262t) of amino acid residues and carbohydrate groups (Rodwell et al. (1986) *PNAS USA* 83:2632-2636; Quadri et al. (1993) *Nucl. Med. Biol.* 20:559-570).

**[0245]** Further, the antibody fragment, antigen binding fragment or polypeptide of the present disclosure may be conjugated to a therapeutic agent. Suitable therapeutic agents include antibiotics or antimicrobials for example, or host defense peptides (e.g., specifically targeted antimicrobial peptides (STAMPs)).

**[0246]** Additional suitable conjugated molecules include ribonuclease (RNase), DNase, an antisense nucleic acid, an inhibitory RNA molecule such as a siRNA molecule, an immunostimulatory nucleic acid, aptamers, ribozymes, triplex forming molecules, and external guide sequences. Aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets, and can bind small molecules, such as ATP (U.S. Pat. No. 5,631,146) and theophiline (U.S. Pat. No. 5,580,737), as well as large molecules, such as reverse transcriptase (U.S. Pat. No. 5,786,462) and thrombin (U.S. Pat. No. 5,543,293). Ribozymes

are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intra-molecularly or inter-molecularly. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. Triplex forming function nucleic acid molecules can interact with double-stranded or single-stranded nucleic acid by forming a triplex, in which three strands of DNA form a complex dependent on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules can bind target regions with high affinity and specificity. Suitable conjugated molecules may further include any protein that binds to DNA provided that it does not create or stabilize biofilm architecture; it is envisioned that at least a subset of such proteins may facilitate the kinetics of binding for the agents disclosed herein.

**[0247]** The functional nucleic acid molecules may act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules may possess a de novo activity independent of any other molecules.

**[0248]** The therapeutic agents can be linked to the fragment or polypeptide directly or indirectly, using any of a large number of available methods. For example, an agent can be attached at the hinge region of the reduced antibody component via disulfide bond formation, using cross-linkers such as N-succinyl 3-(2-pyridyldithio) propionate (SPDP), or via a carbohydrate moiety in the Fc region of the antibody (Yu et al. (1994) *Int. J. Cancer* 56: 244; Upeslacis et al., "Modification of Antibodies by Chemical Methods," in *Monoclonal antibodies: principles and applications*, Birch et al. (eds.), pages 187-230 (Wiley-Liss, Inc. 1995); Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in *Monoclonal antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), pages 60-84 (Cambridge University Press 1995)).

**[0249]** Techniques for conjugating therapeutic agents to antibodies and antibody fragments are well known (Amon et al., "Monoclonal Antibodies For Immunotargeting of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al, (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody in Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection*

And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al. (1982) *Immunol. Rev.* 62:119-58).

**[0250]** The fragments or polypeptides can be linked to another functional molecule such as another antibody or ligand for a receptor to generate a bi-specific or multi-specific molecule that binds to at least two or more different binding sites or target molecules. Linking of the antibody to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, can be done, for example, by chemical coupling, genetic fusion, or non-covalent association. Multi-specific molecules can further include a third binding specificity, in addition to the first and second target epitope.

**[0251]** Bi-specific and multi-specific molecules can be prepared using methods known in the art. For example, each binding unit of the bi-specific molecule can be generated separately and then conjugated to one another. When the binding molecules are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitroberizoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) (Karpovsky et al. (1984) *J. Exp. Med.* 160:1686; Liu et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). When the binding molecules are antibodies, they can be conjugated by sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains.

**[0252]** The antibody fragment, antigen binding fragment or polypeptide as disclosed herein may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

**[0253]** The antibody fragment, antigen binding fragment or polypeptide also can be bound to many different carriers. Thus, this disclosure also provides compositions containing the fragments or polypeptides and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural and modified cellulose, polyacrylamide, agarose, and magnetite. The nature of the carrier can be either soluble or insoluble for purposes disclosed herein. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

**[0254]** Compositions comprising, or alternatively consisting essentially of, or yet further consisting of, one or more of the above embodiments are further provided herein. Further provided are polynucleotides that encode the amino acid sequence of the antibody fragment, the antigen binding fragment or polypeptides, as well as methods to produce recombinantly or chemically synthesize the fragments and polypeptides. The antibody polypeptides and fragments can be produced in a eukaryotic or a prokaryotic cell, or by other methods known in the art and briefly described herein.

**[0255]** The fragments and isolated polypeptides comprising the fragments disclosed herein may be selected such that they have a high level of epitope binding specificity and high binding affinity to the biofilm. In general, the higher the binding affinity of an antibody or antibody fragment or isolated polypeptides comprising the antibody fragments, the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing the target. Accordingly, the fragments and isolated polypeptides comprising the antibody fragments of the present technology useful in the disclosed methods usually have binding affinities of at least  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$ ,  $10^{-10}$ ,  $10^{-11}$ , or  $10^{-12}$  M. In certain aspects, the fragments and isolated polypeptides comprising the antibody fragments have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 hours, at least 5 hours, at least 1 hour, or at least 30 minutes. In another aspect, the affinity of the antibody or antigen binding fragment is less than or about 1000 picoMole (pM), less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, or about 10 pM, or about 9 pM, or about 8 pM, or alternatively less than about 4 pM, or alternatively less than about 2 pM.

**[0256]** In any of the above embodiments, a peptide linker can be added to the N-terminus or C-terminus of the antibody fragment, antigen binding fragment or polypeptide as disclosed herein. A “linker” or “peptide linker” refers to a peptide sequence linked to either the N-terminus or the C-terminus of a polypeptide sequence. In one aspect, the linker is from about 1 to about 20 amino acid residues long or alternatively 2 to about 10, about 3 to about 5 amino acid residues long. An example of a peptide linker is Gly-Pro-Ser-Leu-Lys-Leu (SEQ ID NO. 43). Other examples include Gly-Gly-Gly (SEQ ID NO. 44); Gly-Pro-Ser-Leu (SEQ ID NO. 45); Gly-Pro-Ser (SEQ ID NO. 46); Pro-Ser-Leu-Lys (SEQ ID NO. 47); Gly-Pro-Ser-Leu-Lys (SEQ ID NO. 48), and Ser-Leu-Lys-Leu (SEQ ID NO. 49).

*Polynucleotides encoding the polypeptides*

**[0257]** Further provided herein are isolated polynucleotides encoding the above noted antibody fragments, antigen binding fragments and polypeptides, as well as vector and host cells containing the same, as well as methods for recombinant production of the polypeptides using recombinant cell systems as known in the art and described herein. The polynucleotide can be DNA or RNA. The polynucleotides can be operatively linked to regulatory sequences, promoters, enhancers and the like, for the recombinant reproduction of the polynucleotides and/or the recombinant production of polypeptides. The polynucleotides can be inserted into a vector (plasmid or viral, for example) and inserted into an appropriate host cell (eukaryotic or prokaryotic) for recombinant reproduction or expression. Thus, one can recombinantly produce the antibody fragment or polypeptides by culturing the host cell under conditions for the expression of the polynucleotide. In one aspect, the polypeptides are isolated from the cell or the culture medium. Also provided by this application are the polynucleotides described herein conjugated to a detectable agent for use in the diagnostic methods.

**[0258]** Vectors comprising the isolated or recombinant polynucleotides or antibodies are further provided examples of which are known in the art and briefly described herein. In one aspect where more than one isolated or recombinant polynucleotide is to be expressed as a single unit, the isolated or recombinant polynucleotides can be contained within a polycistronic vector. The polynucleotides can be DNA, RNA, mRNA or interfering RNA, such as siRNA, miRNA or dsRNA.

**[0259]** The disclosure further provides the isolated or recombinant polynucleotide operatively linked to a promoter of RNA transcription, as well as other regulatory sequences for replication and/or transient or stable expression of the DNA or RNA. As used herein, the term "operatively linked" means positioned in such a manner that the promoter will direct transcription of RNA off the DNA molecule. Examples of such promoters are SP6, T4 and T7. In certain embodiments, cell-specific promoters are used for cell-specific expression of the inserted polynucleotide. Vectors which contain a promoter or a promoter/enhancer, with termination codons and selectable marker sequences, as well as a cloning site into which an inserted piece of DNA can be operatively linked to that promoter are known in the art and commercially available. For general methodology and cloning strategies, see Gene Expression Technology (Goeddel ed., Academic Press, Inc. (1991)) and references cited therein and Vectors: Essential Data Series (Gacesa and Ramji, eds., John Wiley & Sons, N.Y.

(1994)) which contains maps, functional properties, commercial suppliers and a reference to GenEMBL accession numbers for various suitable vectors.

**[0260]** In one embodiment, polynucleotides derived from the polynucleotides disclosed herein encode polypeptides or proteins having diagnostic and therapeutic utilities as described herein as well as probes to identify transcripts of the protein that may or may not be present. These nucleic acid fragments can be prepared, for example, by restriction enzyme digestion of larger polynucleotides and then labeled with a detectable marker. Alternatively, random fragments can be generated using nick translation of the molecule. For methodology for the preparation and labeling of such fragments, see Sambrook et al. (1989) *supra*.

**[0261]** Expression vectors containing these nucleic acids are useful to obtain host vector systems to produce proteins and polypeptides. It is implied that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Non-limiting examples of suitable expression vectors include plasmids, yeast vectors, viral vectors and liposomes. Adenoviral vectors are particularly useful for introducing genes into tissues *in vivo* because of their high levels of expression and efficient transformation of cells both *in vitro* and *in vivo*. When a nucleic acid is inserted into a suitable host cell, e.g., a prokaryotic or a eukaryotic cell and the host cell replicates, the protein can be recombinantly produced. Suitable host cells will depend on the vector and can include mammalian cells, animal cells, human cells, simian cells, insect cells, yeast cells, and bacterial cells constructed using known methods. See Sambrook et al. (1989) *supra*. In addition to the use of viral vector for insertion of exogenous nucleic acid into cells, the nucleic acid can be inserted into the host cell by methods known in the art such as transformation for bacterial cells; transfection using calcium phosphate precipitation for mammalian cells; or DEAE-dextran; electroporation; or microinjection. See, Sambrook et al. (1989) *supra*, for methodology. Thus, this disclosure also provides a host cell, e.g., a mammalian cell, an animal cell (rat or mouse), a human cell, or a prokaryotic cell such as a bacterial cell, containing a polynucleotide encoding a protein or polypeptide or antibody.

**[0262]** A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any

embodiment disclosed herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

**[0263]** This disclosure also provides genetically modified cells that contain and/or express the polynucleotides disclosed herein. The genetically modified cells can be produced by insertion of upstream regulatory sequences such as promoters or gene activators (see, U.S. Pat. No. 5,733,761).

**[0264]** The polynucleotides can be conjugated to a detectable and/or purification marker, e.g., an enzymatic label or a radioisotope for detection of nucleic acid and/or expression of the gene in a cell. A wide variety of appropriate detectable markers are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In one aspect, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, calorimetric indicator substrates can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples. Thus, this disclosure further provides a method for detecting a single-stranded polynucleotide or its complement, by contacting target single-stranded polynucleotide with a labeled, single-stranded polynucleotide (a probe) which is a portion of the polynucleotide disclosed herein under conditions permitting hybridization (optionally moderately stringent hybridization conditions) of complementary single-stranded polynucleotides, or optionally, under highly stringent hybridization conditions. Hybridized polynucleotide pairs are separated from un-hybridized, single-stranded polynucleotides. The hybridized polynucleotide pairs are detected using methods known to those of skill in the art and set forth, for example, in Sambrook et al, (1989) *supra*.

**[0265]** The polynucleotide embodied in this disclosure can be obtained using chemical synthesis, recombinant cloning methods, PCR, or any combination thereof. Methods of chemical polynucleotide synthesis are known in the art and need not be described in detail herein. One of skill in the art can use the sequence data provided herein to obtain a desired polynucleotide by employing a DNA synthesizer or ordering from a commercial service.

**[0266]** The polynucleotides disclosed herein can be isolated or replicated using PCR. The PCR technology is the subject matter of U.S. Pat. Nos. 4,683,195; 4,800,159; 4,754,065; and

4,683,202 and described in PCR: The Polymerase Chain Reaction (Mullis et al. eds., Birkhauser Press, Boston (1994)) or MacPherson et al. (1991) and (1995) *supra*, and references cited therein. Alternatively, one of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to replicate the DNA. Accordingly, this disclosure also provides a process for obtaining the polynucleotides disclosed herein by providing the linear sequence of the polynucleotide, nucleotides, appropriate primer molecules, chemicals such as enzymes and instructions for their replication and chemically replicating or linking the nucleotides in the proper orientation to obtain the polynucleotides. In a separate embodiment, these polynucleotides are further isolated. Still further, one of skill in the art can insert the poly-nucleotide into a suitable replication vector and insert the vector into a suitable host cell (prokaryotic or eukaryotic) for replication and amplification. The DNA so amplified can be isolated from the cell by methods known to those of skill in the art. A process for obtaining polynucleotides by this method is further provided herein as well as the polynucleotides so obtained.

**[0267]** RNA can be obtained by first inserting a DNA polynucleotide into a suitable host cell. The DNA can be delivered by any appropriate method, e.g., by the use of an appropriate gene delivery vehicle (e.g., liposome, plasmid or vector) or by electroporation. When the cell replicates and the DNA is transcribed into RNA; the RNA can then be isolated using methods known to those of skill in the art, for example, as set forth in Sambrook et al. (1989) *supra*. For instance, mRNA can be isolated using various lytic enzymes or chemical solutions according to the procedures set forth in Sambrook et al. (1989) *supra*, or extracted by nucleic-acid-binding resins following the accompanying instructions provided by manufacturers.

**[0268]** Polynucleotides exhibiting sequence complementarity or homology to a polynucleotide disclosed herein are useful as hybridization probes or as an equivalent of the specific polynucleotides identified herein. Since the full coding sequence of the transcript is known, any portion of this sequence or homologous sequences, can be used in the methods disclosed herein.

**[0269]** It is known in the art that a “perfectly matched” probe is not needed for a specific hybridization. Minor changes in probe sequence achieved by substitution, deletion or insertion of a small number of bases do not affect the hybridization specificity. In general, as much as 20% base-pair mismatch (when optimally aligned) can be tolerated. In some embodiments, a probe useful for detecting the aforementioned mRNA is at least about 80% identical to the homologous region. In some embodiments, the probe is 85% identical to the

corresponding gene sequence after alignment of the homologous region; in some embodiments, it exhibits 90% identity.

**[0270]** These probes can be used in radioassays (e.g., Southern and Northern blot analysis) to detect, prognose, diagnose or monitor various cells or tissues containing these cells. The probes also can be attached to a solid support or an array such as a chip for use in high throughput screening assays for the detection of expression of the gene corresponding a polynucleotide disclosed herein. Accordingly, this disclosure also provides a probe comprising or corresponding to a polynucleotide disclosed herein, or its equivalent, or its complement, or a fragment thereof, attached to a solid support for use in high throughput screens.

**[0271]** The total size of fragment, as well as the size of the complementary stretches, will depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between at least 5 to 10 to about 100 nucleotides, or even full length according to the complementary sequences one wishes to detect.

**[0272]** Nucleotide probes having complementary sequences over stretches greater than 5 to 10 nucleotides in length are generally well suited, so as to increase stability and selectivity of the hybrid, and thereby improving the specificity of particular hybrid molecules obtained. In certain embodiments, one can design polynucleotides having gene-complementary stretches of 10 or more or more than 50 nucleotides in length, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology with two priming oligonucleotides as described in U.S. Pat. No. 4,603,102 or by introducing selected sequences into recombinant vectors for recombinant production. In one aspect, a probe is about 50-75 or more alternatively, 50-100, nucleotides in length.

**[0273]** The polynucleotides of the present disclosure can serve as primers for the detection of genes or gene transcripts that are expressed in cells described herein. In this context, amplification means any method employing a primer-dependent polymerase capable of replicating a target sequence with reasonable fidelity. Amplification may be carried out by natural or recombinant DNA-polymerases such as T7 DNA polymerase, Klenow fragment of

*E. coli* DNA polymerase, and reverse transcriptase. For illustration purposes only, a primer is the same length as that identified for probes.

**[0274]** One method to amplify polynucleotides is PCR and kits for PCR amplification are commercially available. After amplification, the resulting DNA fragments can be detected by any appropriate method known in the art, e.g., by agarose gel electrophoresis followed by visualization with ethidium bromide staining and ultraviolet illumination.

**[0275]** Methods for administering an effective amount of a gene delivery vector or vehicle to a cell have been developed and are known to those skilled in the art and described herein. Methods for detecting gene expression in a cell are known in the art and include techniques such as in hybridization to DNA microarrays, in situ hybridization, PCR, RNase protection assays and Northern blot analysis. Such methods are useful to detect and quantify expression of the gene in a cell. Alternatively expression of the encoded polypeptide can be detected by various methods. In particular it is useful to prepare polyclonal or monoclonal antibodies that are specifically reactive with the target polypeptide. Such antibodies are useful for visualizing cells that express the polypeptide using techniques such as immunohistology, ELISA, and Western blotting. These techniques can be used to determine expression level of the expressed polynucleotide.

**[0276]** As noted above, the antibodies, antibody fragments, proteins and polypeptides are obtainable by a number of processes known to those of skill in the art, which include purification, chemical synthesis and recombinant methods. Polypeptides can be isolated from preparations such as host cell systems by methods such as immunoprecipitation with antibody, and standard techniques such as gel filtration, ion-exchange, reversed-phase, and affinity chromatography. For such methodology, see for example Deutscher et al. (1999) *Guide To Protein Purification: Methods In Enzymology* (Vol. 182, Academic Press). Accordingly, this disclosure also provides the processes for obtaining these polypeptides as well as the products obtainable and obtained by these processes.

**[0277]** The polypeptides also can be obtained by chemical synthesis using a commercially available automated peptide synthesizer such as those manufactured by Perkin/Elmer/Applied Biosystems, Inc., Model 430A or 431A, Foster City, Calif., USA. The synthesized polypeptide can be precipitated and further purified, for example by high performance liquid chromatography (HPLC). Accordingly, this disclosure also provides a process for chemically synthesizing the proteins disclosed herein by providing the sequence of the protein and

reagents, such as amino acids and enzymes and linking together the amino acids in the proper orientation and linear sequence.

### *Compositions*

**[0278]** Compositions are further provided. The compositions comprise a carrier and one or more of an antibody fragment or antigen binding fragment or isolated polypeptide as disclosed herein, an isolated polynucleotide disclosed herein, a vector disclosed herein, an isolated host cell disclosed herein, a small molecule, an antibody, or an antibody fragment (e.g., a Fab (fragment antigen binding) fragment) disclosed herein. The carriers can be one or more of a solid support or a pharmaceutically acceptable carrier. The compositions can further comprise an adjuvant or other components suitable for administrations as vaccines. In one aspect, the compositions are formulated with one or more pharmaceutically acceptable excipients, diluents, carriers and/or adjuvants. In addition, embodiments of the compositions of the present disclosure include one or more of an isolated polypeptide disclosed herein, an isolated polynucleotide disclosed herein, a vector disclosed herein, a small molecule, an isolated host cell disclosed herein, an antibody, or an antibody fragment (e.g., a Fab (fragment antigen binding) fragment) of the disclosure, formulated with one or more pharmaceutically acceptable substances.

**[0279]** For oral preparations, any one or more of an isolated or recombinant polypeptide as described herein, an isolated or recombinant polynucleotide as described herein, a vector as described herein, an isolated host cell as described herein, a small molecule or an antibody as described herein can be used alone or in pharmaceutical formulations disclosed herein comprising, or consisting essentially of, the compound in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide;

a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

**[0280]** Pharmaceutical formulations and unit dose forms suitable for oral administration are particularly useful in the treatment of chronic conditions, infections, and therapies in which the patient self-administers the drug. In one aspect, the formulation is specific for pediatric administration.

**[0281]** The disclosure provides pharmaceutical formulations in which the one or more of an antibody fragment, an antigen binding domain, an isolated polypeptide each as disclosed herein, an isolated polynucleotide as disclosed herein, a vector as disclosed herein, an isolated host cell as disclosed herein, or an antibody as disclosed herein, that are formulated into preparations for injection in accordance with the disclosure by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers (e.g., acacia, alginate, and sodium alginate) and preservatives or other antimicrobial agents. A non-limiting example of such is another therapeutic agent is an antibody to other vaccine components such as surface antigens, e.g., an OMP P5, OMP 26, OMP P2, or a vaccine component such as Type IV Pilin protein (see Jurcisek and Bakaletz (2007) *J. of Bacteriology* 189(10):3868-3875 and Murphy, T.F. et al. (2009) *The Pediatric Infectious Disease Journal* 28:S121-S126) and antibacterial agents. For intravenous administration, suitable carriers include physiological bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, a composition for parenteral administration must be sterile and should be fluid to the extent that easy syringability exists.

**[0282]** Aerosol formulations provided by the disclosure can be administered via inhalation and can be propellant or non-propellant based. For example, embodiments of the pharmaceutical formulations disclosed herein comprise a compound disclosed herein formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like. For administration by inhalation, the compounds can be delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. A non-limiting example of a non-propellant is a pump spray that is ejected from a closed container by means of mechanical force (i.e., pushing down a piston with one's finger or by compression of the

container, such as by a compressive force applied to the container wall or an elastic force exerted by the wall itself (e.g., by an elastic bladder)).

**[0283]** Suppositories disclosed herein can be prepared by mixing a compound disclosed herein with any of a variety of bases such as emulsifying bases or water-soluble bases. Embodiments of this pharmaceutical formulation of a compound disclosed herein can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

**[0284]** Unit dosage forms for oral or rectal administration, such as syrups, elixirs, and suspensions, may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more compounds disclosed herein. Similarly, unit dosage forms for injection or intravenous administration may comprise a compound disclosed herein in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

**[0285]** Embodiments of the pharmaceutical formulations disclosed herein include those in which one or more of an isolated polypeptide disclosed herein, an isolated polynucleotide disclosed herein, a vector disclosed herein, a small molecule for use in the disclosure, an isolated host cell disclosed herein, or an antibody disclosed herein is formulated in an injectable composition. Injectable pharmaceutical formulations disclosed herein are prepared as liquid solutions or suspensions; or as solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles in accordance with other embodiments of the pharmaceutical formulations disclosed herein.

**[0286]** In an embodiment, one or more of an isolated polypeptide disclosed herein, an isolated polynucleotide disclosed herein, a vector disclosed herein, an isolated host cell disclosed herein, an antibody, or an antibody fragment (e.g., a Fab (fragment antigen binding) fragment) disclosed herein is formulated for delivery by a continuous delivery system. The term “continuous delivery system” is used interchangeably herein with “controlled delivery system” and encompasses continuous (e.g., controlled) delivery devices (e.g., pumps) in combination with catheters, injection devices, and the like, a wide variety of which are known in the art.

**[0287]** Mechanical or electromechanical infusion pumps can also be suitable for use with the present disclosure. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852; 5,820,589; 5,643,207; 6,198,966; and the like. In general, delivery of a compound disclosed herein can be accomplished using any of a variety of refillable, pump systems. Pumps provide consistent, controlled release over time. In some embodiments, a compound disclosed herein is in a liquid formulation in a drug-impermeable reservoir, and is delivered in a continuous fashion to the individual.

**[0288]** In one embodiment, the drug delivery system is an at least partially implantable device. The implantable device can be implanted at any suitable implantation site using methods and devices well known in the art. An implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to, a subdermal, subcutaneous, intramuscular, or other suitable site within a subject's body. Subcutaneous implantation sites are used in some embodiments because of convenience in implantation and removal of the drug delivery device.

**[0289]** Drug release devices suitable for use in the disclosure may be based on any of a variety of modes of operation. For example, the drug release device can be based upon a diffusive system, a convective system, or an erodible system (e.g., an erosion-based system). For example, the drug release device can be an electrochemical pump, osmotic pump, an electro osmotic pump, a vapor pressure pump, or osmotic bursting matrix, e.g., where the drug is incorporated into a polymer and the polymer provides for release of drug formulation concomitant with degradation of a drug-impregnated polymeric material (e.g., a biodegradable, drug-impregnated polymeric material). In other embodiments, the drug release device is based upon an electro diffusion system, an electrolytic pump, an effervescent pump, a piezoelectric pump, a hydrolytic system, etc.

**[0290]** Drug release devices based upon a mechanical or electromechanical infusion pump can also be suitable for use with the present disclosure. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852, and the like. In general, a subject treatment method can be accomplished using any of a variety of refillable, non-exchangeable pump systems. Pumps and other convective systems may be utilized due to their generally more consistent, controlled release over time. Osmotic pumps are used in some embodiments due to their combined advantages of more

consistent controlled release and relatively small size (see, e.g., PCT International Pat. Application Publication No. WO 97/27840 and U.S. Pat. Nos. 5,985,305 and 5,728,396). Exemplary osmotically-driven devices suitable for use in the disclosure include, but are not necessarily limited to, those described in U.S. Pat. Nos. 3,760,984; 3,845,770; 3,916,899; 3,923,426; 3,987,790; 3,995,631; 3,916,899; 4,016,880; 4,036,228; 4,111,202; 4,111,203; 4,203,440; 4,203,442; 4,210,139; 4,327,725; 4,627,850; 4,865,845; 5,057,318; 5,059,423; 5,112,614; 5,137,727; 5,234,692; 5,234,693; 5,728,396; and the like. A further exemplary device that can be adapted for the present disclosure is the Synchromed infusion pump (Medtronic).

**[0291]** In some embodiments, the drug delivery device is an implantable device. The drug delivery device can be implanted at any suitable implantation site using methods and devices well known in the art. As noted herein, an implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to a subdermal, subcutaneous, intramuscular, or other suitable site within a subject's body.

**[0292]** Suitable excipient vehicles for a compound disclosed herein are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Methods of preparing such dosage forms are known, or will be apparent upon consideration of this disclosure, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of the compound adequate to achieve the desired state in the subject being treated.

**[0293]** Compositions of the present disclosure include those that comprise a sustained-release or controlled release matrix. In addition, embodiments of the present disclosure can be used in conjunction with other treatments that use sustained-release formulations. As used herein, a sustained-release matrix is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid-based hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. A sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic

acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone.

Illustrative biodegradable matrices include a polylactide matrix, a polyglycolide matrix, and a polylactide co-glycolide (co-polymers of lactic acid and glycolic acid) matrix.

**[0294]** In another embodiment, antibody fragment (as well as combination compositions) is delivered in a controlled release system. For example, a compound disclosed herein may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (Sefton (1987) CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al. (1980) Surgery 88:507; Saudek et al. (1989) N. Engl. J. Med. 321:574). In another embodiment, polymeric materials are used. In yet another embodiment a controlled release system is placed in proximity of the therapeutic target, i.e., the liver, thus requiring only a fraction of the systemic dose. In yet another embodiment, a controlled release system is placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic. Other controlled release systems are discussed in the review by Langer (1990) Science 249:1527-1533.

**[0295]** In another embodiment, the compositions of the present disclosure (as well as combination compositions separately or together) include those formed by impregnation of an inhibiting agent described herein into absorptive materials, such as sutures, bandages, and gauze, or coated onto the surface of solid phase materials, such as surgical staples, zippers and catheters to deliver the compositions. Other delivery systems of this type will be readily apparent to those skilled in the art in view of the instant disclosure.

**[0296]** The present disclosure provides methods and compositions for the administration of a one or more of an antibody fragment for the treatment of a microbial infection. In various embodiments, these methods disclosed herein span almost any available method and route suitable for drug delivery, including in vivo and ex vivo methods, as well as systemic and localized routes of administration.

**[0297]** In specific aspects, the disclosure provides for formulations or co-formulations comprising antibodies or antibody fragments (e.g., Fab (fragment antigen binding) fragments) that specifically recognize or bind a DNABII protein or polypeptide, or an isolated or recombinant polypeptide consisting essentially of an amino acid sequence selected from: SEQ ID NOS. 12 to 17, SEQ ID NO. 33, or an equivalent each thereof. Antibodies or

antibody fragments (e.g., Fab (fragment antigen binding) fragments) disclosed herein may be selected such that they have a high level of epitope binding specificity and high binding affinity to the biofilm. In general, the higher the binding affinity of an antibody or an antibody fragment (e.g., Fab (fragment antigen binding) fragments), the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing the target. Accordingly, the antibodies or antibody fragments (e.g., Fab (fragment antigen binding) fragments) of the present technology useful in the disclosed methods usually have binding affinities of at least  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$ ,  $10^{-10}$ ,  $10^{-11}$ , or  $10^{-12}$  M. In certain aspects, the antibodies or antibody fragments (e.g., Fab (fragment antigen binding) fragments) have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 hours, at least 5 hours, at least 1 hour, or at least 30 minutes. In another aspect, the affinity of the antibody or antigen binding fragment is less than or about 1000 picoMole (pM), 900 pM, 800 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM, about 100 pM, 50 pM, 40 pM, 30 pM, 20 pM, 10 pM, 9 pM, 8 pM, 7 pM, 6 pM, 5 pM, or 4 pM.

**[0298]** In some embodiments, the antibodies are present in the formulation at a concentration from about 0.1 mg/mL to about 200 mg/mL, or alternatively from about 1 to about 150 mg/mL, or alternatively about 2 mg/mL to about 100 mg/mL, or alternatively about 3 mg/mL to about 80 mg/mL, or alternatively about 4 mg/mL to about 50 mg/mL, or alternatively about 5 mg/mL to about 20 mg/mL. In some embodiments, the antibodies are present at a concentration of at least about 1 mg/mL, or alternatively at least about 2 mg/mL, at least about 3 mg/mL, or alternatively at least about 4 mg/mL, or alternatively at least about 5 mg/mL, or alternatively at least about 6 mg/mL, or alternatively at least about 7 mg/mL, or alternatively at least about 8 mg/mL, or alternatively at least about 9 mg/mL, or alternatively at least about 10 mg/mL, or alternatively at least about 15 mg/mL, or alternatively at least about 20 mg/mL, or alternatively at least about 30 mg/mL, or alternatively at least about 40 mg/mL, or alternatively at least about 50 mg/mL, or alternatively at least about 60 mg/mL, or alternatively at least about 70 mg/mL, or alternatively at least about 80 mg/mL, or alternatively at least about 90 mg/mL, or alternatively at least about 100 mg/mL, or alternatively at least about 120 mg/mL, or alternatively at least about 150 mg/mL or alternatively at least about 200 mg/mL. In some embodiments, at least one of the plurality of antibodies is present at a concentration of at least about 1 mg/mL, or alternatively at least about 2 mg/mL, or alternatively at least about 3 mg/mL, or alternatively at least about 4 mg/mL, or alternatively at least about 5 mg/mL, or alternatively at least about 6 mg/mL, or

alternatively at least about 7 mg/mL, or alternatively at least about 8 mg/mL, or alternatively at least about 9 mg/mL, or alternatively at least about 10 mg/mL, or alternatively at least about 15 mg/mL, or alternatively at least about 20 mg/mL, or alternatively at least about 30 mg/mL, or alternatively at least about 40 mg/mL, or alternatively at least about 50 mg/mL, or alternatively at least about 60 mg/mL, or alternatively at least about 70 mg/mL, or alternatively at least about 80 mg/mL, or alternatively at least about 90 mg/mL, or alternatively at least about 100 mg/mL, or alternatively at least about 120 mg/mL, or alternatively at least about 150 mg/mL, or alternatively at least about 200 mg/mL.

**[0299]** In some embodiments, wherein multiple different antibodies are included an antibody co-formulation, the different antibodies may be present in substantially equal concentrations. In another aspect of such embodiments, the different antibodies one or more of the antibodies may be present in a substantially higher concentration than the other antibodies, e.g., ratios of about 1.5:1, or alternatively about 1.5:1:1, or alternatively about 1.5:1:1:1, or alternatively about 2:1, or alternatively about 2:1:1, or alternatively about 2:1:1:1, or alternatively at least about 2.5:1, or alternatively at least about 2.5:1:1, or alternatively at least about 2.5:1:1:1.

Methods of stably formulating antibody formulations and co-formulations can be made according to techniques disclosed in the art – see, e.g., U.S. Pat. Application No. 12/875,083 (published as US 2011/0059079).

### **Diagnostic and Therapeutic Methods**

**[0300]** Also provided are methods for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA, by contacting the DNABII polypeptide or protein or the microbial DNA with an antibody fragment, antigen binding fragment or composition as described herein, thereby inhibiting, competing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA. In a further aspect, the DNABII polypeptide and the microbial DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The contacting can be performed *in vitro* or *in vivo*.

**[0301]** In another aspect, a method for inhibiting, preventing or breaking down a microbial biofilm is provided by contacting the biofilm with an antibody fragment or antigen binding fragment thereof, or polypeptide as described herein thereby inhibiting, preventing or breaking down the microbial biofilm. In a further aspect, the DNABII polypeptide and the microbial

DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The contacting can be performed *in vitro* or *in vivo*.

**[0302]** When practiced *in vitro*, the methods are useful to screen for or confirm antibody fragments and polypeptides having the same, similar or opposite ability as the polypeptides, polynucleotides, antibodies, antibody fragments (e.g., Fab (fragment antigen binding) fragment), host cells, small molecules and compositions disclosed herein. Alternatively, they can be used to identify which antibody fragment or polypeptide is best suited to treat a microbial infection. For example, one can screen for new agents or combination therapies by having two samples containing for example, the DNABII polypeptide and microbial DNA and the agent to be tested. The second sample contains the DNABII polypeptide and microbial DNA and an agent known to active, e.g., an anti-IHF antibody or a small molecule to serve as a positive control. In a further aspect, several samples are provided and the agents are added to the system in increasing dilutions to determine the optimal dose that would likely be effective in treating a subject in the clinical setting. As is apparent to those of skill in the art, a negative control containing the DNABII polypeptide and the microbial DNA can be provided. In a further aspect, the DNABII polypeptide and the microbial DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The samples are contained under similar conditions for an effective amount of time for the agent to inhibit, compete or titrate the interaction between the DNABII polypeptide and microbial DNA and then the sample is assayed for emission of signal from the luminescent molecules. If the sample emits a signal, then the agent is not effective to inhibit binding.

**[0303]** In another aspect, the *in vitro* method is practiced in a miniaturized chamber slide system wherein the microbial (such as a bacterial) isolate causing an infection could be isolated from the human/animal then cultured to allow it to grow as a biofilm *in vitro*, see for example Experiment 1 below. The agent (such as anti-IHF antibody) or potential agent biofilm is added alone or in combination with another agent to the culture with or without increasing dilutions of the potential agent or agent such as an anti-IHF (or other antibody, small molecule, agent, etc.) to find the optimal dose that would likely be effective at treating that patient when delivered to the subject where the infection existed. As apparent to those of skill in the art, a positive and negative control can be performed simultaneously.

**[0304]** In a further aspect, the method is practiced in a high throughput platform with the antibody fragment and/or potential agent (alone or in combination with another agent) in a flow cell. The anti-IHF antibody or fragment thereof, or potential agent is added alone or in combination with another agent to the culture with or without increasing dilutions of the potential agent or agent such as an anti-IHF (or other antibody, small molecule, agent, etc.) to find the optimal dose that would likely be effective at treating that patient when delivered to the subject where the infection existed. Biofilm isolates are sonicated to separate biofilm bacteria from DNABII polypeptide such as IHF bound to microbial DNA. The DNABII polypeptide—DNA complexes are isolated by virtue of the anti-IHF antibody on the platform. The microbial DNA is then released with e.g., a salt wash, and used to identify the biofilm bacteria added. The freed DNA is then identified, e.g., by PCR sequenced. If DNA is not freed, then the agent(s) successfully performed or bound the microbial DNA. If DNA is found in the sample, then the agent did not interfere with DNABII polypeptide-microbial DNA binding. As is apparent to those of skill in the art, a positive and/or negative control can be simultaneously performed.

**[0305]** In another aspect one or more of the antibody fragments, antigen binding fragments polypeptides or compositions disclosed herein are used in a method of detecting a biofilm *in vivo*. In further embodiments, the antibody fragments, polypeptides or compositions are detectably labeled, for example with a luminescent or fluorescent molecule. Further applications of the methods disclosed herein include methods of use of such antibody fragments, polypeptides or compositions to image a biofilm using, for example, a detectably labeled primary antibody fragments, polypeptides or compositions which provides a detectable signal upon binding to the biofilm or a detectably labeled secondary antibody fragments, polypeptides or compositions which binds to the primary antibody fragments, polypeptides or compositions when it is bound to the biofilm.

**[0306]** The above methods also can be used as a diagnostic test since it is possible that a given bacterial species will respond better to reversal of its biofilm by one agent more than another, this rapid high throughput assay system could allow one skilled the art to assay a panel of possible anti-IHF-like agents to identify the most efficacious of the group.

**[0307]** The advantage of these methods is that most clinical microbiology labs in hospitals are already equipped to perform these sorts of assays (i.e., determination of MIC, MBC values) using bacteria that are growing in liquid culture (or planktonically). As is apparent to those of skill in the art, bacteria generally do not persist planktonically when they are causing

diseases. Instead they are growing as a stable biofilm and these biofilms are significantly more resistant to treatment by antibiotics, antibodies or other therapeutics. This resistance is why most MIC/MBC values fail to accurately predict efficacy *in vivo*. Thus, by determining what “dose” of agent could reverse a bacterial biofilm *in vitro* (as described above) Applicants' pre-clinical assay would be a more reliable predictor of clinical efficacy, even as an application of personalized medicine.

**[0308]** In addition to the clinical setting, the methods can be used to identify the microbe causing the infection and/or confirm effective alternative agents in an industrial setting, such as in a pipe.

**[0309]** In a further aspect of the above methods, an antibiotic or antimicrobial known to inhibit growth of the underlying infection is added sequentially or concurrently, to determine if the infection can be inhibited. It is also possible to add the antibody fragments, polypeptides or compositions to the microbial DNA or DNABII polypeptide before adding the complex to assay for biofilm inhibition.

**[0310]** When practiced *in vivo* in a non-human animal such as a chinchilla, the method provides a pre-clinical screen to identify antibody fragments, polypeptides or compositions that can be used alone or in combination with other agents to break down biofilms.

**[0311]** In another aspect, provided herein is a method of inhibiting, preventing or breaking down a biofilm in a subject by administering to the subject an effective amount of an antibody fragment, polypeptide or composition, thereby inhibiting, preventing or breaking down the microbial biofilm.

**[0312]** Alternatively or additionally, methods of inhibiting, preventing or breaking down a biofilm may be practiced *in vitro* and/or *ex vivo* and involve providing a sample of the biofilm – taken from a subject or generated *in vitro* – and administering an effective amount of an antibody fragment, polypeptide or composition, thereby inhibiting, preventing or breaking down the microbial biofilm. Similarly, the antibody fragments, polypeptides or compositions disclosed herein may be used in method embodiments for inhibiting, preventing, or breaking down microbial biofilms on surfaces colonized by biofilms such as, but not limited to, hospital instruments, industrial equipment, and other materials not comprised of living tissue.

**[0313]** In a further aspect, the methods further comprise, or alternatively consist essentially of, or yet further consist of administering to the subject an effective amount of one or more of an antimicrobial, an antigenic peptide or an adjuvant.

**[0314]** A non-limiting example of an antimicrobial agent are antibodies directed against vaccine component such as a surface antigen, e.g., an OMP P5, rsPilA, OMP 26, OMP P2, or Type IV Pilin protein (see Jurcisek and Bakaletz (2007) *J. Bacteriology* 189(10):3868-3875; Murphy et al. (2009) *The Pediatric Infectious Disease Journal* 28:S121-S126; Novotny et al. (2015) *Mol Microbiol.* 96(2):276-92).

**[0315]** The agents and compositions disclosed herein can be concurrently or sequentially administered with other antimicrobial agents and/or surface antigens. In one particular aspect, administration is locally to the site of the infection by direct injection or by inhalation for example. Other non-limiting examples of administration include by one or more method comprising transdermally, urethrally, sublingually, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, intranasally, by inhalation or orally.

**[0316]** Microbial infections and disease that can be treated by the methods disclosed herein include but are not limited to infection by various organisms associated with biofilm formation, including but not limited to those disclosed in Examples 4 through 9. Non-limiting examples of relevant organisms (and exemplary strains thereof in parentheses) include: *Aggregatibacter actinomycetemcomitans*, *Borrelia burgdorferi* (e.g., B31), *Bordetella pertussis* (e.g., Tohama I), *Burkholderia pseudomallei* (e.g., 668), *Burkholderia cenocepacia* (e.g., HI2424), *Escherichia coli* (e.g., K12 MG1655), *Enterococcus faecalis* (e.g., V583), *Haemophilus influenzae* (e.g., Rd KW20), *Helicobacter pylori* (e.g., 26695), *Klebsiella pneumoniae*, *Moraxella catarrhalis* (e.g., RH4), *Mycobacterium smegmatis* (e.g., MC2), *Mycobacterium tuberculosis* (e.g., CDC1551), *Neisseria gonorrhoeae* (e.g., FA1090), *Neisseria meningitidis* (e.g., MC58), *Pseudomonas aeruginosa*, *Porphyromonas gingivalis* (e.g., W83), *Prevotella intermedia* (e.g., 17), *Prevotella melaninogenica* (e.g., ATCC ® 25845), *Staphylococcus aureus* (e.g., MW2), *Staphylococcus epidermidis* (e.g., RP62A), *Streptococcus agalactiae* (e.g., 2603V/R), *Streptococcus bovis*, *Streptococcus gallolyticus* (e.g., UCN34), *Streptococcus gordonii* (e.g., NCTC 7868 (Challis)), *Streptococcus mutans* (e.g., UA159), *Streptococcus pneumoniae* (e.g., R6), *Streptococcus pyogenes* (e.g., MGAS10270), *Streptococcus sobrinus* (e.g., 6715), *Salmonella enterica* (e.g., *typhi*, CT18), *Treponema denticola* (e.g., ATCC ® 35405), *Treponema palladum* (e.g., Nichols), *Vibrio cholera* (e.g., El Tor, N16961). Additional organisms

known to associate with and/or form biofilms include but are not limited to *Campylobacter* spp., *Candida* spp., *Legionella pneumophila*, and *Listeria monocytogenes*. For example, cystic fibrosis patients have *Pseudomonas* infections that often result in antibiotic resistant biofilms. Exemplary diseases associated with biofilms include, but are not limited to, lung infections of cystic fibrosis patients, otitis media, native valve infectious endocarditis, osteomyelitis, rhinosinusitis, prostatitis, recurrent urinary tract infection, wounds, dental caries and periodontitis. Conditions such as an infected artificial device, joint, catheter, stent or other surgical implant are also associated with biofilm formation.

**[0317]** These microbial infections may be present in the upper, mid and lower airway (otitis, sinusitis, bronchitis but also exacerbations of chronic obstructive pulmonary disease (COPD), chronic cough, complications of and/or primary cause of cystic fibrosis (CF) and community acquired pneumonia (CAP). Thus, by practicing the *in vivo* methods disclosed herein, these diseases and complications from these infections can also be prevented or treated.

**[0318]** Infections might also occur in the oral cavity (*caries, periodontitis*) and caused by *Streptococcus mutans*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*. Infections might also be localized to the skin (abscesses, ‘staph’ infections, impetigo, secondary infection of burns, Lyme disease) and caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Borrelia burdorferi*. Infections of the urinary tract (UTI) can also be treated and are typically caused by *Escherichia coli*. Infections of the gastrointestinal tract (GI) (diarrhea, cholera, gall stones, gastric ulcers) are typically caused by *Salmonella enterica* serovar, *Vibrio cholerae* and *Helicobacter pylori*. Infections of the genital tract include and are typically caused by *Neisseria gonorrhoeae*. Infections can be of the bladder or of an indwelling device caused by *Enterococcus faecalis*. Infections associated with implanted prosthetic devices, such as artificial hip or knee replacements, or dental implants, or medical devices such as pumps, catheters, stents, or monitoring systems, typically caused by a variety of bacteria, can be treated by the methods disclosed herein. These devices can be coated or conjugated to an agent as described herein. Thus, by practicing the *in vivo* methods disclosed herein, these diseases and complications from these infections can also be prevented or treated.

**[0319]** Infections caused by *Streptococcus agalactiae* can also be treated by the methods disclosed herein and it is the major cause of bacterial septicemia in newborns. Infections caused by *Neisseria meningitidis* which can cause meningitis can also be treated.

**[0320]** Thus, routes of administration applicable to the methods disclosed herein include intranasal, intramuscular, urethrally, intratracheal, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral, inhalation, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. An active agent can be administered in a single dose or in multiple doses. Embodiments of these methods and routes suitable for delivery, include systemic or localized routes. In general, routes of administration suitable for the methods disclosed herein include, but are not limited to, direct injection, enteral, parenteral, or inhalational routes.

**[0321]** Parenteral routes of administration other than inhalation administration include, but are not limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be conducted to effect systemic or local delivery of the inhibiting agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

**[0322]** The antibody fragments, polypeptides or compositions disclosed herein can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not limited to, oral and rectal (e.g., using a suppository) delivery.

**[0323]** Methods of administration of the active through the skin or mucosa include, but are not limited to, topical application of a suitable pharmaceutical preparation, transcutaneous transmission, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. Iontophoretic transmission may be accomplished using commercially available "patches" that deliver their product continuously via electric pulses through unbroken skin for periods of several days or more.

**[0324]** In various embodiments of the methods disclosed herein, the antibody fragments, polypeptides or compositions will be administered by inhalation, injection or orally on a continuous, daily basis, at least once per day (QD), and in various embodiments two (BID),

three (TID), or even four times a day. Typically, the therapeutically effective daily dose will be at least about 1 mg, or at least about 10 mg, or at least about 100 mg, or about 200—about 500 mg, and sometimes, depending on the compound, up to as much as about 1 g to about 2.5 g.

**[0325]** This disclosure provides methods and compositions to inhibit or prevent infection of a host or host cell by a bacteria that exports an DNABII protein, the methods and compositions comprising, or alternatively consisting essentially of, or yet further consisting of, administering to a tissue exposed to or infected with the bacteria an effective amount of an bacteria-relevant antibody fragments, antigen binding fragments thereof, polypeptides or compositions that specifically recognizes and binds the DNABII protein, thereby inhibiting or preventing infection of the host or host cell by the bacteria. Multiple antibody fragments, polypeptides or compositions can be administered concurrently or sequentially along with supporting therapies as noted herein.

**[0326]** This disclosure provides a method to inhibit or prevent infection of a cell by a bacteria that exports an DNABII protein. The method comprises, or alternatively consists essentially of, or yet further consists of, administering to a tissue infected with the bacteria an effective amount of an antibody fragment, polypeptide or composition that specifically recognizes and binds the DNABII protein, thereby inhibiting or preventing infection of the bacteria. Multiple antibody fragments, polypeptides or compositions can be administered concurrently or sequentially along with supporting therapies as noted herein.

**[0327]** The administration can be *in vitro* in a culture or *in vivo*, by administration to a patient infected with the bacteria. When practiced *in vivo*, the method can be used to treat a subject infected with the bacteria by administering to the infected subject an effective amount of the antibody. In addition, when the subject is a non-human animal, the method can be used to test possible therapies or combination therapies prior to administration to a human. When practiced *in vitro*, the method is useful to screen for other therapeutic agents and combination therapies, such as small molecule drugs, that inhibit or prevent infection of the bacteria in a tissue.

**[0328]** Also provided are methods to treat a bacterial infection in subject in need thereof, wherein the subject is infected with a bacteria that comprises an DNABII protein, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of an antibody fragment, polypeptide or

composition that specifically recognizes and binds the DNABII protein, thereby inhibiting or preventing infection by the bacteria. The antibody fragment can be generated from a polyclonal, monoclonal or a derivative of an antibody that recognizes and binds the bacteria-relevant DNABII protein. The source of antibody against the DNABII protein can be elicited by either active vaccination of the host with the DNABII protein or passive transfer of antiserum or an antibody against proteins of the DNABII protein. Multiple antibody fragments, polypeptides or compositions can be administered concurrently or sequentially along with supporting therapies as noted herein.

**[0329]** Yet further provided are methods to treat a condition in a subject in need thereof, wherein the condition is associated with a bacterial infection wherein the bacteria expresses an DNABII protein, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of an antibody fragment, polypeptide or composition that specifically recognizes and binds the DNABII protein, thereby inhibiting or preventing infection of the bacteria. The source of antibody that produced the antibody fragment, polypeptide or composition against the DNABII protein can be elicited by either active vaccination of the host with the DNABII protein or passive transfer of antiserum or an antibody against the DNABII protein. The antibody can be polyclonal, monoclonal or a derivative of an antibody that recognizes and binds the bacteria-relevant DNABII protein. Multiple antibody fragments, polypeptides or compositions can be administered concurrently or sequentially along with supporting therapies as noted herein.

**[0330]** Any of the above methods can further comprise, or alternatively consist essentially of, or yet further consist of, administering to the subject or the tissue or cell culture *in vitro*, an effective amount of one or more of an antimicrobial, an antigenic peptide or an adjuvant. The subject, in some aspects, is a non-human animal or a human patient.

**[0331]** The antibody fragment, polypeptide or composition is administered locally or systemically by any appropriate method, e.g., to the site of infection or biofilm, topically, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, urethrally, intranasally, by inhalation or orally.

**[0332]** In some aspects, the subject is a pediatric patient and the antibody is administered in a formulation for the pediatric patient.

**[0333]** A screen to identify potential therapeutic agents that inhibit or prevent infection of a cell by a bacteria that exports a DNABII protein and/or that disrupt or prevent biofilm

formation is also disclosed. The screening method comprises, or alternatively consists essentially of, or yet consists of, contacting *in vitro* or administering *in vivo* to a tissue infected with the bacteria an agent and determining if the agent binds the DNABII protein. Methods to determining binding are known in the art and several non-limiting examples are described herein. In one aspect, if the agent binds the protein, the agent is a potential therapeutic agent and if the agent does not bind the protein, the agent is not a potential therapeutic agent. In another aspect, if the infection or biofilm is inhibited, disrupted, or prevented *in vivo*, the agent is a potential therapeutic agent and if infection is not inhibited or prevented, the agent is not a potential therapeutic agent. Methods of determining if the infection is inhibited or prevented are known in the art and several non-limiting examples are described herein; methods of determining if a biofilm is disrupted or prevented are known in the art and further disclosed herein. Non-limiting examples of potential therapeutic agents are from the group of: an antibody, an antibody derivative, a polypeptide or a small molecule. Multiple antibodies can be administered concurrently or sequentially along with supporting therapies as noted herein.

**[0334]** In a further aspect, the agent binds the protein and the binding is compared to the binding of anti-DNABII antisera to the antibody fragment or polypeptide.

**[0335]** It should be appreciated that any of the general properties contemplated with respect of the antibody fragments, polypeptides or compositions for inhibiting, titrating, or competing the binding of a DNABII protein to a microbial DNA should likewise apply to the above disclosed methods relating to bacterial infection.

**[0336]** Dosing can be accomplished in accordance with the methods disclosed herein using capsules, tablets, oral suspension, suspension for intra-muscular injection, suspension for intravenous infusion, gel or cream for topical application, or suspension for intra-articular injection.

**[0337]** Dosage, toxicity and therapeutic efficacy of compositions described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, to determine the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. In certain embodiments, compositions exhibit high therapeutic indices. While compounds that exhibit toxic side effects may be used, care should be taken to design a

delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

**[0338]** The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies (in certain embodiments, within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

**[0339]** In some embodiments, an effective amount of a composition sufficient for achieving a therapeutic or prophylactic effect, ranges from about 0.000001 mg per kilogram body weight per administration to about 10,000 mg per kilogram body weight per administration. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per administration to about 100 mg per kilogram body weight per administration. Administration can be provided as an initial dose, followed by one or more “booster” doses. Booster doses can be provided a day, two days, three days, a week, two weeks, three weeks, one, two, three, six or twelve months after an initial dose. In some embodiments, a booster dose is administered after an evaluation of the subject's response to prior administrations.

**[0340]** The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to, the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the therapeutic compositions described herein can include a single treatment or a series of treatments.

### **Functional Analysis with Antibodies**

**[0341]** Antibodies and antibody fragments (e.g., Fab (fragment antigen binding) fragment) disclosed herein can be used to purify the polypeptides disclosed herein and to identify biological equivalent polypeptide and/or polynucleotides. They also can be used to identify

agents that modify the function of the polypeptides disclosed herein, e.g., aptamer, polynucleotides and small molecules. These antibodies include polyclonal antisera, monoclonal antibodies, and various reagents derived from these preparations that are familiar to those practiced in the art and described above.

**[0342]** Antibodies and antibody fragments (e.g., Fab (fragment antigen binding) fragment) that neutralize the activities of proteins encoded by identified genes can also be used *in vivo* and *in vitro* to demonstrate function by adding such neutralizing antibodies into *in vivo* and *in vitro* test systems. They also are useful as pharmaceutical agents to modulate the activity of polypeptides disclosed herein.

**[0343]** Various antibody preparations and antibody fragments (e.g., Fab (fragment antigen binding) fragment) can also be used in analytical methods such as ELISA assays or Western blots to demonstrate the expression of proteins encoded by the identified genes by test cells *in vitro* or *in vivo*. Fragments of such proteins generated by protease degradation during metabolism can also be identified by using appropriate polyclonal antisera with samples derived from experimental samples.

**[0344]** Further, in some embodiments, the antibodies and antibody fragments (e.g., Fab (fragment antigen binding) fragment) disclosed herein may be used to visualize and/or detect biofilms. In such embodiments, the antibodies may be detectably labeled, e.g., with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like or conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member of biotin-avidin specific binding pair), and the like. Detectably labeled antibodies may then be introduced to the sample suspected of being colonized by a biofilm and visualized and/or detected through microscopy or other methods known to detect the relevant label, e.g., spectroscopy, cytometry, or other common techniques. Conjugated antibodies or unlabeled antibodies may likewise be identified through known analytical methods targeting the conjugated moiety or antibody, respectively. For example, in some embodiments, a detectably labeled secondary antibody specific to the isotype of the antibodies and/or antibody fragments (e.g., Fab (fragment antigen binding) fragment) disclosed herein may be used in the visualization and/or detection of a biofilm; such embodiments may entail the administration of the antibody specific to the “tail” of a DNABII protein to the surface suspected of being colonized by a biofilm, followed by the administration of the detectably labeled secondary antibody and the subsequent detection of the relevant label.

**[0345]** The antibody fragments, antigen binding domains, polypeptides or compositions disclosed herein may be used for vaccination or to boost vaccination, alone or in combination with peptides or protein-based vaccines or dendritic-cell based vaccines.

### Screening Assays

**[0346]** The present disclosure provides methods for screening for equivalent agents, such as equivalent monoclonal antibodies and fragments thereof to exemplified antibodies as described herein and various agents that modulate the activity of the active agents and pharmaceutical compositions disclosed herein or the function of a polypeptide or peptide product encoded by the polynucleotide disclosed herein. For the purposes of this disclosure, an “agent” is intended to include, but not be limited to a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein (e.g., antibody), a polynucleotide (anti-sense) or a ribozyme. A vast array of compounds can be synthesized, for example polymers, such as polypeptides and polynucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term “agent.” In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. It should be understood, although not always explicitly stated that the agent is used alone or in combination with another agent, having the same or different biological activity as the agents identified by the inventive screen.

**[0347]** For the purposes of this disclosure, a “binding moiety” is intended to include, but not be limited to a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein (e.g., antibody), a polynucleotide (anti-sense) or a ribozyme that has affinity with respect to at least one DNABII protein. A vast array of compounds can be synthesized, for example polymers, such as polypeptides and polynucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term “binding moiety.” In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like.

**[0348]** To practice the screening method *in vitro*, suitable cell culture or tissue infected with the microbial to be treated are first provided. The cells are cultured under conditions (temperature, growth or culture medium and gas (CO<sub>2</sub>) and for an appropriate amount of time to attain exponential proliferation without density dependent constraints. It also is desirable to maintain an additional separate cell culture that is not infected as a control.

**[0349]** As is apparent to one of skill in the art, suitable cells can be cultured in micro-titer plates and several agents can be assayed at the same time by noting genotypic changes, phenotypic changes or a reduction in microbial titer.

**[0350]** When the agent is a composition other than a DNA or RNA, such as a small molecule as described above, the agent can be directly added to the cell culture or added to culture medium for addition. As is apparent to those skilled in the art, an “effective” amount must be added which can be empirically determined.

**[0351]** When the agent is an antibody or antigen binding fragment, the agent can be contacted or incubated with the target antigen and polyclonal antibody as described herein under conditions to perform a competitive ELISA. Such methods are known to the skilled artisan.

**[0352]** The assays also can be performed in a subject. When the subject is an animal such as a rat, chinchilla, mouse or simian, the method provides a convenient animal model system that can be used prior to clinical testing of an agent in a human patient. In this system, a candidate agent is a potential drug if symptoms of the disease or microbial infection is reduced or eliminated, each as compared to untreated, animal having the same infection. It also can be useful to have a separate negative control group of cells or animals that are healthy and not treated, which provides a basis for comparison.

**[0353]** Additional screening assays specific to antibodies contemplated herein include those methods suitable for identifying an antibody or agent which specifically binds to one or more of the polypeptides disclosed herein. This method involves identifying an antibody binding to a site on the antigen to which the antibody of the present invention binds.

**[0354]** An antibody binding site can be determined through methods known to those skilled in the art. For example, when the antibody binds to or recognizes the partial conformation of the antigen, the binding site for the antibody can be determined by identifying amino acid residues on the antigen adjacent to the antibody using X-ray structural analysis. For example, the antibody or its fragment and the antigen or its fragment can be bound to each other and crystallized, followed by structural analysis to identify each amino acid residue on the antigen having an interactional distance with the antibody. The interactional distance is 8 angstroms or shorter, preferably 6 angstroms or shorter, more preferably 4 angstroms or shorter. One or more such amino acid residues having an interactional distance with the antibody can constitute a site (epitope) on the antigen to which the antibody binds. Two or more such

amino acid residues may not be adjacent to each other on the primary sequence. Such structural analysis can provide the three-dimensional structure of the protein, therefore yielding both the sequence and conformation of the identified epitope.

**[0355]** A test antibody or agent may be contacted with one or more of the polypeptides disclosed herein. Subsequently, the distance is measured between the substance and amino acid residues constituting the epitope to which any one of the antibodies disclosed herein bind. The test antibody or agent can be determined to specifically bind if it has an appropriate interaction distance with each of the residues. In some embodiments, the test antibody is not a polyclonal antibody.

**[0356]** In some embodiments, the test antibody or agent is analyzed based on its interaction with a the conformational epitope identified by structural analysis of the antibody-antigen interaction. A non-limiting example of such a conformational epitope is a  $\beta$  hairpin, which may be comprised in one or more of the polypeptides disclosed herein. An additional non-limiting exemplary epitope is an amino acid sequence that conforms to a sharp turn as part of an anti-parallel beta ribbon, such as the consensus amino acid sequence **NPXT**, wherein “X” refers to any amino acid. In some embodiments, X is selected from the amino acids Q, R, K, S, or T. Non-limiting examples of such are disclosed herein with the consensus amino acid sequence **NPXT**, in underlined and bolded text. A skilled artisan will appreciate that in any one of these sequences, the residue in the X position (marked with a **double underline** in the below examples) may be substituted with any amino acid – e.g., Q, R, K, S, or T. Examples include:

SEQ ID NO. 13: *Haemophilus influenzae* IhfA, A5 fragment:

**RPGRNPKTGDVVPVSARRVV.**

SEQ ID NO. 14: *Haemophilus influenzae* HU, A5 fragment: **RTGRNPQTGAEIQIAASKVP.**

SEQ ID NO. 17: *Haemophilus influenzae* IhfB, modified B4 (mB4) fragment:

**FSLHHRQPRLGRNPKTGDSV.**

SEQ ID NO. 26: *Haemophilus influenzae* IhfA, A tip fragment:

**NFELRDKSSRPGRNPKTGDVV.**

SEQ ID NO. 27: *Haemophilus influenzae* IhfB, B tip fragment:

**SLHHRQPRLGRNPKTGDSVNL.**

SEQ ID NO. 31 *Haemophilus influenzae* HU, fragment: **VNERAARTGRNPQTGAEIQIAA.**

**[0357]** In some embodiments, the polypeptide comprising **NPXT** is at least about 20 amino acids long and the NPXT is located approximately centrally in the sequence. Non-limiting examples of such sequences include SEQ ID NOS. 13, 14, and 31.

**[0358]** The agents and compositions can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

### **Combination Therapy**

**[0359]** The compositions and related methods of the present disclosure may be used in combination with the administration of other therapies. These include, but are not limited to, the administration of DNase enzymes, antibiotics, antimicrobials, vaccine adjuvants, or other antibodies.

**[0360]** In some embodiments, the methods and compositions include a deoxyribonuclease (DNase) enzyme that acts synergistically with the anti-DNABII antibody. A DNase is any enzyme that catalyzes the cleavage of phosphodiester linkages in the DNA backbone. Three non-limiting examples of DNase enzymes that are known to target not only cruciform structures, but also a variety of secondary structure of DNA include DNase I, T4 EndoVII and T7 Endo I. In certain embodiments, the effective amount of anti-DNABII antibody needed to destabilize the biofilm is reduced when combined with a DNase. When administered *in vitro*, the DNase can be added directly to the assay or in a suitable buffer known to stabilize the enzyme. The effective Unit dose of DNase and the assay conditions may vary, and can be optimized according to procedures known in the art.

**[0361]** In other embodiments, the methods and compositions can be combined with antibiotics and/or antimicrobials. Antimicrobials are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi, or protozoans. Although biofilms are generally resistant to the actions of antibiotics, compositions and methods described herein can be used to sensitize the infection involving a biofilm to traditional therapeutic methods for treating infections. In other embodiments, the use of antibiotics or antimicrobials in combination with methods and compositions described herein allow for the reduction of the effective amount of the antimicrobial and/or biofilm reducing agent. Some non-limiting examples of antimicrobials and antibiotics useful in combination with methods of the current disclosure include amoxicillin, amoxicillin-clavulanate, cefdinir, azithromycin, and sulfamethoxazole-trimethoprim. The therapeutically effective dose of the antimicrobial and/or antibiotic in

combination with the biofilm reducing agent can be readily determined by traditional methods. In some embodiments the dose of the antimicrobial agent in combination with the biofilm reducing agent is the average effective dose which has been shown to be effective in other bacterial infections, for example, bacterial infections wherein the etiology of the infection does not include a biofilm. In other embodiments, the dose is 0.1, 0.15, 0.2, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, 0.8, 0.85, 0.9, 0.95, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0 or 5 times the average effective dose. The antibiotic or antimicrobial can be added prior to, concurrent with, or subsequent to the addition of the anti-DNABII antibody.

**[0362]** In other embodiments, the methods and compositions can be combined with antibodies that treat the bacterial infection. One example of an antibody useful in combination with the methods and compositions described herein is an antibody directed against an unrelated outer membrane protein (i.e., OMP P5). Treatment with this antibody alone does not debulk a biofilm *in vitro*. Combined therapy with this antibody and a biofilm reducing agent results in a greater effect than that which could be achieved by either reagent used alone at the same concentration. Other antibodies that may produce a synergistic effect when combined with a biofilm reducing agent or methods to reduce a biofilm include anti-rsPilA anti-OMP26, anti-OMP P2, and anti-whole OMP preparations.

**[0363]** The compositions and methods described herein can be used to sensitize the bacterial infection involving a biofilm to common therapeutic modalities effective in treating bacterial infections without a biofilm but are otherwise ineffective in treating bacterial infections involving a biofilm. In other embodiments, the compositions and methods described herein can be used in combination with therapeutic modalities that are effective in treating or preventing bacterial infections involving a biofilm, but the combination of such additional therapy and biofilm reducing agent or method produces a synergistic effect such that the effective dose of either the biofilm reducing agent or the additional therapeutic agent can be reduced. In other instances the combination of such additional therapy and biofilm reducing agent or method produces a synergistic effect such that the treatment is enhanced. An enhancement of treatment can be evidenced by a shorter amount of time required to treat the infection.

**[0364]** The additional therapeutic treatment can be added prior to, concurrent with, or subsequent to methods or compositions used to reduce the biofilm, and can be contained within the same formulation or as a separate formulation.

## Kits

**[0365]** Kits containing the agents and instructions necessary to perform the *in vitro* and *in vivo* methods as described herein also are claimed. Accordingly, the disclosure provides kits for performing these methods which may include an antibody fragment or antigen binding fragment thereof as disclosed herein as well as instructions for carrying out the methods disclosed herein such as collecting tissue and/or performing the screen, and/or analyzing the results, and/or administration of an effective amount of an antibody fragment or antigen binding fragment thereof, as defined herein. These can be used alone or in combination with other suitable antimicrobial agents.

**[0366]** For example, a kit can comprise, or alternatively consist essentially of, or yet further consist of any one or more antibody fragments, polypeptides or compositions of this disclosure and instructions for use. The kit can further comprising one or more of an adjuvant, an antigenic peptide or an antimicrobial. Examples of carriers include a liquid carrier, a pharmaceutically acceptable carrier, a solid phase carrier, a pharmaceutically acceptable carrier, a pharmaceutically acceptable polymer, a liposome, a micelle, an implant, a stent, a paste, a gel, a dental implant, or a medical implant.

**[0367]** The following examples are intended to illustrate, and not limit the embodiments disclosed herein.

## Examples

### Example 1: Generation of IHF and HU antibodies and Fab fragments

**[0368]** IHF and HU antibodies, proteins and polypeptides were generated to IHF and HU as a whole and to specific fragments.

**[0369]** The methods to produce them are well known to the skilled artisan, e.g., as described in Granston and Nash (1993) *J. Mol. Biol.* 234:45-59; Nash et al. (1987) *Journal of Bacteriology* 169(9):4124-4127; and Rice et al. (1996) *Cell*, 87:1295-1306. Briefly, to overproduce IHF- $\alpha$  (SEQ ID NO. 6), the himA gene was inserted downstream from the P<sub>L</sub> promoter in the bacterial plasmid pAD284. Transformants of strain K5607, a lambda lysogen of strain C600himA42 that had received the desired plasmid, were identified by screening ampicillin-resistant transformants for the ability to grow bacteriophage Mu. DNA was prepared from himA<sup>+</sup> transformants according to standard DNA isolation techniques, and the orientation of the himA gene was determined by restriction enzyme cleavage. Plasmid pP<sub>L</sub>himA-1, which has the himA gene in the proper orientation for expression by the

$P^L$  promoter, was transformed into strain N5271, which contains a cryptic lambda prophage expressing the cI857 thermoinducible repressor, to yield strain K5770.

**[0370]** To overproduce IHF- $\beta$  (SEQ ID NO. 7), plasmid pKT23-hip323, which contains a fusion of the IHF-betacoding sequence to the bacteriophage lambda  $P_L$  promoter was used. pKT23-hip323 was introduced into N5271 to give strain E443. To facilitate the selection of pKT23-hip323 in the presence of another plasmid, changed its selectable marker was changed from ampicillin resistance ( $bla^+$ ) to chloramphenicol resistance ( $cat^-$ ). A  $cat^-$  containing fragment was isolated from plasmid pBR325 as described by Flamm and Weisberg and was inserted into the unique  $ScaI$  site in  $bla$ . The ligated DNA was introduced into strain E403, which carries a hip mutation and which synthesizes temperature-sensitive X repressor, and chloramphenicol-resistant transformants were selected at low temperature. One such transformant (E735) was  $hip^+$  and ampicillin sensitive; it therefore appears to carry a  $bla$   $cat^+$  derivative of pKT23-hip323 (pE735).

**[0371]** To generate a strain that overproduces both subunits of IHF, E735 was transformed with plasmid p $P_L$ himA-1, selecting transformant (E738) that had become ampicillin resistant and had retained chloramphenicol resistance. The generation of a second strain that overproduces both subunits of depended on the construction of plasmid p $P_L$ hip himA-5, which was made by ligating blunted ( $SstII$  restriction enzyme site) containing the  $pheT$  and  $himA$  genes into the pKT23-hip323 plasmid. This is described in further detail in (Nash et al. (1987) *J. Bacteriology* 169(9):4124-4127.  $himA^+$  transformants of strain K5607 were identified by screening for  $HimA^+$ , and the plasmid DNA was analyzed by restriction digestion. In all cases where the plasmid structure was obvious, two copies of  $himA$  had been ligated as a tandem direct repeat into the vector. It is not known if the presence of two copies of the  $himA$  gene on this plasmid is demanded by the selection, but it should be recalled that a single copy of the  $himA$  gene in plasmid p $P_L$ himA-1 is sufficient to complement a  $himA$  mutant. Plasmid p $P_L$ hiphimA-5 was used to transform strain N5271 to yield strain K5746.

**[0372]** The same procedure was replicated to overproduce HU (SEQ ID NO. 3).

**[0373]** Cells were grown in shaking water bath at 31° C. in TBY medium (10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride per liter). At mid-log phase (optical density at 650 nm, ca. 0.6), the cells were shifted to a 42° C. water bath and shaking was continued. Typically, 300 ml of culture was centrifuged and suspended in 0.6 to 0.9 ml of TEG (20 mM Tris hydrochloride (pH 7.4), 1 mM sodium EDTA, 10% glycerol) containing 20 mM NaCl.

The cells were disrupted with six 20-s bursts of sonication, with 40 s between each burst. A portion of the sonic extract was centrifuged in a Somali SS34 rotor for 20 min at 15,000 rpm. Samples of the sonic extract were analyzed by sodium dodecyl sulfate (SDS) gel electrophoresis according to standard molecular biology techniques.

**[0374]** Purification of was done according to the following: A 3.6-liter batch of cells was induced for 3 h. All subsequent steps were carried out at 0 to 4° C. The cell pellet from 3.3-liters was suspended in 10 ml of TEG containing 20 mM NaCl to give a total volume of 29 ml; this suspension was disrupted in two batches, each receiving six bursts of 3 min of sonication separated by 90-s intervals. The sonic extract was centrifuged for 20 min at 15,000 rpm, yielding 16.9 ml of clarified extract. A 10% (vol/vol) solution (1.1 ml) of polymin P (BDH Chemicals Ltd.) was added slowly to the clarified extract; after being stirred for 20 mm, the mixture was centrifuged for 30 mm at 10,000 rpm. The resulting pellet was suspended in 10 ml of TEG containing 500 mM NaCl; after being stirred for 15 min, the mixture was centrifuged for 20 min at 12,000 rpm. The supernatant (10.3 ml) was adjusted to 50% saturation by the addition of 3.2 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting supernatant was adjusted to 70% saturation by the addition of 1.64 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting pellet was suspended in 1 ml of TG (50 mM Tris hydrochloride (pH 7.4) containing 10% glycerol) and dialyzed against two changes of TG. The dialyzed material (2.0 ml) was loaded onto a 1-ml column (0.5 by 5.8 cm) of phosphocellulose (P11; Whatman, Inc.) that had been equilibrated with TG. The column was washed with 3 ml of TG and developed with 20 ml of a linear gradient (0 to 1.2 M) of KCl in TG. Fractions of 0.5 ml were collected, stored at -20° C., and assayed for IHF activity.

**[0375]** Polyclonal anti-IHF was prepared as follows. Rabbits were injected with 250 µg of purified IHF with Freund's complete adjuvant. Booster immunizations of 250 µg of IHF with Freund's incomplete adjuvant were given 1, 7, and 12 weeks later. As determined by immunoblotting of IHF, sera collected 13 weeks after the initial injection had a high titer of IHF-reactive material. The animals were maintained for several years and, when necessary, given further booster immunization in order to maintain a high titer of anti-IHF in their sera. The antibody was not purified further. Crude sera was stored at -70° C. Polyclonal anti-HU was prepared according to the same manner.

**[0376]** Hybridomas producing murine monoclonal antibodies specific to each subunit of IHF or HU were generated by using synthetic polypeptides corresponding to SEQ ID NOS.

12-17 were ordered from Rockland Immmonchemicals, Limerick, PA. Three of the hybridomas were deposited and validated for viability by ATCC, see Table 1, above.

**[0377]** Rabbit polyclonal anti-IhfB2<sub>NTHI</sub> and anti-mIhfB4<sub>NTHI</sub> were prepared as follows. Rabbits were injected with 250 µg of IhfB2<sub>NTHI</sub> peptide or mIhfB4<sub>NTHI</sub> peptide, with Freund's complete adjuvant. Two booster immunizations of 250 µg of IhfB2<sub>NTHI</sub> or mIhfB4<sub>NTHI</sub> with Freund's incomplete adjuvant were given at 21-day intervals. As determined by ELISA of IhfB2<sub>NTHI</sub> or mIhfB4<sub>NTHI</sub>, sera collected 21 days after the third injection had a reciprocal titer of  $\geq 40,000$  of IhfB2<sub>NTHI</sub> or mIhfB4<sub>NTHI</sub> -reactive material. The antibody was not purified further. Crude sera was stored at  $-70^{\circ}\text{C}$ .

**[0378]** Mouse Fab fragments were generated by digestion of monoclonal antibodies. In particular, murine Fab fragments were generated by digestion of murine monoclonal antibodies mIhfB4<sub>NTHI</sub> (clone 12E6.F8.D12.D5), IhfB2<sub>NTHI</sub> (clone 7A4.E4.G4) and IgG1 κ (clone P3.6.2.8.1, Affymetric) for 5 hr with agarose-immobilized ficin + 25 mM cysteine (Thermo Scientific), as shown in FIG. 1 (Mariani *et al.*, A new enzymatic method to obtain high-yield F(ab)2 suitable for clinical use from mouse IgG1, *Molec. Immunol.* (1991) Jan-Feb; 28(1-2):69-77. PMID: 2011130). Rabbit Fab fragments were generated from IgG-enriched polyclonal rabbit anti-IhfB2<sub>NTHI</sub>, anti-mIhfB4<sub>NTHI</sub>, and naive rabbit serum by 4 hr digestion with agarose-immobilized papain protease + cysteine-HCl (ThermoScientific). Fragments were then purified via Protein A spin column, dialyzed versus 10 mM phosphate buffered saline, pH 7.4 and purity of each preparation was confirmed by 10% Bis-Tris PAGE (BioRad) and SYPRO Orange Protein Gel stain (Invitrogen), as shown in **FIG. 1** and **FIG. 10**. The concentration of each Fab fragment preparation was determined via Nanodrop using an extinction coefficient of 1.4.

Example 2: Reduction of biofilm analysis using murine Fab

**[0379]** This experiment describes an *in vitro* model for disruption of an established biofilm in 8-well chamber slide. The materials used in this experiment were: Chocolate Agar; sBHI (BHI with 2 mg heme/mL and 2 mg b-NAD/mL); 8-well Chamber slides (Nunc\* Lab-Tek\* Fisher catalog #12-565-18); Sterile 0.9% saline; LIVE/DEAD BacLight Bacterial Viability Kit (Fisher catalog #NC9439023) and Formalin.

**[0380]** On day 1, NTHI was struck for isolation on chocolate agar. It was then incubated for 20 hrs at  $37^{\circ}\text{C}$ . and 5% CO<sub>2</sub>. The next day, bacteria were suspended in 5 mL equilibrated ( $37^{\circ}\text{C}$ ., 5% CO<sub>2</sub>) and optical density was adjusted to OD<sub>490</sub> $\approx 0.65$  in sBHI. Bacteria were

diluted 1:6 in equilibrated sBHI (1 mL bacterial suspension+5 mL sBHI). Bacteria were then incubated for 3 hours at 37° C. in 5% CO<sub>2</sub>, static (OD<sub>490</sub> should be approx 0.65). Next, the bacteria were again diluted 1:2500 in equilibrated sBHI and 200 mL of the bacterial suspension was added to each well of the chamber slide. For dilution, 10 µL bacteria was added to 990 µL sBHI in an eppendorf tube and 8 µL dilution was added to 192 µL sBHI in each chamber and incubated at 37° C., 5% CO<sub>2</sub>, static.

**[0381]** On the third day after 16 hours of incubation medium was aspirated from chamber by aspirating medium from the corner of the well so as not to disturb biofilm. Then 200 mL of equilibrated sBHI was added to each chamber and incubated for 37° C., 5% CO<sub>2</sub>, static for 8 hours. After 8 hours, the medium was aspirated and 200 mL equilibrated sBHI was added to each untreated chamber; and 40 nM or 440 nM antibody or Fab (each of the following: murine IgG1, murine IgG1 Fab, mAb IHFB2<sub>NTHI</sub>, mAb IhfB2<sub>NTHI</sub> Fab, mAb mIHFB4<sub>NTHI</sub>, and mAb mIHFB4<sub>NTHI</sub> Fab) in sBHI per well was added. They were then incubated at 37° C. and 5% CO<sub>2</sub>, static.

**[0382]** On day 4, after approximately 16 hours of incubation, aspirate sBHI was aspirated and the biofilm was washed twice with 200 mL sterile saline. The saline was aspirated and 200 mL Live/Dead stain was added. Next, 3 mL component A plus 3 mL component B in 1 mL sterile 10 mM phosphate buffered saline was added. It was then incubated for 15 minutes at room temperature, static, protected from light. Stain was aspirated and biofilm was washed twice with 200 mL sterile saline. Saline was aspirated and 200 mL formalin was added to fix biofilm. It was then incubated 15 minutes at room temperature, static, protected from light. Formalin was aspirated and biofilm was washed twice with 200 mL sterile saline. Gasket was removed and coverslip were placed on slide; coverslip were sealed with nail polish and dried prior to viewing by confocal microscopy.

**[0383]** In a separate experiment, NTHI 86-028NP colonies were collected from overnight culture on chocolate agar and suspended in brain heart infusion broth supplemented with 2 µg β-NAD and heme per ml medium (sBHI). The optical density at 490nm was then adjusted to 0.65 and the culture diluted 1:6 in sBHI prior to incubation at 37°C with 5% CO<sub>2</sub> for 3 hr, static. Next, the culture was diluted 1:2500 in fresh sBHI and 200 µl of the suspension aliquotted into each well of an 8-well chamber slide. The slide was then incubated at 37°C with 5% CO<sub>2</sub> for 3 hr, static. After 16 hr, 200 µl fresh sBHI was added to each well, and the slide incubated an additional 8 hr. At this time point, medium was aspirated from each well and treatments (polyclonal antibody, monoclonal antibody or Fab fragments) at 171 nM

added. The biofilms were incubated an additional 16 hr. Biofilms were then washed and stained with FM1-43FX bacterial cell membrane stain (Invitrogen) and fixed overnight at 4 °C in 16% paraformaldehyde, 2.5% glutaraldehyde, 4.0% acetic acid in 0.1 M phosphate buffer (pH 7.4). Fixative was aspirated an 200 µl 0.9% saline was added to each well prior to viewing of biofilms on a Zeiss 510 Meta-laser scanning confocal microscope. Images were compiled with Zeiss Zen software and biofilm biomass calculated with COMSTAT2 software.

**[0384]** Monoclonal antibodies directed against *Haemophilus influenzae* IHF were prepared in mice according to standard techniques using, individually, each of IhfB2<sub>NTHI</sub> and mIhfB4<sub>NTHI</sub>. Example 1 describes the generation of Fab (fragment antigen binding) fragments from monoclonal antibodies directed against the *Haemophilus influenzae* IhfB2<sub>NTHI</sub> and mIhfB4<sub>NTHI</sub>. Fabs generated from murine monoclonal antibodies directed against *Haemophilus influenzae* mIhfB4<sub>NTHI</sub> polypeptides were demonstrated to have robust disruption of biofilms relative to medium, mAb IhfB2<sub>NTHI</sub>, and murine IgG1 Fab controls (**FIG. 2**). Specifically, mAb mIhfB4<sub>NTHI</sub> Fab demonstrated robust disruption of biofilms, showing a 74% reduction in biomass relative to mAb IgG1 Fab control. mAb IhfB2<sub>NTHI</sub> Fab demonstrated a 2% increase in biomass relative to control (**FIG. 2**).

**[0385]** The ability of mAb mIhfB4<sub>NTHI</sub> Fab to disrupt biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP, *S. aureus* 29213, *P. aeruginosa* 27853, and *B. cenocepacia* K56-2 was examined (**FIG. 3**). In particular, biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP, *S. aureus* 29213, *P. aeruginosa* 27853, and *B. cenocepacia* K56-2 were incubated with 440 nM per well murine IgG1, Murine IgG1 Fab, mAb IhfB2<sub>NTHI</sub>, mAb IhfB2<sub>NTHI</sub> Fab, mAb mIhfB4<sub>NTHI</sub>, and mAb mIhfB4<sub>NTHI</sub> Fab (**FIG. 3**). mAb mIhfB4<sub>NTHI</sub> and mAb mIhfB4<sub>NTHI</sub> Fab demonstrated robust disruption of biofilms formed by all bacterial species tested (**FIG. 3**).

Example 3: Otitis Media analysis using murine Fabs

**[0386]** Middle ear infection (or otitis media, OM) is a highly prevalent disease worldwide, with the most severe form (called chronic suppurative OM or CSOM) afflicting 50-330 million children globally each year. The socioeconomic burden of OM is also great, with cost estimates between \$5-6 billion in the United States alone annually. All three of the predominant bacterial pathogens of OM are known to form biofilms both *in vitro* and *in vivo*

and recently, clinicians have come to appreciate that the chronicity and recurrence of OM is due, at least in part, to the formation of bacterial biofilms within the middle ear cavity.

**[0387]** In fact, results of labeling of otorrhea solids from pediatric patients with tympanostomy tubes and persistent otorrhea for eDNA and IHF in combination with microbiological culture indicate that biofilms play a role in chronic otorrhea. Specifically, of 15 pediatric otorrhea samples analyzed, 9 (60%) contained solids positive for labeling IHF in association with a lattice of eDNA (labeled using rabbit anti-IHF, detected with goat anti-rabbit IgG conjugated to AlexaFlour 594) and 75% yielded positive bacterial cultures. Bacterial culture results demonstrated the presence of *H. influenzae*, MRSA, *S. pneumonia*, *M. catarrhalis*, and *P. aeruginosa*. These data suggest that DNABII proteins may serve as a therapeutic target in post-tympanostomy tube otorrhea among other otic disease.

**[0388]** In one chinchilla model of OM, juvenile chinchillas are first given a viral “cold” followed a week later by their being challenged intranasally with an inoculum viable bacteria. Similar to the human condition wherein “my child has a cold and a week later gets an ear infection” chinchillas will also develop a bacterial OM approximately one week after a challenge, and while experiencing the viral upper respiratory tract infection. Once bacteria gain access to the middle ear (either via ascension of the Eustachian tube or following direct challenge to the middle ear space), they will form a robust biofilm. Applicants thus contemplate and indeed have already used chinchilla models as reported herein to demonstrate the protective efficacy of IHF immunization which results in rapid resolution of existing biofilms. This model is also useful for therapeutic approaches via either passive delivery of anti-DNABII antibody or via delivery of a small molecule or other agent known to bind to IHF or other DNABII family members.

**[0389]** Because the chinchilla model is used for development and pre-clinical testing of human vaccines, it is important to establish meaningful immunological parallels with the human host, particularly the child. Applicants have shown that effusions recovered from children with AOM due to NTHI, and middle ear fluids from chinchillas with experimental NTHI-induced OM, recognized immunodominant regions of OMP P5 in a similar hierarchical manner (see for e.g., Novotny et al. (2000) Infect 68(4):2119-2128; Novotny et al. (2007) 9<sup>th</sup> International Symposium on Recent Advances in Otitis Media; St. Pete Beach, Fla.; Novotny et al. (2002) Vaccine 20(29-30):3590-3597). Applicants have also shown that chinchillas with experimental OM, children with natural OM, and adults with exacerbations of COPD, all recognized peptides representing PilA in a highly analogous manner (see, e.g.,

Adams et al. (2007) 107th General Meeting, American Society for Microbiology, 2007, Toronto, ON; Adams et al. (2007) 9th International Symposium on Recent Advances in Otitis Media, St. Pete Beach, Fla.). Thus, chinchillas with experimental OM and children with natural disease respond similarly immunologically to at least two unrelated NTHI protein adhesins. This parallel was put to the ultimate test recently, when the chinchilla AV-NTHI superinfection model was used to conduct pre-clinical efficacy testing of a novel 11-valent Protein D-pneumococcal polysaccharide conjugate vaccine. Data obtained in the chinchilla predicted an efficacy of 34% whereas, when tested in children, the efficacy obtained against *H. influenzae*-induced OM was 35.6% (see, e.g., Novotny et al. (2006) Vaccine 24(22):4804-11 and Prymula et al. (2006) Lancet. 367(9512):740-8), thus lending strong support to the relevancy of this model for the development and testing of OM vaccine candidates.

*Methods:*

**[0390]** In order to determine the efficacy of the generated antibodies, NTHI bacteria were injected into the middle ear space of the chinchillas and allowed to form a biofilm. In particular, adult chinchillas (*Chinchilla lanigera*) with no evidence of middle ear disease were procured (Rauscher's Chinchilla Ranch, LLC) and rested for 7 days prior to transbullar challenge with 1000 colony-forming units (CFU) of nontypeable *Haemophilus influenzae* #86-028NP per bulla diluted in sterile, pyrogen-free saline (Day zero (0)) (**FIG. 4**). On day 4 and on day 5, a 342 nM Fab fragment solution was infused into each middle ear space (100  $\mu$ l per bulla; 342 nM) (**FIG. 4**). On day six (6), animals were sacrificed. Experiments included cohorts using murine mAb IgG1 Fab fragments (4 chinchillas), murine mAb IhfB2<sub>NTHI</sub> Fab fragments (4 chinchillas), and murine mAb mIhfB4<sub>NTHI</sub> Fab fragments (4 chinchillas).

**[0391]** Middle ear fluids were collected and an aliquot serially diluted and plated on to chocolate agar to quantitate the relative planktonic bacterial load. Remaining fluids were centrifuged at 1000  $\times$  g for 5 min, supernatants separated and both cellular pellet and fluid fractions snap-frozen prior to storage at -80°C. The middle ear mucosa and adherent bacterial biomass were digitally imaged, collected into pre-weighed microcentrifuge tubes and homogenized in 1.0 ml sterile 0.9% sodium chloride. Homogenates were also serially diluted and plated, as before, to quantitate the population of bacteria adherent within the middle ear space per mg tissue/biomass. Remaining sample was snap-frozen prior to storage at -80°C. Culture plates were incubated for 24 h at 37°C in a humidified atmosphere prior to enumeration of bacterial colonies via Protocol2 instrument (Symbiosis).

**[0392]** Video otoscopy and tympanometry. Video otoscopy using a 0-degree, 3-inch probe connected to a digital camera system (MedRx, Largo, FL) was utilized to monitor signs of OM (e.g. tympanic membrane inflammation and/or presence of fluid in the middle ear space). Tympanometry was performed with a MADSEN Otoflex tympanometer and data analyzed with OTOSuite software (Otometrics, Schaumburg, IL). Overall signs of OM were blindly rated on an established 0 to 4+ scale and middle ears with a score of  $\geq 2.0$  were considered positive for OM if middle ear fluid was visible behind the tympanic membrane. If the tympanic membrane could not be visualized due to an obstruction within the ear canal (i.e. due to cerumen accumulation), that ear was excluded from the day's count. Per established protocol, each middle ear was considered independent, and for each cohort, the percentage of middle ears with OM was calculated.

**[0393]** To rank the residual biofilm within the middle ear space, middle ear images were scrambled and reviewed blindly by 7 individuals. Using an established rubric, a score of 0 to 4 was assigned to each image, whereby 0: no biofilm visible, 1: biofilm fills  $\leq 25\%$  of middle ear space, 2: biofilm fills  $>25\%$  to  $\leq 50\%$  of middle ear space, 3: biofilm fills  $>50\%$  to  $\leq 75\%$  middle ear space, 4: biofilm fills  $>75\%$  to  $\leq 100\%$  middle ear space.

**[0394]** This ranking/scoring scale is described in detail in Table 4 below, indicating the relative amount of biomass remaining within the middle ear of each animal.

**Table 4**

Score	Criteria
0	No evidence of biomass.
1+	Biomass fills $\leq 25\%$ of middle ear space. Junction of the bony septa to inferior bulla is visible.
2+	Biomass fills $>25\%$ to $\leq 50\%$ of middle ear space. Unable to visualize where the bony septa meet the inferior bulla.
3+	Biomass fills $>50\%$ to $\leq 75\%$ of middle ear space. Biomass covers $>50\%$ of the length of bony septa.
4+	Biomass fills $>75\%$ to $\leq 100\%$ of middle ear space. Bony septa not visible; obscured by biomass.

**[0395]** The relative quantity of a panel of pro- and anti-inflammatory cytokines in clarified middle ear fluids (TNF, IL-1 $\beta$ , IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and IL-17A) was

determined using BD Cytometric Bead array (BD Biosciences) according to manufacturer's instructions and samples assessed on a BD Accuri C6 cytometer. Data were analyzed with FloJo V\_10 software.

**[0396]** To perform histologic analyses of middle ear biofilms and mucosae, bullae were placed into 10% neutral buffered formalin after imaging and stored at 4°C. Bullae were rinsed with water for 2 h at room temperature, then placed into fresh 0.1M Tris (pH 6.95) overnight followed by an additional water rinse. Bullae were then placed into 0.35M EDTA- 0.1M Tris (pH 6.95) and decalcified with an H2800 Energy Beam Sciences microwave processor set at 37°C, 1000 watts for 2 hr cycles until negative ammonium oxalate test achieved. Bullae were then embedded in paraffin, sectioned to 5  $\mu$ m thickness and stained with hematoxylin and eosin.

*Results:*

**[0397]** Mean otoscopy scores were determined at days 4, 5, and 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab, as shown in **FIG. 5(A)**. Using a WelchAllyn MacroView otoscope, the tympanic membrane of each animal was visualized and blindly ranked on a 0 – 4+ scale, wherein a score of  $\geq 2.0$  indicated the presence of fluid within the middle ear space, i.e. otitis media. (Novotny *et al.*, (2006) *Vaccine* 24:4804). Administration of mAb mIhfB4<sub>NTHI</sub> Fab resulted in a lower mean otoscopy score compared to IgG1 Fab and IhfB2<sub>NTHI</sub> Fab controls (\* $P \leq 0.05$  and \*\*  $P \leq 0.01$ ).

**[0398]** **FIG. 5(B)** shows representative otoscopy images at day 0, day 4, and day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab. By day 4, middle ear fluid is seen behind the tympanic membrane. On day 4 and on day 5, chinchillas were administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab. At day 6, middle ear fluid is seen in chinchillas administered IgG1 Fab (control) and thickening of the tympanic membrane can be seen in chinchillas administered IhfB2<sub>NTHI</sub> Fab, both of which indicate the presence of biofilm. At day 6, a non-inflamed tympanic membrane is seen in chinchillas administered mAb mIhfB4<sub>NTHI</sub> Fab, indicating resolution of biofilm.

**[0399]** Quantitation of colony forming units (CFU) of *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of mucosal biofilm was performed in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus*

*influenzae* (NTHI) 86-028NP challenge and biofilm formation, as shown in **FIG. 6(A)**. Quantitations were performed on day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge (treatments were performed on days 4 and 5). Significantly fewer *Haemophilus influenzae* (NTHI) 86-028NP were adherent within the middle ear biofilms & to mucosa 1 day after receipt of two treatments with mIhfB4<sub>NTHI</sub> Fab compared to IgG1 Fab and IhfB2<sub>NTHI</sub> Fab controls (**FIG 6(A)**) ( $p \leq 0.01$ ).

**[0400]** In addition, quantitation of colony forming units (CFU) of *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of middle ear fluid was performed in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation, as shown in **Fig. 6(B)**. No difference between relative concentration of recoverable *Haemophilus influenzae* (NTHI) 86-028NP in middle ear fluids was observed between IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab (**Fig. 6(B)**). Without being bound by any particular theory, it is thought that a difference may not have been observed because this study was performed in a short time frame (only 6 days from challenge to sample recovery), as antibody directed against DNABII protein tips induces biofilm collapse and release of resident bacteria. It is possible that this short time frame may not have been long enough to observe the clearance of the released (planktonic) bacteria.

**[0401]** Quantitation of mass (grams, g) of middle ear mucosa and mucosal biofilm collected from chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation was also performed, as shown in **FIG. 7(A)**. Quantitations were performed on day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge. As shown in **FIG. 7(A)**, less biomass was present within the middle ear 1 day after two treatments with mIhfB4<sub>NTHI</sub> Fab.

**[0402]** **FIG. 7(B)** shows quantitations of volume (microliters, mL) of middle ear fluid (MEF) retrieved from chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed on day 6 after *Haemophilus influenzae* (NTHI) 86-028NP challenge. No difference between volume of MEF observed between chinchillas administered IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab. Without being bound by any particular theory, it is thought that a difference may not have been observed because this study was performed in a short time frame (only 6 days from challenge to sample recovery) antibody directed against DNABII protein tips induces biofilm collapse and

release of resident bacteria. It is possible that this short time frame may not have been long enough to observe a difference in relative volume of recovered middle ear fluids between chinchillas treated with IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab.

**[0403]** Mucosal biomass analysis was also performed to assess the ability of mIhfB4<sub>NTHI</sub> Fab to disrupt biofilm formed in the middle ears of chinchillas. **FIG. 8(A)** shows mucosal biomass scores reviewed blindly by 7 individuals for chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. A mucosal biofilm score scale was used to rank the residual biofilm within the middle ear space. Using an established rubric, a score of 0 to 4 was assigned to each image, as follows: zero (0): no biofilm visible; 1: biofilm fills >0 to  $\leq$ 25% of middle ear space; 2: biofilm fills >25% to  $\leq$ 50% of middle ear space; 3: biofilm fills >50% to  $\leq$ 75% middle ear space; and 4: biofilm fills >75% to 100% middle ear space. A significantly lower mucosal biomass score was observed in chinchillas treated with mIhfB4<sub>NTHI</sub> Fab compared to chinchillas administered IgG1 Fab or IhfB2<sub>NTHI</sub> Fab controls (\* $P \leq 0.05$  and \*\*\* $P \leq 0.001$ ).

**[0404]** Representative middle ear images obtained by otoscopy 1 day after receipt of final dose of either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or murine mAb mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation are shown in **FIG. 8(B)**. Treatment with mIhfB4<sub>NTHI</sub> Fab shows little to no biofilm remaining, whereas treatment with IgG1 Fab or IhfB2<sub>NTHI</sub> Fab (controls) shows abundant biofilm remaining in the middle ear.

**[0405]** Pro- and anti-inflammatory cytokines were measured in clarified middle ear fluids to assess the effects of mIhfB4<sub>NTHI</sub> Fab on inflammation. **FIG. 9** shows the relative quantity of a panel of pro- and anti-inflammatory cytokines in clarified middle ear fluids (IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17A, IL-12p70, and TNF) in chinchillas administered either IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitation was performed to provide picogram (pg) cytokine per milliliter (ml) MEF. A greater quantity of pro-inflammatory cytokines in middle ears treated with IhfB2<sub>NTHI</sub> Fab and IgG1 Fab controls were seen relative to and mIhfB4<sub>NTHI</sub> Fab. The greatest amount of anti-inflammatory cytokines IL-4 (data not shown), IL-10 (shown in **FIG. 9**), and IL-13 (data not shown) were observed in middle ears treated with mIhfB4<sub>NTHI</sub> Fab, compared to IgG1 Fab and IhfB2<sub>NTHI</sub> Fab controls.

**[0406]** **FIG. 10** shows a histologic analysis of the chinchilla middle ear. Chinchillas were administered either murine IhfB2<sub>NTHI</sub> Fab or murine mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Staining was performed with hematoxylin and eosin stains. Images were obtained using a 10X objective. The scale bar represents 100  $\mu$ m. The image on the left shows the middle ear from an animal administered murine IhfB2<sub>NTHI</sub> Fab control. This tissue section shows the presence of a robust biofilm present within the middle ear space as well as signs of significant inflammation as evidenced by the influx of PMNs, submucosal edema and focal bleeding. Overall, there was no remediation of disease or disruption of the pre-existing bacterial biofilm following administration of the murine IhfB2<sub>NTHI</sub> Fab control. Conversely, the image on the right shows the middle ear from an animal administered murine mIhfB4<sub>NTHI</sub> Fab. This tissue section shows the absence of any trace of a biofilm within the middle ear lumen. In addition, whereas there is some disorganization of the mucosal epithelium with limited submucosal focal bleeding, these changes are exceptionally mild compared to the image on the left. Overall, administration of murine mIhfB4<sub>NTHI</sub> Fab resulted in both complete eradication of the pre-existing bacterial biofilm and significantly reduced signs of inflammation fully in keeping with other data obtained in this pre-clinical study.

Example 4: Otitis Media analysis using rabbit Fab.

Methods:

**[0407]** Example 1 describes the generation of Fab (fragment antigen binding) fragments from rabbit polyclonal antibodies directed against *Haemophilus influenzae* IhfB2<sub>NTHI</sub> and mIhfB4<sub>NTHI</sub>, as well as from naive rabbit serum IgG. The purity of each Fab preparation (rabbit polyclonal naïve Fab, rabbit polyclonal IhfB2 Fab, and rabbit polyclonal mIhfB4 Fab) was confirmed by PAGE (**FIG. 11**). **FIG. 11** shows the purity of each Fab preparation (3 separate preparations: naïve, IhfB2 and mIhfB4), as confirmed by 10% Bis-Tris PAGE (BioRad) and SYPRO Orange Protein Gel stain (Invitrogen).

**[0408]** **FIG. 11** depicts the method by which the disruption of biofilms formed by *Haemophilus influenzae* (NTHI) 86-028NP was analyzed in the middle ear of adult chinchillas using Fab fragments generated from polyclonal rabbit anti-IhfB2<sub>NTHI</sub>, anti-mIhfB4<sub>NTHI</sub>, and naive rabbit serum. Experiments included cohorts using native rabbit serum IgG Fab fragments, rabbit anti-IhfB2<sub>NTHI</sub> Fab fragments, and rabbit anti-mIhfB4<sub>NTHI</sub> Fab fragments.

**[0409]** In order to determine the efficacy of the generated Fab (fragment antigen binding) fragments from rabbit polyclonal antibodies, NTHI bacteria were injected into the middle ear space of the chinchillas and allowed to form a biofilm. In particular, adult chinchillas (*Chinchilla lanigera*) with no evidence of middle ear disease were procured (Rauscher's Chinchilla Ranch, LLC) and rested for 7 days prior to transbullar challenge with 1000 colony-forming units (CFU) of nontypeable *Haemophilus influenzae* #86-028NP per bulla diluted in sterile, pyrogen-free saline (Day zero (0)) (**FIG. 11**). Then, either on days 4 and 5 or on days 4, 5, and 6, a 342 nM Fab fragment solution was infused into each middle ear space (100  $\mu$ l per bulla; 342 nM). For chinchillas administered the Fab solution on days 4 and 5, 3 chinchillas per cohort were sacrificed either on day 6 or day 12 (two dose experiments) (**FIG. 11**). For those administered on day 4, 5, and 6, 3 chinchillas per cohort were sacrificed on day 13 (three dose experiments). Following sacrifice, chinchillas were imaged, middle ear mucosa was collected, adherent biofilm was assessed, middle ear fluids were collected, quantitation of bacteria was performed, and middle ear fluids were assessed using a cytokine multiplex assay.

**[0410]** Methods for preparation of middle ear fluids, video otoscopy and tympanometry, residual biofilm ranking, and quantification of cytokines were performed as described in Example 3.

**[0411]** The ability of rabbit polyclonal mIhfB4<sub>NTHI</sub> Fab to reduce mucosal biofilm was determined. **FIG. 12** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of mucosal biofilm in chinchillas administered either rabbit IgG1 Fab, rabbit IhfB2<sub>NTHI</sub> Fab, or rabbit mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed for the following dose/sacrifice combinations: (1) two doses of the respective rabbit Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective rabbit Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective rabbit Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge.

**[0412]** In all three dose/sacrifice combinations, significantly fewer *Haemophilus influenzae* (NTHI) 86-028NP were adherent to the middle ear mucosa and present within biofilms after administration of mIhfB4<sub>NTHI</sub> Fab compared to nonspecific IgG1 Fab and IhfB2<sub>NTHI</sub> Fab controls (**FIG. 12**). Administration of two doses of mIhfB4 Fab fragments

was comparable to three doses in eradicating established middle ear biofilms (**FIG. 12**). At 7 days following administration of two treatment doses, animals that had received Fab fragments to mIhfB4 immunogen had significantly fewer bacteria within this biological sample from the middle ear (\*\*  $P \leq 0.01$ ), as shown in **FIG. 12**.

**[0413]** **FIG. 13** shows quantitations of colony forming units (CFU) *Haemophilus influenzae* (NTHI) 86-028NP per milligram (mg) of middle ear mucosa in chinchillas administered either rabbit polyclonal IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed for the following dose/sacrifice combinations: (1) two doses of the respective Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge. Because antibodies directed against DNABII protein tips can induce biofilm collapse and release of resident bacteria, a difference in relative concentration of recoverable NTHI in middle ear fluids one day after administration of the final dose was not expected and was not observed. However, one week after receipt of two or three doses of mIhfB4 Fab fragments, a significant reduction in NTHI within middle ear fluids compared to either the cohort that received IgG1 Fab or IhfB2<sub>NTHI</sub> Fab was observed (**FIG. 13**) ( $P \leq 0.01$  and  $P \leq 0.05$ , respectively). Administration of Fab fragments directed against mIhfB4 continued to mediate a therapeutic effect for at least one week following administration of the last dose of Fab and data suggest this dosing regime is flexible and optimizable.

**[0414]** Mean mucosal biofilm scores were determined to assess the ability of rabbit polyclonal mIhfB4NTHI Fab to disrupt biofilm. **FIG. 14** shows mean mucosal biofilm scores for chinchillas administered either rabbit polyclonal IgG1 Fab, IhfB2<sub>NTHI</sub> Fab, or mIhfB4<sub>NTHI</sub> Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Quantitations were performed for the following dose/sacrifice combinations: (1) two doses of the respective Fabs were administered, followed by sacrifice 1 day later; (2) two doses of the respective Fabs were administered, followed by sacrifice 7 days later; and (3) three doses of the respective Fabs were administered, followed by sacrifice 7 days later. Doses of Fabs were administered on days 4 and 5 (two doses) or on days 4, 5, and 6 (three doses) after *Haemophilus influenzae* (NTHI) 86-028NP challenge. A mucosal biofilm score

scale was used to rank the residual biofilm within the middle ear space. Using an established rubric, a score of 0 to 4 was assigned to each image, as follows: zero (0): no biofilm visible; 1: biofilm fills  $>0$  to  $\leq 25\%$  of middle ear space; 2: biofilm fills  $>25\%$  to  $\leq 50\%$  of middle ear space; 3: biofilm fills  $>50\%$  to  $\leq 75\%$  middle ear space; and 4: biofilm fills  $>75\%$  to 100% middle ear space (**FIG. 14**).

**[0415]** As shown in **FIG. 14**, a significantly lower mean mucosal biomass score was observed in chinchillas treated with rabbit polyclonal mIhfB4<sub>NTHI</sub> Fab compared to chinchillas administered rabbit polyclonal IgG1 Fab or IhfB2<sub>NTHI</sub> Fab controls ( $*P \leq 0.05$  and  $**P \leq 0.01$ ). For data in which two doses of the respective Fabs were administered, followed by sacrifice 1 day later, a significantly lower mean mucosal biomass score was observed in chinchillas treated with mIhfB4<sub>NTHI</sub> Fab compared to either the cohort that received IgG1 Fab or IhfB2<sub>NTHI</sub> Fab ( $P \leq 0.05$ ) (**FIG. 14**). For data in which two doses of the respective Fabs were administered, followed by sacrifice 7 days later, a significantly lower mean mucosal biomass score was observed in chinchillas treated with mIhfB4<sub>NTHI</sub> Fab compared to the cohort that received IgG1 Fab ( $P \leq 0.05$ ) and compared to the cohort that received IhfB2<sub>NTHI</sub> Fab ( $P \leq 0.01$ ) (**FIG. 14**). For data in which three doses of the respective Fabs were administered, followed by sacrifice 7 days later, a significantly lower mean mucosal biomass score was observed in chinchillas treated with mIhfB4<sub>NTHI</sub> Fab compared to either the cohort that received IgG1 Fab or IhfB2<sub>NTHI</sub> Fab ( $P \leq 0.05$ ) (**FIG. 14**). Because antibody directed against DNABII protein tips induces biofilm collapse, a significant decrease in mucosal biofilm in the middle ear of animals administered mIhfB4 Fab fragments one day after receipt of two doses was observed. This decrease was maintained one week after receipt of two or three doses of mIhfB4 Fab fragments. Administration of Fab fragments directed against mIhfB4 continued to mediate a therapeutic effect for at least one week after receipt of the last dose and data suggest this dosing regime is flexible and optimizable.

**[0416]** Pro- and anti-inflammatory cytokines were measured in clarified middle ear fluids to assess the effects of mIhfB4<sub>NTHI</sub> Fab on inflammation. **FIG. 15** shows the relative quantity of a panel of pro- and anti-inflammatory cytokines in clarified middle ear fluids in chinchillas administered either naïve rabbit IgG Fab, rabbit anti-IhfB2<sub>NTHI</sub> IgG Fab, or rabbit anti-mIhfB4<sub>NTHI</sub> IgG Fab after *Haemophilus influenzae* (NTHI) 86-028NP challenge and biofilm formation. Fluids were collected from chinchillas that were administered two doses of Fab fragments and sacrificed one day after the receipt of the second dose. The pro-inflammatory

cytokines measured included: IL-1 $\beta$ , IL-6, IL-8, IL-12p70, IL-17A, TNF, and IFN $\gamma$ . The anti-inflammatory cytokines measured included: IL-4, IL-10, and IL-13. A greater relative quantity of pro-inflammatory cytokines were observed in middle ears treated with rabbit anti-IhfB2<sub>NTHI</sub> Fab or nonspecific rabbit IgG Fab compared to middle ears treated with rabbit anti-mIhfB4<sub>NTHI</sub> IgG Fab (shown in **FIG. 15**). The greatest quantity of anti-inflammatory cytokines (IL-4, IL-10, and IL-13) were observed in middle ears treated with rabbit anti-mIhfB4<sub>NTHI</sub> Fab (shown in **FIG. 15**).

**[0417]** A summary of the efficacies of IgG Fab fragments versus intact rabbit polyclonal and mouse monoclonal IgG is shown in Table 5 below.

**Table 5**

Characteristics:		<i>In vitro</i> vs NTHI Biofilms		<i>In vivo</i> (Chinchilla model of NTHI induced otitis media)			
Host/ Target	Clonality of IgG/ IgG or Fab Fragment	Concentra- tion applied to pre-formed biofilms	Reduction in Biomass <sup>1</sup>	Concentra- tion infused into middle ear space	Log reduction in CFU NTHI/ mg middle ear mucosal biofilm <sup>2</sup>	Middle ear biofilm score <sup>3</sup>	<i>in</i> <i>vivo</i> study code <sup>4</sup>
Mouse/I hfB2	Mono- clonal/ IgG	171 nM	0	342.5 nM	0	3.3	Po Mo
Mouse/I hfB2	Mono- clonal/ Fab	171 nM	0	342.5 nM	0	2.7	mAb Fab
Mouse/ mIhfB4	Mono- clonal/ IgG	171 nM	71%	342.5 nM	1.8-log <sub>10</sub>	1.4	Po Mo
Mouse/ mIhfB4	Mono- clonal/ Fab	171 nM	77%	342.5 nM	3.9-log <sub>10</sub>	1.0	mAb Fab

Rabbit/ IhfB2	Poly-clonal/ IgG	171 nM	0	342.5 nM	ND	ND	Po Mo
Rabbit/ IhfB2	Poly-clonal/ Fab	171 nM	0	342.5 nM	0.4- $\log_{10}$	3.7	Rab pAb Fab
Rabbit/ mIhfB4	Poly-clonal/ IgG	171 nM	75%	342.5 nM	ND	ND	Po Mo
Rabbit/ mIhfB4	Poly-clonal/ Fab	171 nM	78%	342.5 nM	5.5- $\log_{10}$	1.0	Rab pAb Fab

<sup>1</sup>Relative to respective naive serum for polyclonal sera or nonspecific murine IgG1 for monoclonal sera determined by COMSTAT2.

<sup>2</sup>One day after receipt of the final dose, relative to respective naive serum for polyclonal sera or nonspecific murine IgG1 for monoclonal sera.

<sup>3</sup>Biomass score: 0= no biomass; 1= 0-25% middle ear space filled with bacterial biomass; 2= >25-50% filled; 3= >50-75% filled; 4=>75-100% filled.

<sup>4</sup>PoMo: rabbit polyclonal and murine monoclonal antibody efficacy study; mAb Fab: murine monoclonal IgG Fab fragments efficacy study; Rab pAb Fab: rabbit polyclonal IgG Fab fragments efficacy study.

<sup>5</sup>ND refers to Not done, that is these antibodies not tested *in vivo*.

**[0418]** Table 5 shows the targets (IhfB2 and mIhfB4) used to generate each of various mouse monoclonals, providing data for IgG and Fab fragments for each of these targets. Table 5 also shows the targets (IhfB2 and mIhfB4) used to generate each of various rabbit polyclonal sera, providing data for IgG and Fab fragments for each of these targets. All experiments shown were performed as highly controlled therapeutic studies. The table summarizes and provides quantitations regarding the experiments presented in Examples 2, 3, and 4, above, as well as presents data (“PoMo” study code) that are published in ‘Monoclonal antibodies against DNA-binding tips of DNABII proteins disrupt biofilms *in vitro* and induce bacterial clearance *in vivo*.’ (2016) EBioMedicine. Aug; 10:33-44. For the *in vitro* vs NTHI biofilms, the methods used were as described in Example 2, above. The exact concentration of antibodies or Fab fragments as indicated was applied to pre-formed biofilms. In particular, the concentration used was 171 nM for all antibodies and fragments used in the *in vitro* analyses. For the *in vivo* studies, the methods used were as described in Examples 3 and 4,

above. The exact inoculum (*Haemophilus influenzae* (NTHI) 86-028NP) delivered to each animal's ear to initiate biofilm formation was controlled and infusion of ears was performed with specific quantities of antibody or fragments as indicated. In particular, the concentration used was 342.5 nM for all antibodies and fragments used in the *in vivo* analyses.

**[0419]** In the *in vitro* biofilm analyses using murine antibodies and Fab fragments, murine monoclonal mIhfB4 IgG showed a 71% reduction in biomass and murine monoclonal mIhfB4 Fab showed a 77% reduction in biomass. For the *in vivo* chinchilla model of NTHI induced otitis media, murine monoclonal mIhfB4 IgG showed a 1.8-log<sub>10</sub> reduction in CFU NTHI/ mg middle ear mucosal biofilm and murine monoclonal mIhfB4 Fab showed a 3.9-log<sub>10</sub> reduction in CFU NTHI/ mg middle ear mucosal biofilm. These measurements were performed one day after receipt of the final dose, and are relative to nonspecific murine IgG1. Murine IhfB2 IgG showed a middle ear biofilm score of 3.3. Murine IhfB2 Fab showed a middle ear biofilm score of 2.7. Murine mIhfB4 IgG showed a middle ear biofilm score of 1.4. Murine mIhfB4 Fab showed a middle ear biofilm score of 1.0.

**[0420]** In the *in vitro* biofilm analyses using rabbit antibodies and Fab fragments, rabbit polyclonal mIhfB4 IgG showed a 75% reduction in biomass and rabbit polyclonal mIhfB4 Fab showed a 78% reduction in biomass. For the *in vivo* chinchilla model of NTHI induced otitis media, rabbit polyclonal mIhfB4 Fab showed 5.5-log<sub>10</sub> reduction in CFU NTHI/ mg middle ear mucosal biofilm, whereas rabbit polyclonal IhfB2 Fab (control) showed 0.4-log<sub>10</sub> reduction in CFU NTHI/ mg middle ear mucosal biofilm. Rabbit polyclonal mIhfB4 Fab showed a middle ear biofilm score of 1.0, whereas rabbit polyclonal IhfB2 Fab (control) showed a middle ear biofilm score of 3.7.

**[0421]** The "PoMo" *in vivo* study code provided in Table 5 refers to data that are published in 'Monoclonal antibodies against DNA-binding tips of DNABII proteins disrupt biofilms *in vitro* and induce bacterial clearance *in vivo*.' (2016) *EBioMedicine*. Aug; 10:33-44.

#### Example 5: Passive Immunity

**[0422]** Methods described herein may be used to confer passive immunity on a non-immune subject. Passive immunity against a given antigen may be conferred through the transfer of antibody fragments or antigen binding fragments that specifically recognize or bind to a particular antigen as disclosed herein. Antibody donors and recipients may be human or non-human subjects. Additionally or alternatively, the antibody composition may

comprise an isolated or recombinant polynucleotide encoding an antibody fragment or antigen binding fragment that specifically recognizes or binds to a particular antigen.

**[0423]** Passive immunity may be conferred in cases where the administration of immunogenic compositions poses a risk for the recipient subject, the recipient subject is immuno-compromised, or the recipient subject requires immediate immunity. Immunogenic compositions may be prepared in a manner consistent with the selected mode of administration. Compositions may comprise antibody fragments and/or antigen binding fragments, alone or in combination with polyclonal antibodies, monoclonal antibodies, antibodies generated *in vivo*, antibodies generated *in vitro*, purified or partially purified antibodies, or whole serum. Administration may comprise a single dose of an antibody composition, or an initial administration followed by one or more booster doses. Booster doses may be provided a day, two days, three days, a week, two weeks, three weeks, one, two, three, six or twelve months, or at any other time point after an initial dose. A booster dose may be administered after an evaluation of the subject's antibody titer. Methods can be used for monitoring the effectiveness of the therapy through biofilm formation.

**[0424]** Video otoscopy and tympanometry can be used for monitoring biofilm formation. Video otoscopy using a 0-degree, 3-inch probe connected to a digital camera system (MedRx, Largo, FL) is utilized to monitor signs of OM (e.g. tympanic membrane inflammation and/or presence of fluid in the middle ear space). Tympanometry is performed with a MADSEN Otoflex tympanometer and data analyzed with OTOSuite software (Otometrics, Schaumburg, IL). Overall signs of OM are blindly rated on an established 0 to 4+ scale and middle ears with a score of  $\geq 2.0$  were considered positive for OM if middle ear fluid is visible behind the tympanic membrane. If the tympanic membrane can not be visualized due to an obstruction within the ear canal (i.e. due to cerumen accumulation), that ear can be excluded from the day's count. Per established protocol, each middle ear is considered independent, and for each cohort, the percentage of middle ears with OM was calculated. The production of pro-inflammatory and anti-inflammatory cytokines can be evaluated using methods as disclosed herein.

#### Example 6: Treatment of Oral Disease

**[0425]** A number of oral bacteria (e.g., *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*) have been implicated in the pathogenesis of inflammatory diseases such as periodontitis and peri-implantitis, which destroy alveolar bone and gingiva.

Investigations of the pathogenesis of these bacteria are hampered by lack of effective animal models. One of the challenges of investigating the pathogenicity of specific bacteria is the difficulty of establishing a biofilm when exogenous bacteria are introduced into the oral cavity of animals. Though animal models of periodontitis have been developed, cultivable bacteria are rarely recovered from the oral cavity of inoculated animals. Developing an effective animal model which can assess the pathogenicity of specific bacteria will greatly aid in elucidating their pathogenic mechanisms.

**[0426]** One can use the compositions as described herein to treat oral disease. To test efficacy, the surface of machined titanium dental implants (1.2×4.5 mm) can be modified by grit blasting with A103 (100  $\mu$ m) and HCl etching (pH 7.8 for 20 min at 80° C.). Machined and nano-textured implants are incubated in TSB medium inoculated with D7S clinical strain of *Aggregatibacter actinomycetemcomitans* (Aa) for 1 to 3 days at 37° C. The bacterial biofilm on the implants are analyzed by SEM, as well as by confocal laser scanning microscopy following staining with LIVE/DEAD® BacLight™. Implants with and without established biofilm are transmucosally placed into the alveolar bone of female rats between premolar and incisor region of the maxillae. To detect the presence of a biofilm on the implants placed *in vivo*, bacterial samples are collected from saliva and the oral surfaces of implants after 2 days. Aa can be detected by culture, as well as by PCR analysis. Micro-CT and histological analysis of peri-implant bone and mucosal tissues can be performed at various time points, e.g., six weeks after implantation. The methods and compositions disclosed herein are developed both therapeutic as well as preventative strategies for reduction and/or elimination of these biofilms using the compositions as described herein. A decrease in redness, inflammation, and bleeding compared to infected controls would indicate biofilm reduction and/or elimination. In addition, reduced or absent inflammatory or pro-inflammatory histology and maintenance of torque removal force for the implant screw compared to infected controls indicates biofilm reduction and/or elimination.

#### Example 7: Lyme Disease

**[0427]** This experiment provides a mouse model for pre-clinical testing of compositions as described to treat lyme disease. See Dresser et al. *Pathogens* 5(12)e1000680, Epub 2009 Dec. 4. Lyme disease is caused by the microorganism *Borrelia burgdorferi*, a spirochete. *B. burgdorferi* is transmitted via the bite of the *Ixodes* tick and subsequently disseminates, via the bloodstream, to other tissues and organs.

**[0428]** In this animal model, C3H/HeN mice are injected with spirochetes via dorsal subcutaneous and intraperitoneal injection, or via intravenous injection. Blood and biopsy specimens are recovered at approximately 7 days post infection for evaluation of microbial burden and assessment of pathology in tissues and organs. The methods and compositions disclosed herein are contemplated to develop both therapeutic as well as preventative strategies for reduction and/or elimination of the resulting *B. burgdorferi* biofilms which form subsequent to challenge and are believed to contribute to both the pathogenesis and chronic nature of the disease.

Example 8: Cystic Fibrosis

**[0429]** This experiment provides a porcine model for pre-clinical testing of agents to treat cystic fibrosis. See Stoltz et al. (2010) *Science Translational Medicine* 2(29):29-31. Cystic fibrosis is an autosomal recessive disease due to mutations in a gene that encodes the CF transmembrane conductance regulator (called CFTR) anion channel. In this model, pigs which have been specifically bred to carry a defect in the genes called “CFTR” and called CF pigs spontaneously develop hallmark features of CF lung disease that includes infection of the lower airway by multiple bacterial species. The pigs can be immunized with the agents as described herein to either: 1) immunize these CF pigs with a polypeptide or other immunogenic agent thereby inducing the formation of antibodies which will eradicate bacterial biofilms in the lungs (similarly to how antibodies to IHF eradicated biofilms resident within the middle ears of chinchillas following active immunization), to deliver agents to the lungs of these animals by nebulization to assess the amelioration of the signs of disease and associated pathologies.

Example 9: Tuberculosis

**[0430]** Applicants also provide a pre-clinical model for tuberculosis (TB). See Ordway et al. (2010) *Anti. Agents and Chemotherapy* 54:1820. The microorganism *Mycobacterium tuberculosis* is responsible for a growing global epidemic. Current figures suggest that there are approximately 8 million new cases of TB and about 2.7 million deaths due to TB annually. In addition to the role of this microbe as a co-infection of individuals with HIV (of the ~45 million infected with HIV, estimates are that  $\sim\frac{1}{3}$  are also co-infected with *M. tuberculosis*), its particularly troublesome that isolates have become highly resistant to multiple drugs and no new drug for TB has been introduced in over a quarter of a century. In this animal model, SPF guinea pigs are maintained in a barrier colony and infected via

aerosolized spray to deliver ~20 cfu of *M. tuberculosis* strain Erdman K01 bacilli into their lungs. Animals are sacrificed with determination of bacterial load and recovery of tissues for histopathological assessment on days 25, 50, 75, 100, 125 and 150 days post-challenge. Unlike mice which do not develop classic signs of TB, guinea pigs challenged in this manner develop well-organized granulomas with central necrosis, a hallmark of human disease. Further, like humans, guinea pigs develop severe pyogranulomatous and necrotizing lymphadenitis of the draining lymph nodes as part of the primary lesion complex. Use of this model will provide a pre-clinical screen to confirm and identify therapeutic as well as preventative strategies for reduction and/or elimination of the resulting *M. tuberculosis* biofilms which have been observed to form in the lungs of these animals subsequent to challenge and are believed to contribute to both the pathogenesis and chronicity of the disease.

#### Example 10: Device Application

**[0431]** Multiple animal models of catheter/indwelling device biofilm infections are known. See Otto (2009) *Nature Reviews Microbiology* 7:555. While typically considered normal skin flora, the microbe *Staphylococcus epidermidis* has become what many regard as a key opportunistic pathogen, ranking first among causative agents of nosocomial infections. Primarily, this bacterium is responsible for the majority of infections that develop on indwelling medical devices which are contaminated by this common skin colonizer during device insertion. While not typically life-threatening, the difficulty associated with treatment of these biofilm infections, combined with their frequency, makes them a serious public health burden. Current costs associated with treatment of vascular catheter associated bloodstream infections alone that are due to *S. epidermidis* amount to \$2 billion annually in the United States. In addition to *S. epidermidis*, *E. faecalis* and *S. aureus* are also contaminations found on indwelling medical devices. There are several animal models of catheter-associated *S. epidermidis* infections including rabbits, mice, guinea pigs and rats all of which are used to study the molecular mechanisms of pathogenesis and which lend themselves to studies of prevention and/or therapeutics. Rat jugular vein catheters have been used to evaluate therapies that interfere with *E. faecalis*, *S. aureus* and *S. epidermidis* biofilm formation. Biofilm reduction is often measured three ways—(i) sonicate catheter and calculate CFUs, (ii) cut slices of catheter or simply lay on a plate and score, or (iii) the biofilm can be stained with crystal violet or another dye, eluted, and OD measured as a proxy for CFUs.

biofilm can be stained with crystal violet or another dye, eluted, and OD measured as a proxy for CFUs.

### Equivalents

**[0432]** Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

**[0433]** The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

**[0434]** Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

**[0435]** The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

**[0436]** In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

**[0437]** All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control. Reference to a publication does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

**[0438]** Other embodiments are set forth within the following claims.

The claims that define the invention are as follows:

1. A method to treat or prevent an inflammatory response caused by an infection with biofilm-producing eubacteria that produce a DNABII protein in a subject, or treat a subject infected with a biofilm or suffering from a condition caused by the presence of a biofilm produced by a biofilm-producing eubacteria that produced the DNABII protein, the method comprising administering to the subject an effective amount of an antibody Fab fragment of an mIhfB4<sub>NTHI</sub> antibody.
2. The use of an antibody Fab fragment of an mIhfB4<sub>NTHI</sub> antibody in the manufacture of a medicament for treating or preventing an inflammatory response caused by an infection with biofilm-producing eubacteria that produce a DNABII protein in a subject, or treating a subject infected with a biofilm or suffering from a condition caused by the presence of a biofilm produced by a biofilm-producing eubacteria that produced the DNABII protein, by administering to the subject an effective amount of the antibody Fab fragment.
3. A method according to claim 1 or a use according to claim 2, wherein:
  - the mIhfB4<sub>NTHI</sub> antibody is derived from a mammalian polyclonal antibody, that is optionally selected from the group of a rabbit polyclonal antibody, a murine polyclonal antibody, a sheep polyclonal antibody, a canine polyclonal antibody, or a human polyclonal antibody;
  - the mIhfB4<sub>NTHI</sub> antibody is derived from monoclonal antibody;
  - the mIhfB4<sub>NTHI</sub> antibody is a humanized antibody or a human antibody,
  - the mIhfB4<sub>NTHI</sub> antibody is derived from a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335,
  - the Fab fragment further comprises a detectable label,
  - the administration of the Fab fragment of the mIhfB4<sub>NTHI</sub> antibody induces the production of an anti-inflammatory cytokine response that comprises one or more of: IL-4, IL-13, or IL-10,
  - the subject is a mammal, optionally a human or a pediatric patient, or
  - the method further comprising administering an effect amount of an antibiotic and/or vaccine adjuvant.

4. An isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335, optionally further comprising a detectable label.
5. A composition comprising the isolated polypeptide of claim 4 and a carrier, optionally wherein the carrier comprises a preservative or stabilizer.
6. An isolated polynucleotide encoding the isolated polypeptide of claim 4, optionally further comprising a detectable label,  
optionally wherein the detectable label is not a conjugated polynucleotide,  
optionally wherein the isolated polynucleotide is operably linked to a regulatory element, that optionally is contained within an expression vector or a host cell.
7. A composition comprising the isolated polynucleotide of claim 6 and a carrier, optionally wherein the carrier comprises a preservative or stabilizer.
8. A vector comprising the isolated polynucleotide of claim 6.
9. An isolated host cell comprising the polypeptide of claim 4 or the isolated polynucleotide of claim 6,  
optionally wherein the host cell is a prokaryotic cell or a eukaryotic cell.
10. A method for producing a polypeptide comprising culturing the isolated host cell of claim 9 under conditions that allow for the expression of polynucleotide to a polypeptide, optionally further comprising isolating the polypeptide.
11. A method to prevent formation of or to disrupt a biofilm associated with an industrial process comprising treating a surface susceptible to or containing a biofilm caused by eubacteria that express IHF DNABII protein an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.
12. A method of identifying if a binding moiety disrupts a biofilm, comprising contacting a biofilm component with the binding moiety and with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited

under ATCC Accession No. PTA-122335 and assaying for the binding of the binding moiety the DNABII in the biofilm as compared to the binding of the isolated polypeptide to the DNABII in the biofilm, wherein a binding moiety with substantially the same or greater binding as compared to the binding of the polypeptide disrupts a biofilm.

13. A method to treat a condition characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein in a subject or detect the formation of a biofilm caused by eubacteria that express IHF DNABII protein in the subject, comprising administering an effective amount of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.

14. Use of the polypeptide of claim 4 in the manufacture of a medicament for treating a condition in a subject or detecting a biofilm in the subject, wherein the condition is characterized by the formation of a biofilm, by administering an effective amount of the polypeptide.

15. The method of claim 13 or use of claim 14, wherein when the biofilm is to be detected, the method further comprises imaging or monitoring biofilm formation or disruption in the subject; when the biofilm is to be detected, the polypeptide is chemically modified; when the biofilm is to be detected, the polypeptide is conjugated to a diagnostic agent,; and/or the condition is selected from the group of: chronic non-healing wounds, including venous ulcers and diabetic foot ulcers, ear infections, sinus infections, urinary tract infections, pulmonary infections, cystic fibrosis, chronic obstructive pulmonary disease, catheter-associated infections, infections associated with implanted prostheses, or periodontal disease.

16. A method to monitor the development or progression of a condition in a subject, wherein the condition is characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein, the method comprising treating the subject with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335,

optionally wherein the method further comprises observing complexation of the isolated polypeptide with any biofilm present.

17. A method to monitor the treatment of a condition in a subject, wherein the condition is characterized by the formation of a biofilm caused by eubacteria that express IHF DNABII protein, the method comprising administering the subject with an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335,

optionally wherein the method further comprises imaging or monitoring biofilm formation and/or disruption in the subject,

optionally wherein the polypeptide is chemically modified, and/or

optionally wherein the polypeptide is conjugated to a diagnostic agent.

18. A method to prevent formation of or to disrupt a biofilm caused by eubacteria that express IHF DNABII protein in a subject in need thereof, comprising administering to the subject an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335.

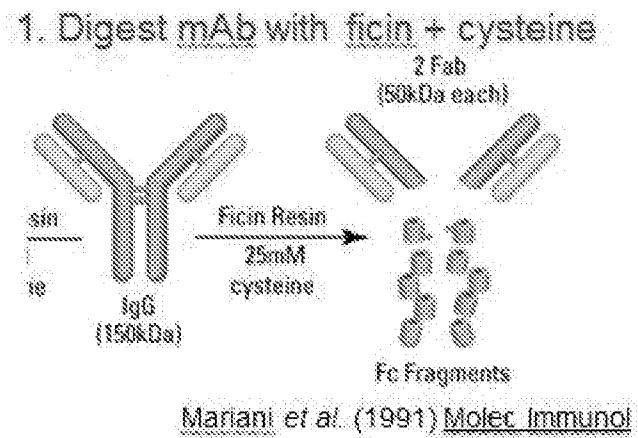
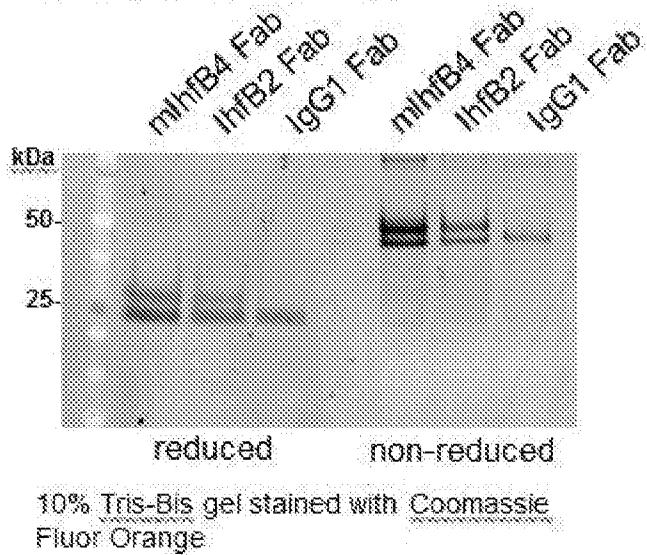
19. An anti-biofilm coating for a non-physiological surface comprising the polypeptide of claim 4, or a non-physiological surface coated with the polypeptide of claim 4, optionally wherein the surface is in an industrial setting.

20. A method to treat or prevent a condition associated with infection of eubacteria that produce a DNABII polypeptide in the subject in a subject, comprising administering to the subject an effective amount of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335 and an antibiotic and/or vaccine adjuvant, optionally wherein the polypeptide increases the potency of the antibiotic.

21. Use of an isolated polypeptide consisting essentially of a Fab fragment of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession No. PTA-122335, in the manufacture of a medicament for treating or preventing a condition associated with infection of eubacteria that produce a DNABII polypeptide in a subject, the treating or preventing comprising administering to the subject an effective amount of the isolated

polypeptide and an antibiotic and/or vaccine adjuvant, optionally wherein the polypeptide increases the potency of the antibiotic.

22. A kit comprising the isolated polypeptide of claim 4 and instructions for use, optionally further comprising an anti-IhfB2<sub>NHTI</sub> antibody or fragment thereof.

**FIG. 1****2. Purify Fab fragments**

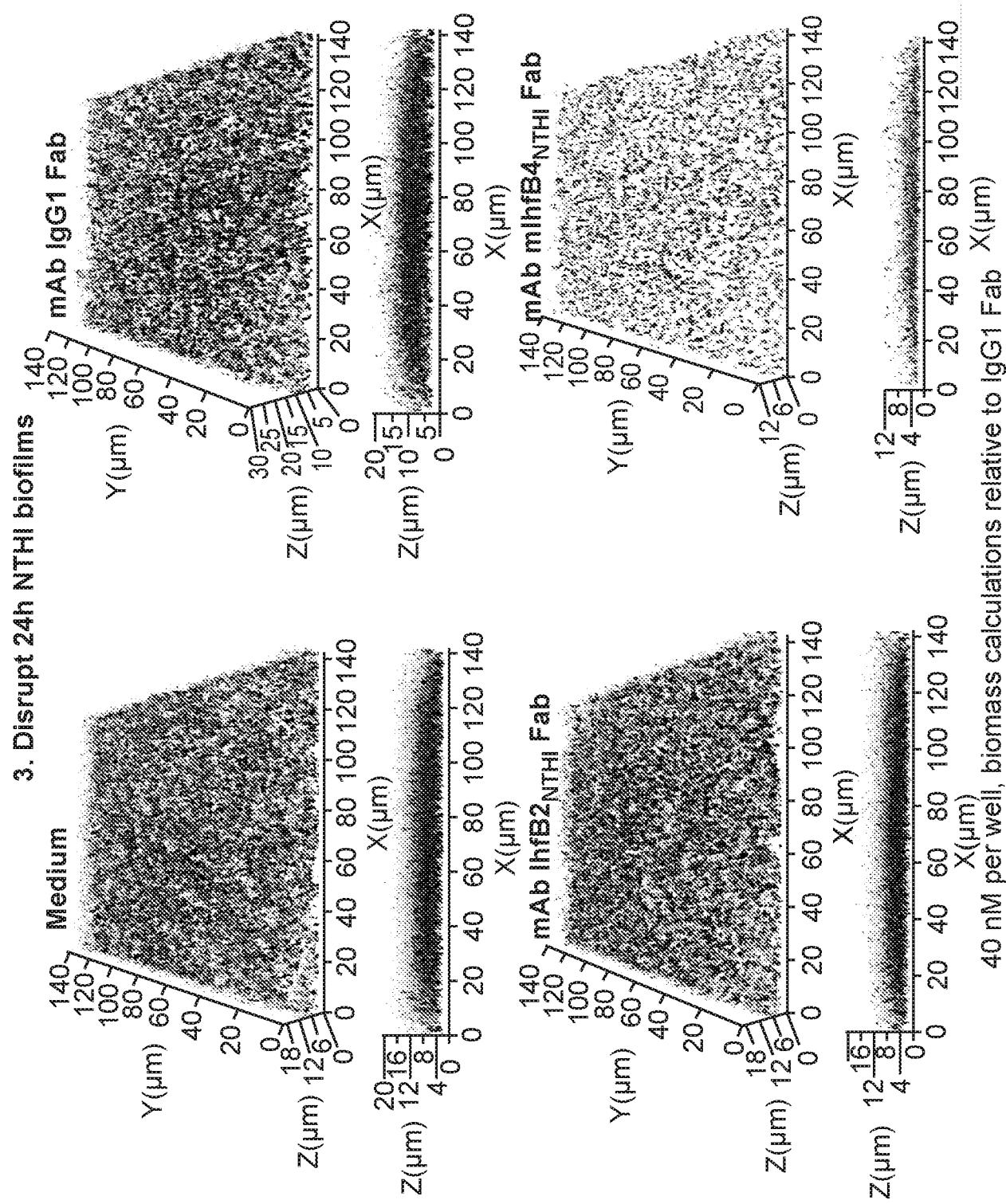
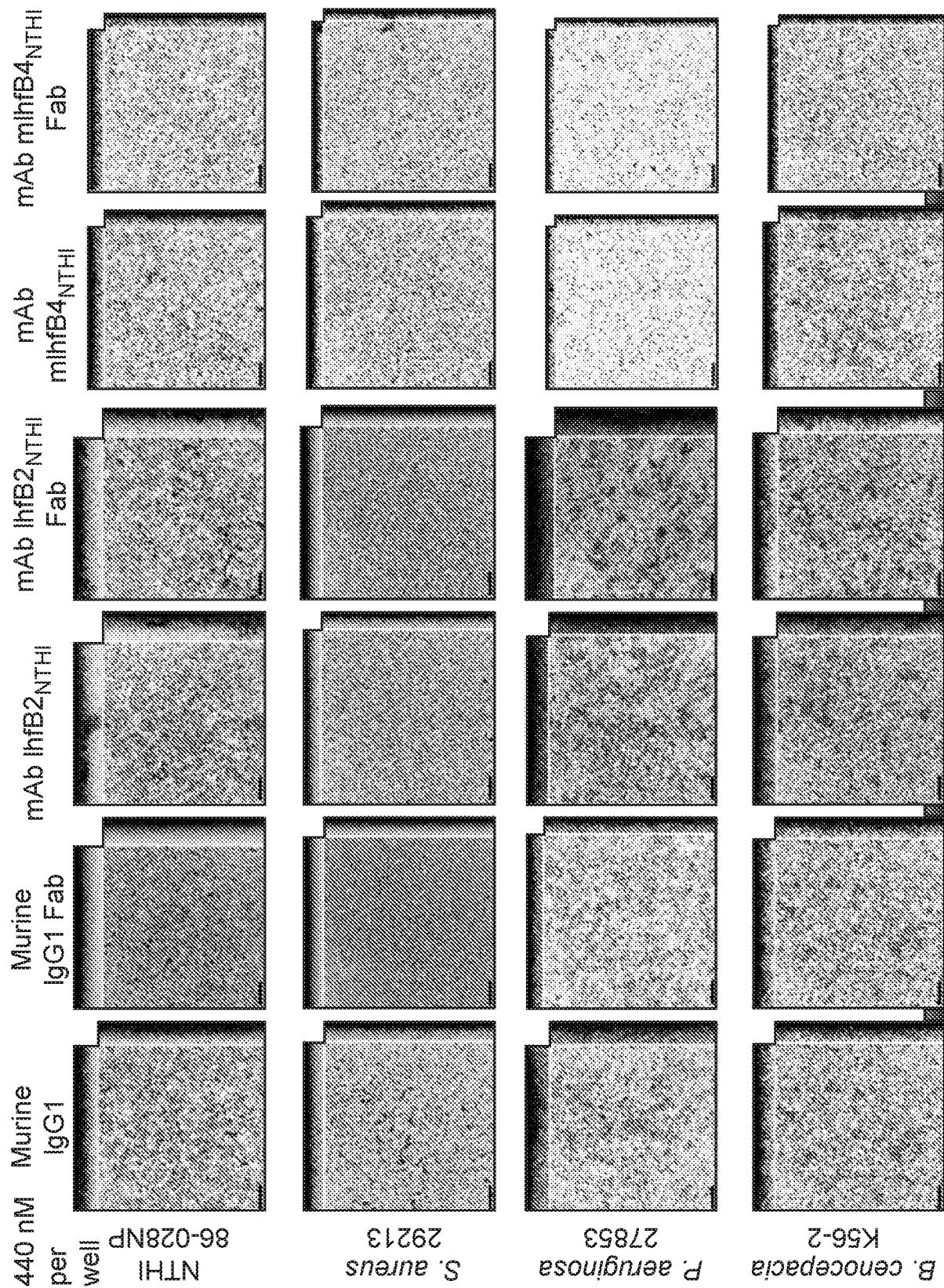


FIG. 3



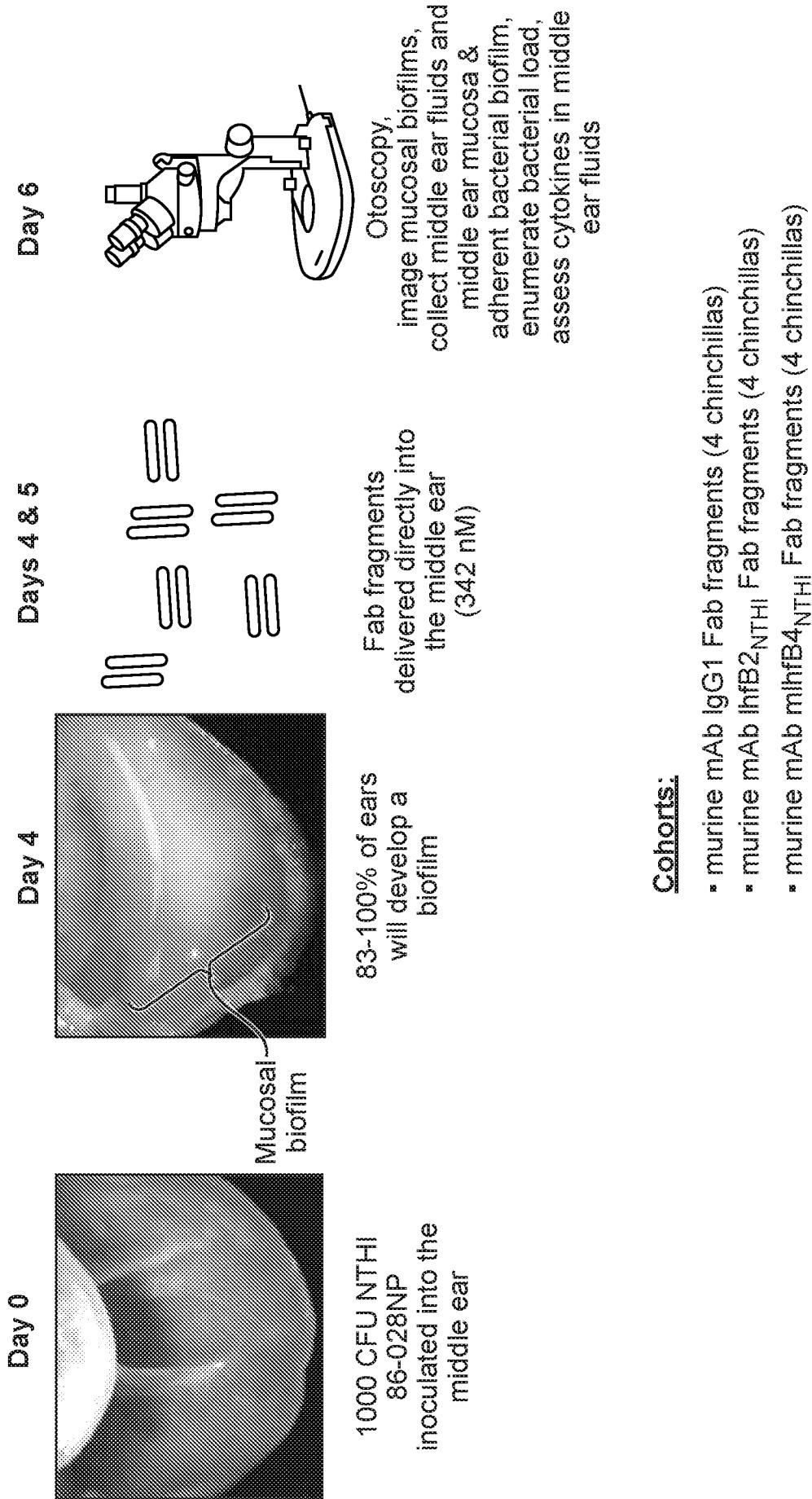


FIG. 4

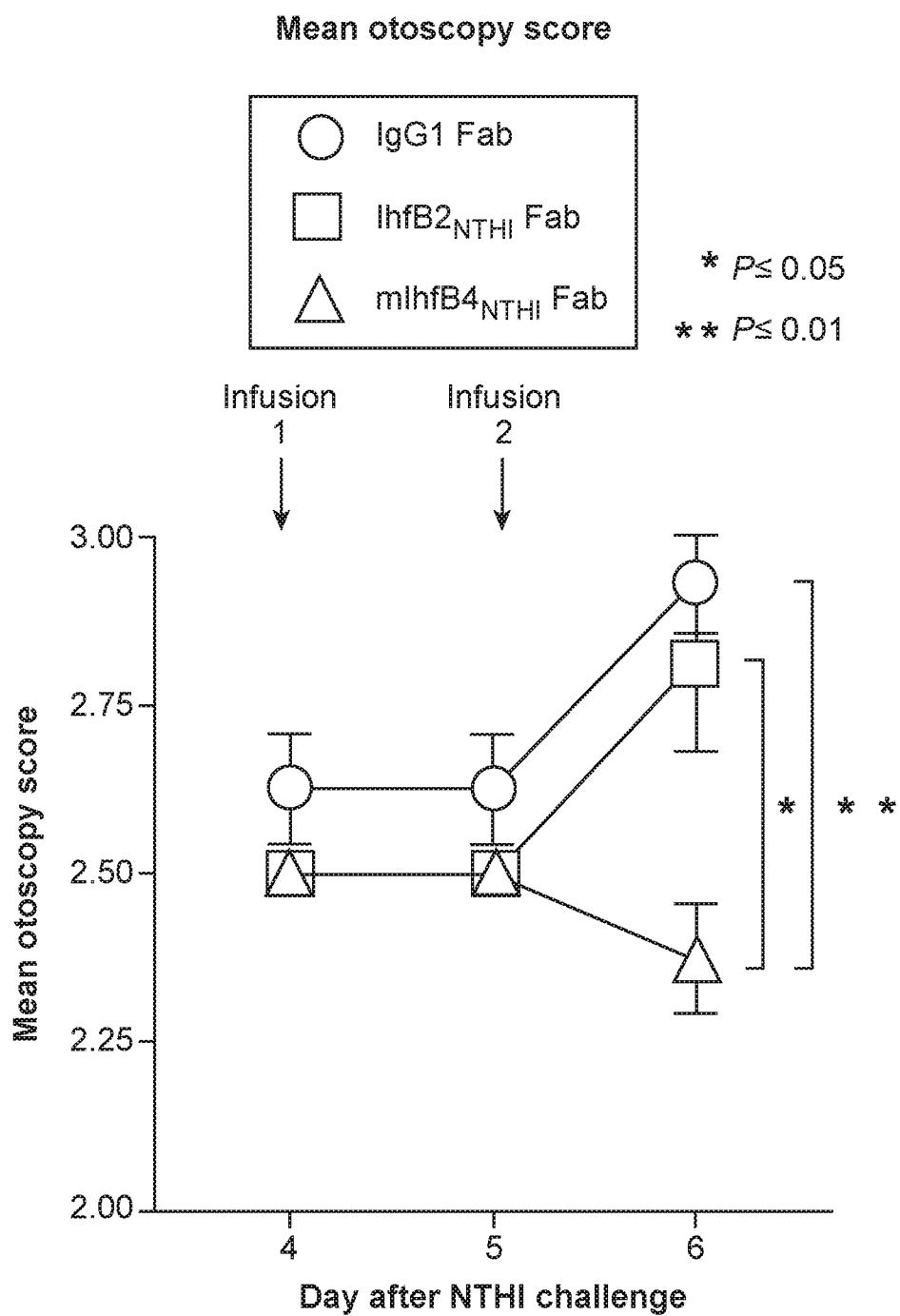


FIG. 5A

## Representative otoscopy images

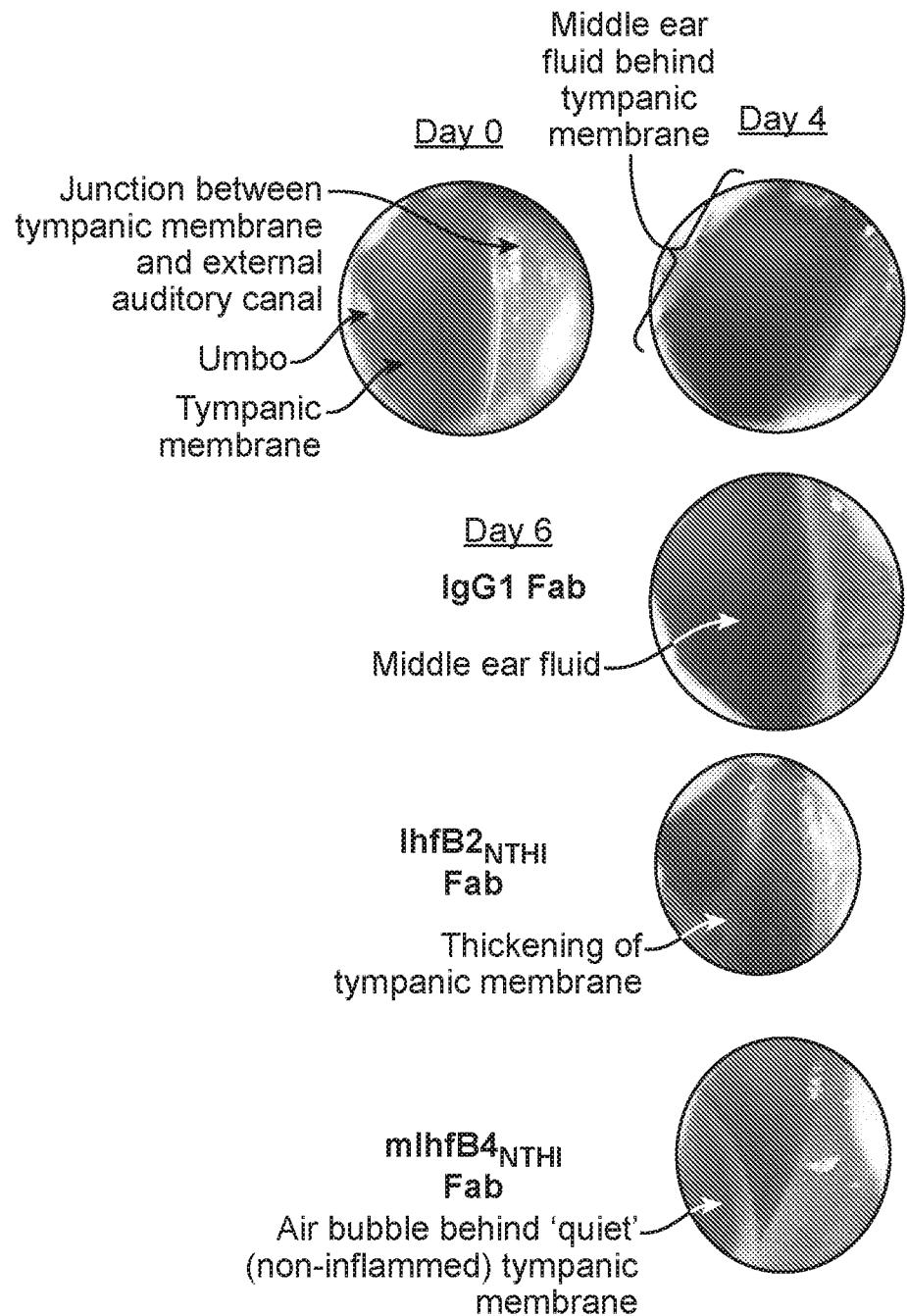


FIG. 5B

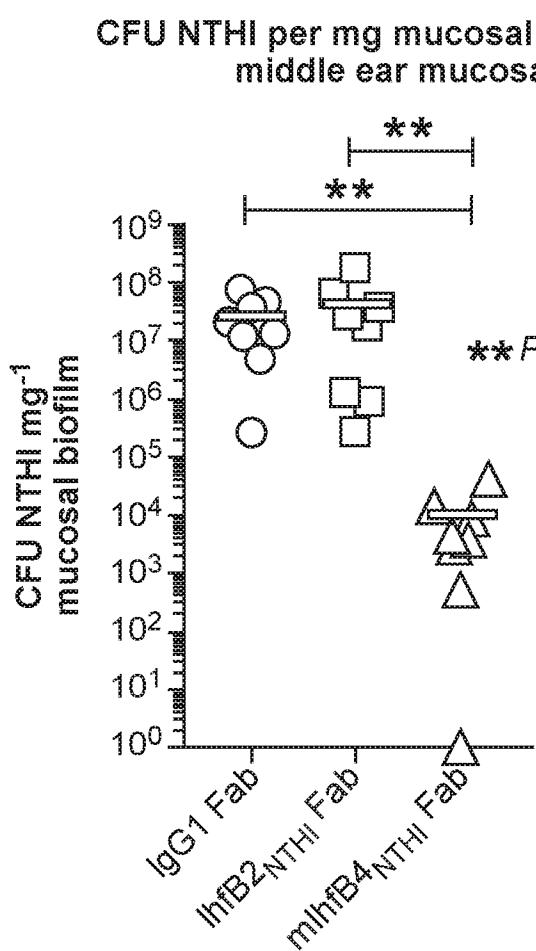


FIG. 6A

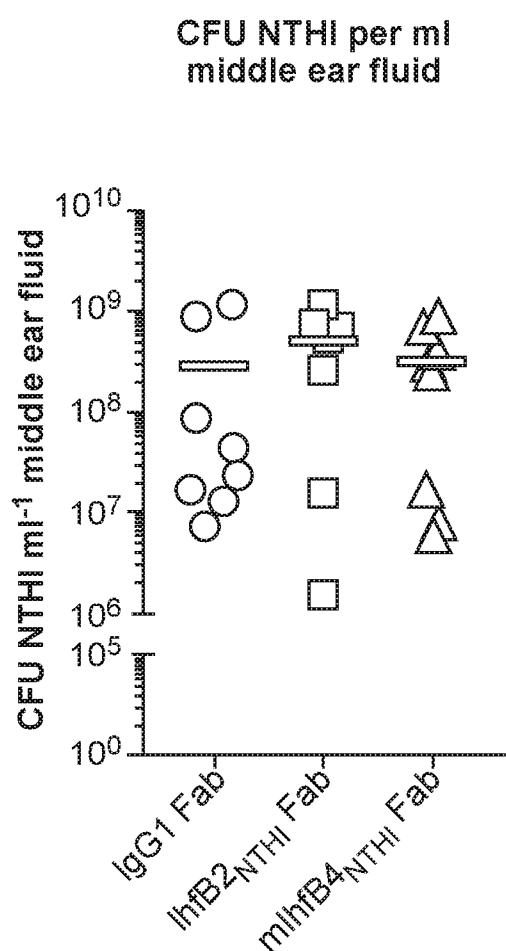


FIG. 6B

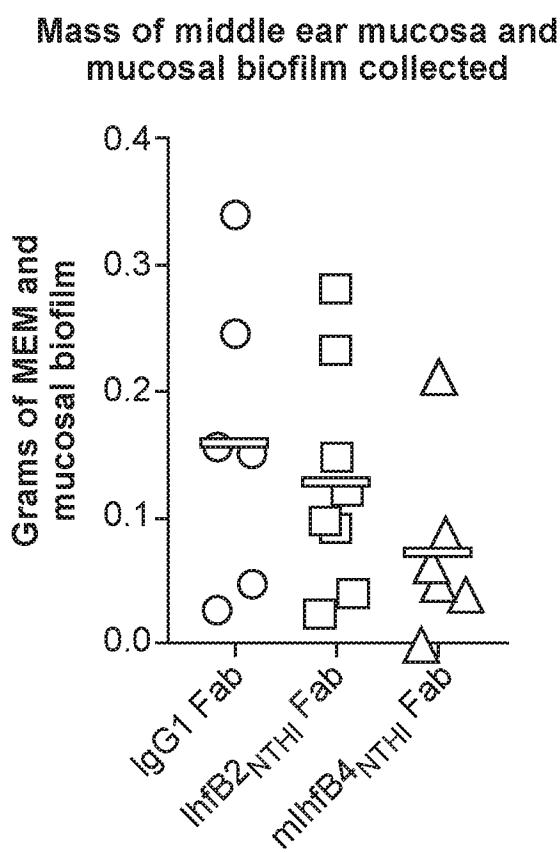


FIG. 7A

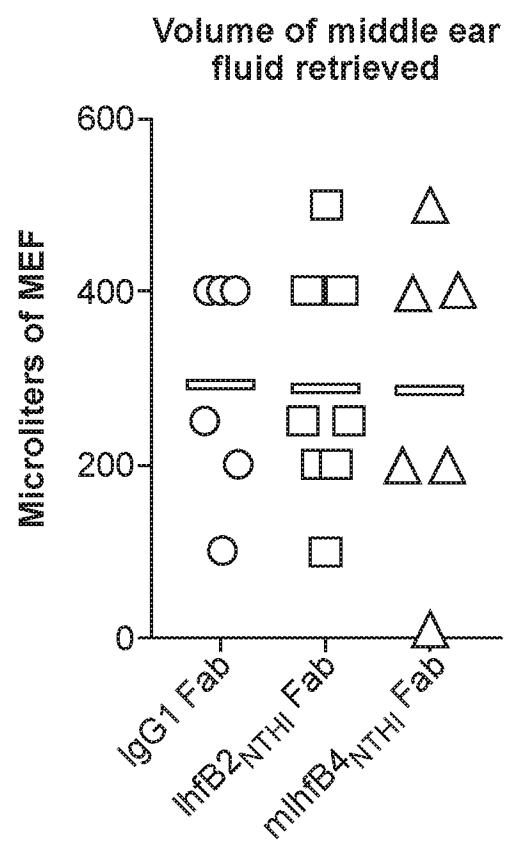


FIG. 7B

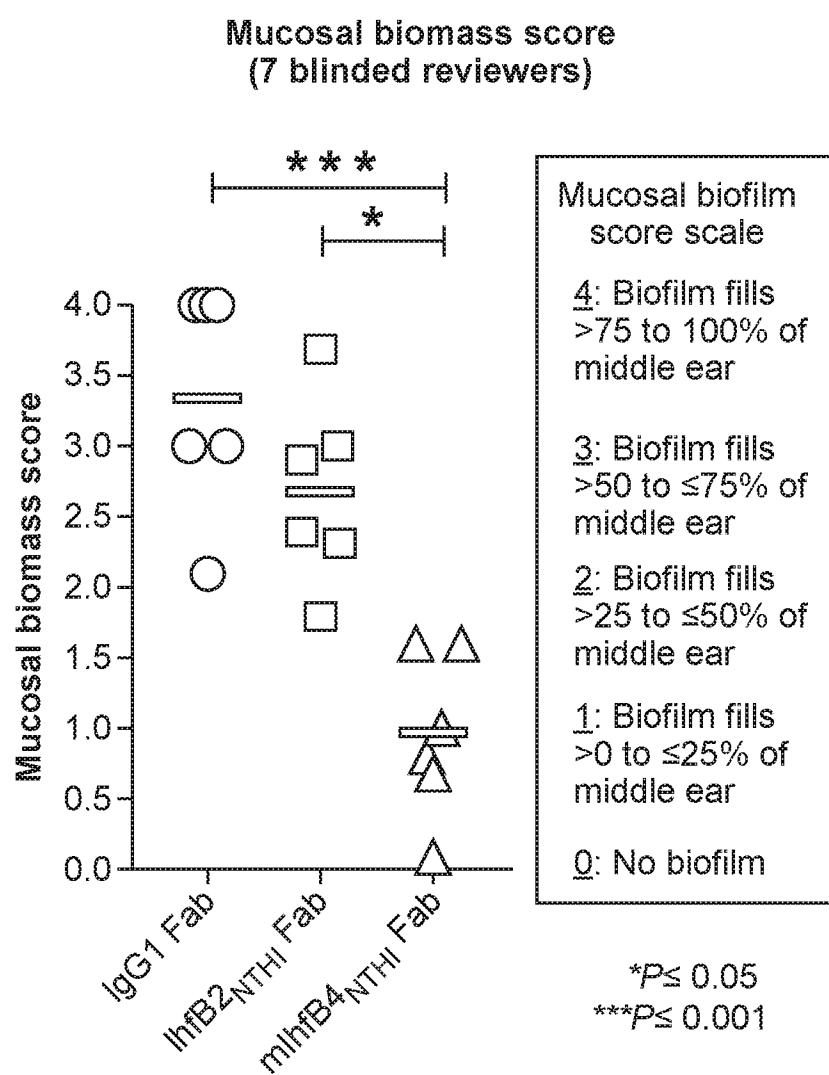


FIG. 8A

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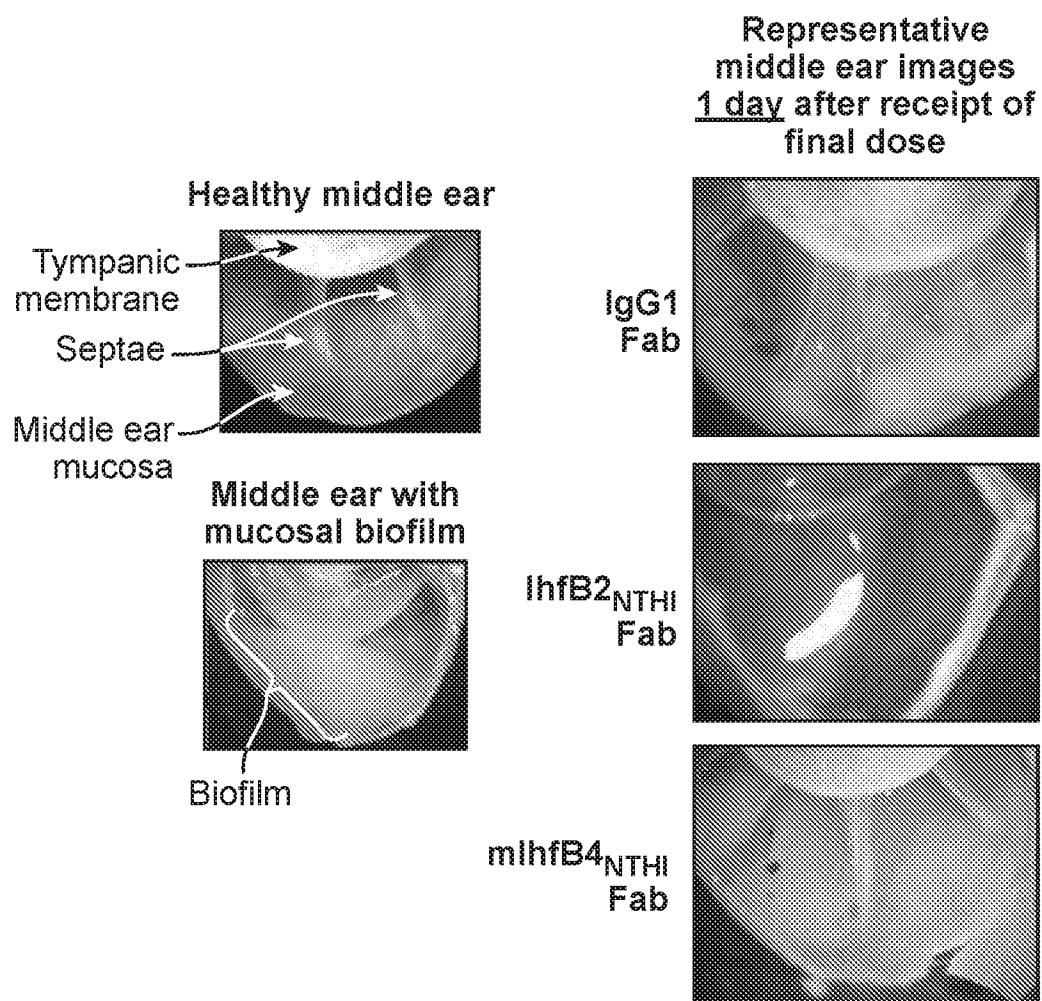


FIG. 8B

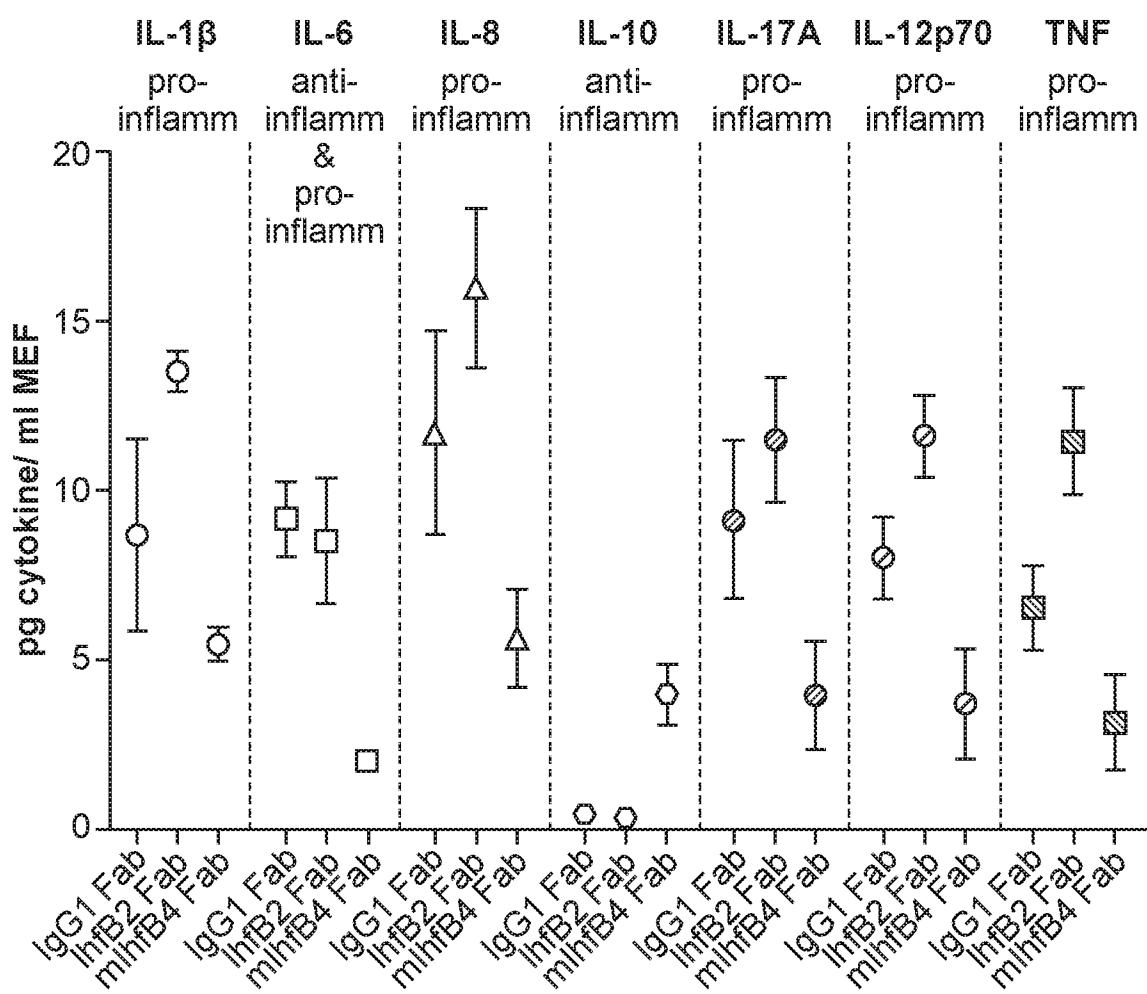


FIG. 9

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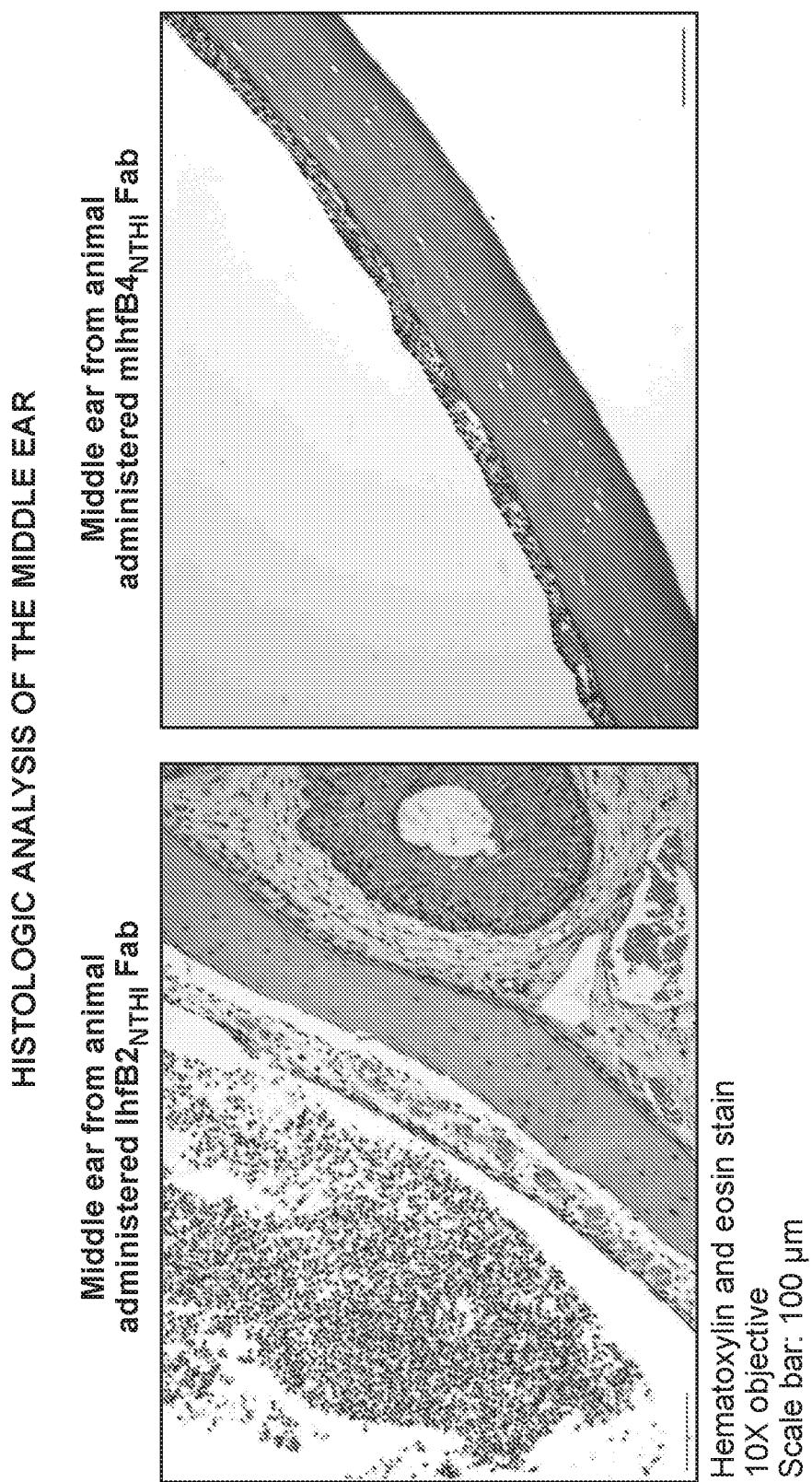
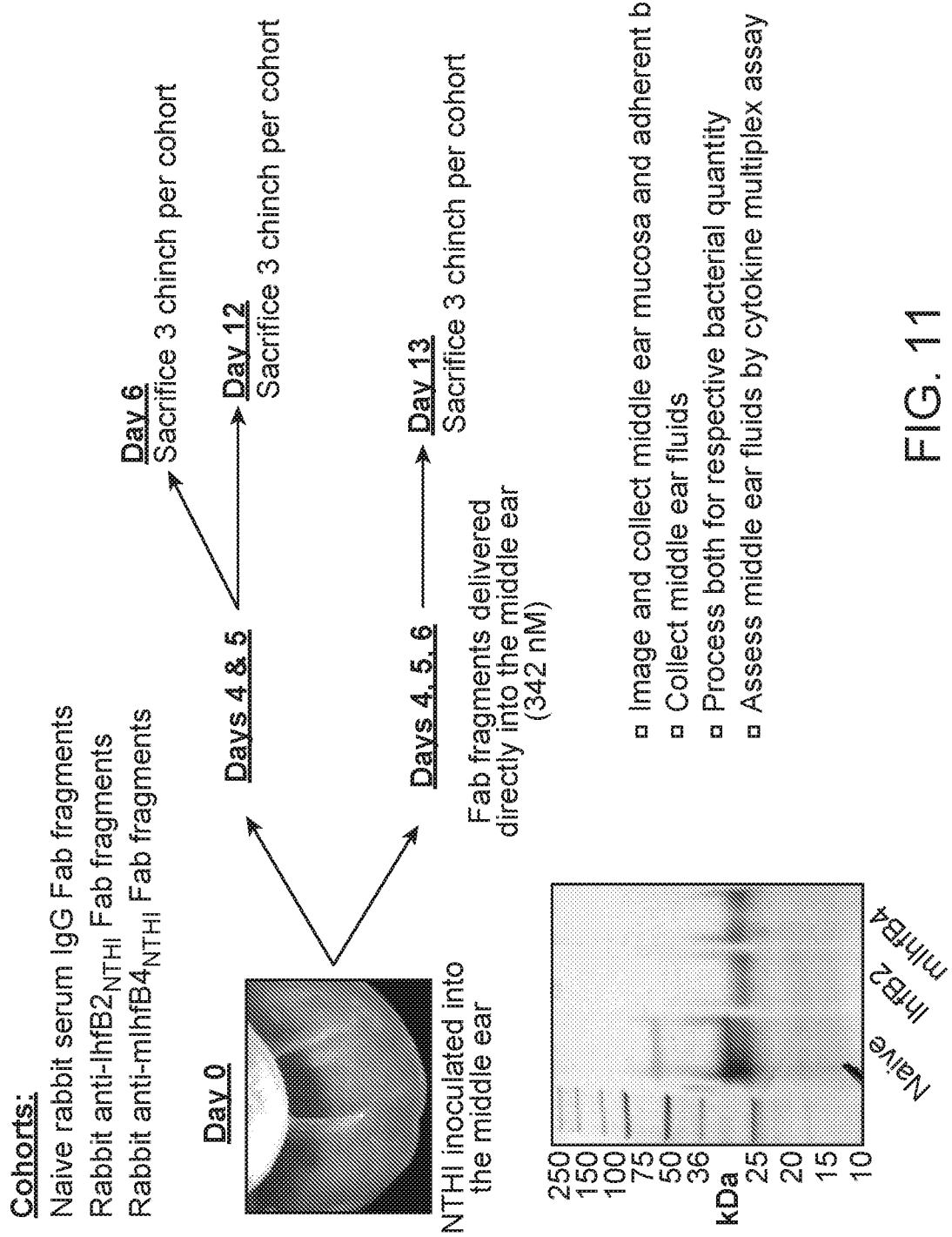


FIG. 10



**RELATIVE QUANTITY OF NTHI ADHERENT TO MIDDLE EAR  
MUCOSA & MUCOSAL BIOFILMS**

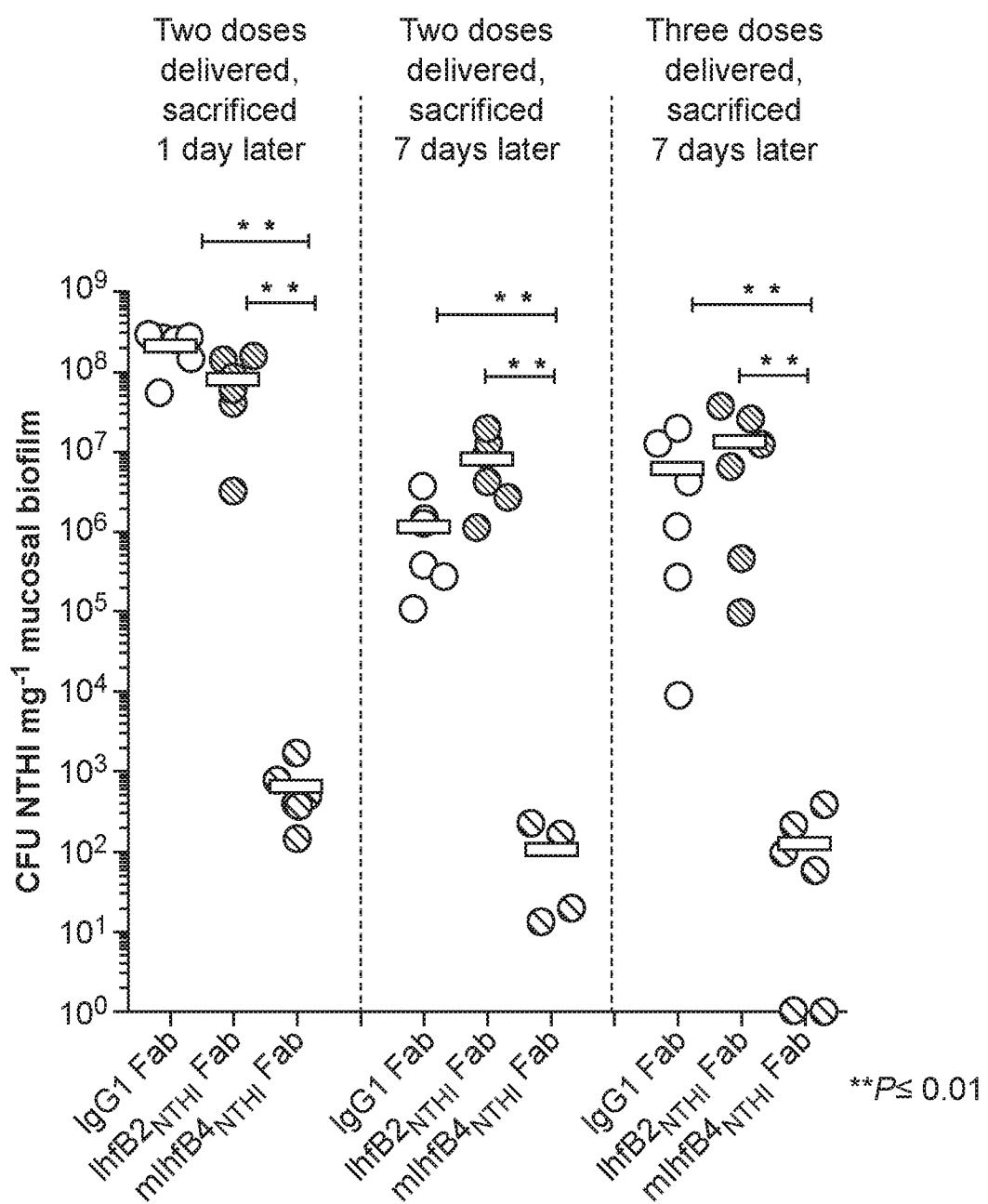


FIG. 12

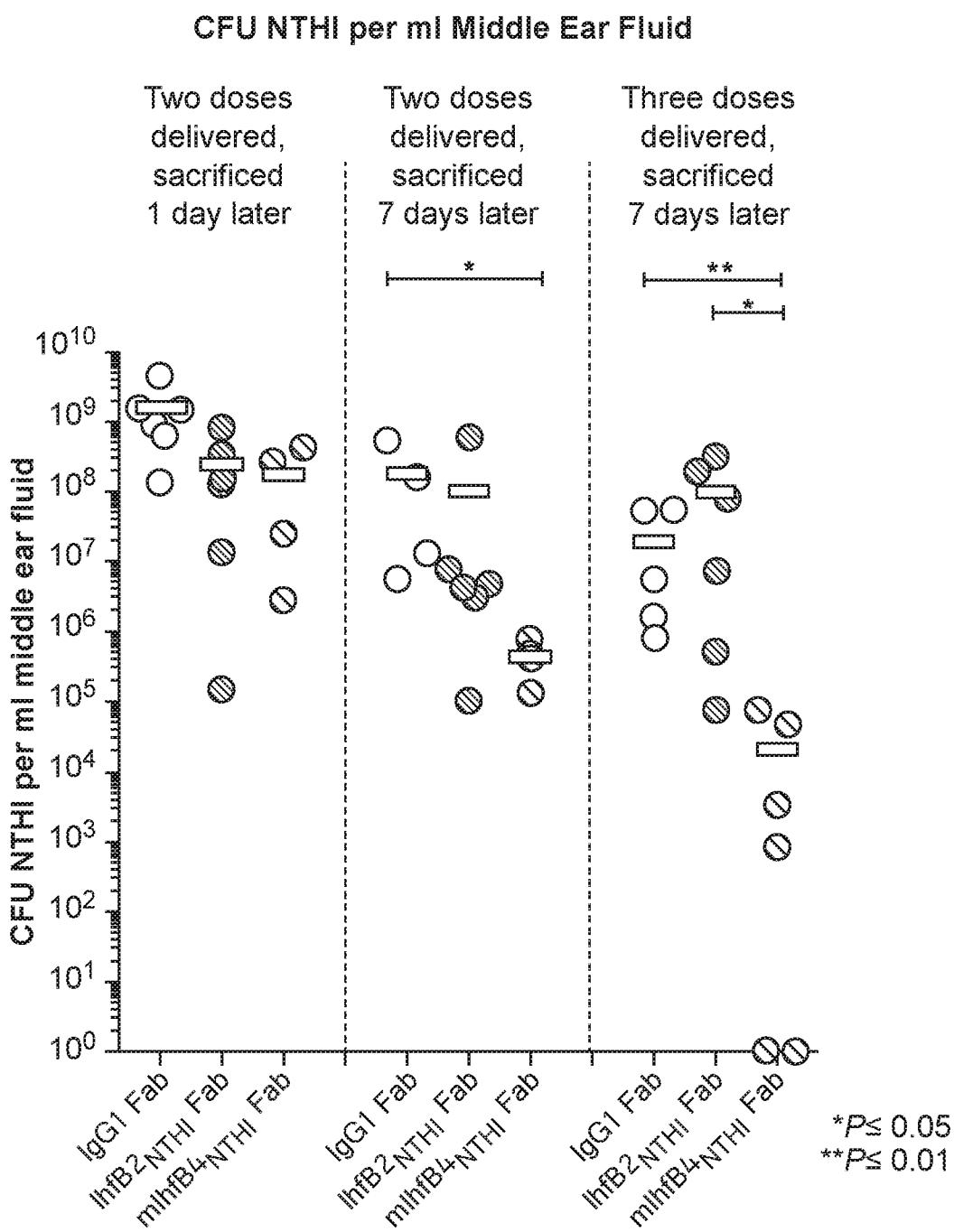


FIG. 13

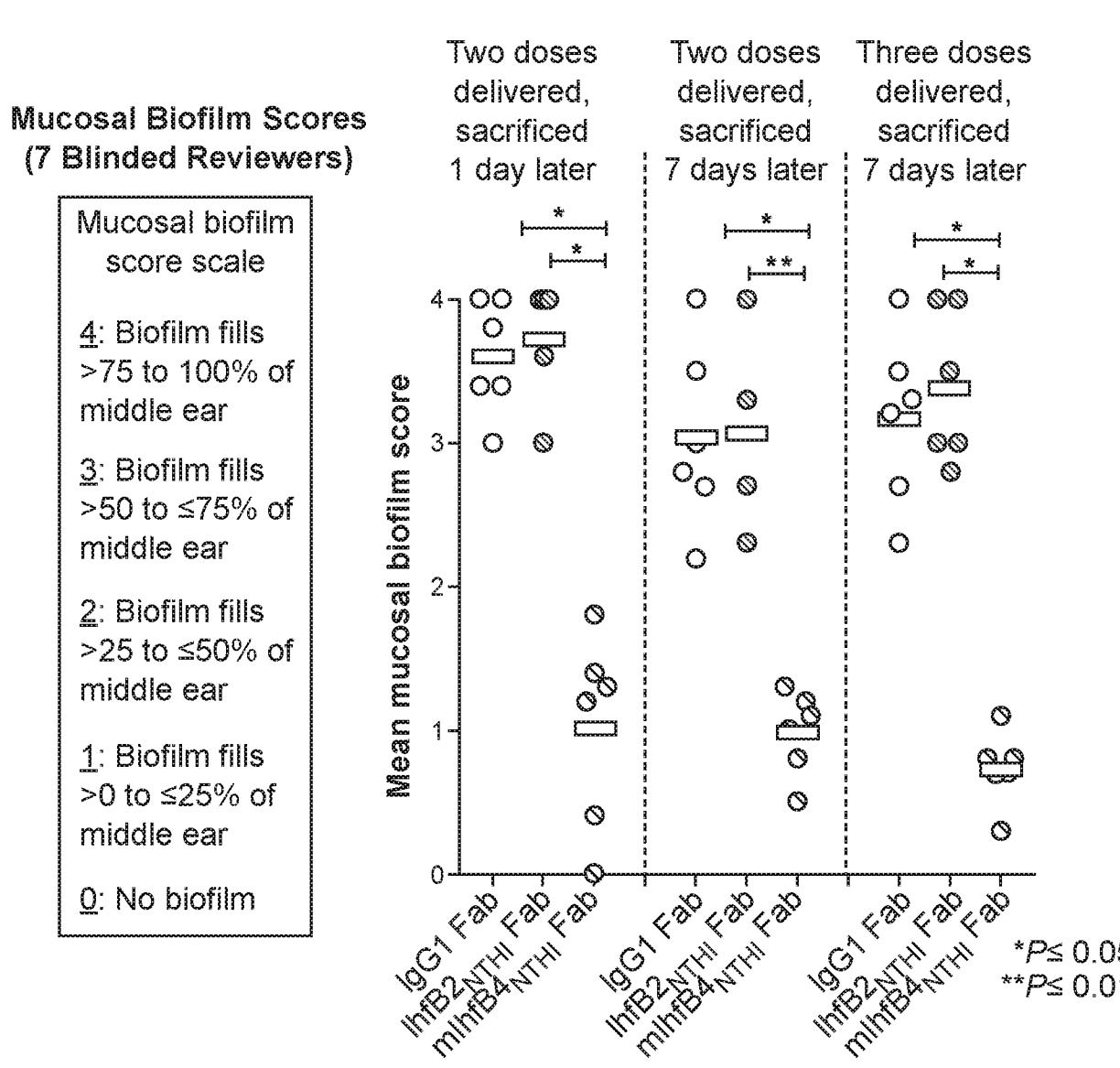


FIG. 14

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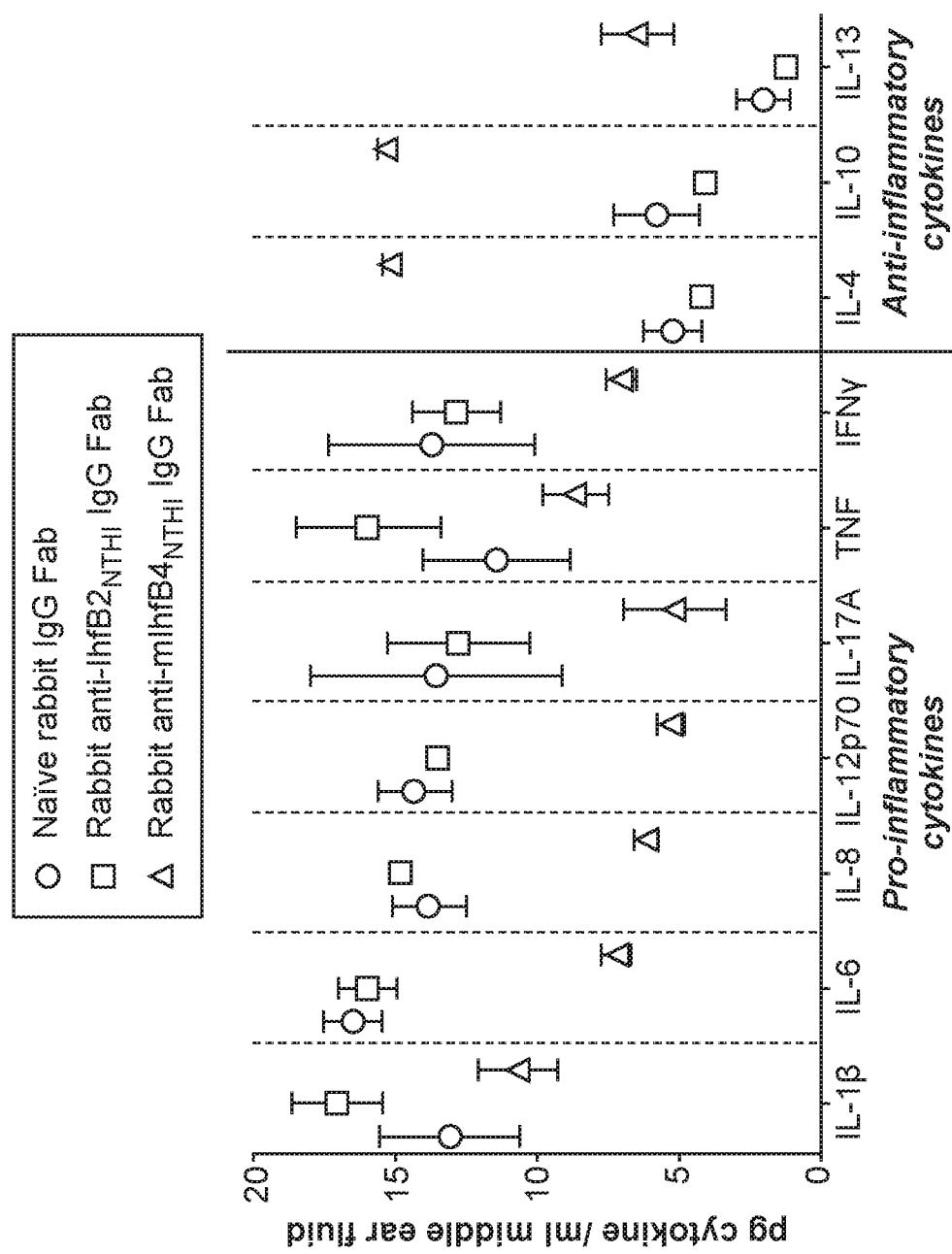


FIG. 15