



(51) International Patent Classification:

A61K 39/125 (2006.01) C07K 14/06 (2006.01)

A61K 39/42 (2006.01) C07K 16/00 (2006.01)

C07K 14/01 (2006.01) C07K 16/08 (2006.01)

(21) International Application Number:

PCT/US2019/048231

(22) International Filing Date:

27 August 2019 (27.08.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/723,155 27 August 2018 (27.08.2018) US

62/723,565 28 August 2018 (28.08.2018) US

(71) Applicants: **VANDERBILT UNIVERSITY** [US/US]; 305 Kirkland Hall, 2201 West End Avenue, Nashville, TN 37240 (US). **BAYLOR COLLEGE OF MEDICINE** [US/US]; One Baylor Plaza, Houston, TX 77030 (US).

(72) Inventors: **CROWE, JR, James, E.**; c/o Vanderbilt University Medical Center, 11475 MRB IV - 2213 Garland Ave., Nashville, TN 37232 (US). **ESTES, Mary**; c/o Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 (US).

(74) Agent: **HIGHLANDER, Steven, L.**; Parker Highlander PLLC, 1120 S. Capital of Texas Highway, Bldg. One, Suite 200, Austin, TX 78746 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: HUMAN MONOCLONAL ANTIBODIES THAT NEUTRALIZE PANDEMIC GII.4 NOROVIRUSES

(57) Abstract: The present disclosure is directed to antibodies binding to and neutralizing norovirus and methods for use thereof. Thus, in accordance with the present disclosure, there is provided a method of detecting a norovirus infection in a subject comprising (a) contacting a sample from the subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; and (b) detecting norovirus in the sample by binding of the antibody or antibody fragment to a norovirus antigen in the sample.

A

Isotype	NORO mAb clone	Blockade EC ₅₀ (μg/mL)	HAI titer (μg/mL)
IgG	115	2.8	1.7
	313.1	7.6	15.0
	246A	7.4	5.0
	260B	2.4	5.0
	279A	>	>
	329A	5.6	5.0
	118	>	5.0
	316	2.5	5.0
	202A.1	>	>
	312A	7.2	5.0
	317	5.3	3.4
	303	>	>
	263	9.7	>
	296A	8.3	5.0
	327A	5.0	5.0
	315B	2.7	3.4
	251A	>	>
	256A	>	>
	278	>	>
	123	23.9	10.0
	310	>	>
IgA	318	3.7	1.7
	320	3.1	5.0
	273A	3.6	5.0
	232	4.4	15.0

FIGS. 1A

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

DESCRIPTION

HUMAN MONOCLONAL ANTIBODIES THAT NEUTRALIZE PANDEMIC GII.4 NOROVIRUSES

PRIORITY CLAIM

5 This application claims benefit of priority to U.S. Provisional Application Serial No. 62/723,155, filed August 27, 2018, and U.S. Provisional Application Serial No. 62/723,565, filed August 28, 2018, the entire contents of both applications being hereby incorporated by reference.

BACKGROUND

10 This invention was made with government support under NIH Grants P30DK058404, P01AI057788, P30CA125123 and T32GM120011 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. Field of the Disclosure

15 The present disclosure relates generally to the fields of medicine, infectious disease, and immunology. More particular, the disclosure relates to human antibodies binding to human norovirus.

2. Background

20 Since the licensure and use of rotavirus vaccines, human noroviruses (HuNoV) have become the major etiologic agent of epidemic and sporadic acute gastroenteritis (Glass *et al.*, 2009). The persistence of HuNoVs is attributed to many factors, such as a low infectious dose, extreme environmental viral stability, high levels of shedding, and prolonged shedding even after symptoms have resolved (Fields *et al.*, 2013). According to the Centers for Disease
25 Control and Prevention, HuNoVs cause on average 19 to 21 million cases of infection and between 570 to 800 deaths in children under the age of five each year in the United States. HuNoVs infect people of all ages and, even though infection is characteristically acute and self-limiting, disease can become life threatening in children, the elderly, and the immunocompromised (Bok and Green, 2012). The correlates of HuNoV immunity in humans
30 are poorly incompletely understood. There are several correlates of protection that have been described; the new capacity to perform in vitro neutralization testing described here may

provide an improved correlate. Antibodies are clearly important to human immunity (Atmar *et al.*, 2018).

One of the challenges for developing antibodies or vaccines to prevent HuNoV-associated disease is the extreme antigenic diversity of field strains. HuNoVs currently are classified phylogenetically into 7 different genogroups (GI-GVII) and at least 41 different genotypes (Vinjé, 2015). Viruses from genogroup I (GI) and the rapidly evolving genogroup II (GII) account for nearly all human infections. The HuNoV genome contains 3 open reading frames (ORF1, ORF2, and ORF3). ORF1 encodes nonstructural proteins, while ORF2 and ORF3 encode the major and minor capsid proteins, respectively. In the past, HuNoVs could not be cultivated in cell culture, but the VP1 and VP2 protein sequences could be expressed using a baculovirus expression system to produce HuNoV virus-like particles (VLPs) (Jiang *et al.*, 1992). These VLP reagents have facilitated the study of HuNoV evolution, antigenicity and the emergence of new virus strains (Richardson *et al.*, 2013; Erdman *et al.*, 1989).

Since the mid-1990s, viruses from genogroup II genotype 4 (GII.4) have caused the majority of outbreaks, with new strains emerging every 2-3 years (Vinjé, 2015). In 2012, the GII.4 Sydney strain emerged and since then has continued to predominate among circulating strains. The molecular basis for antibody-mediated recognition of these strains and their mechanisms of action are not well characterized.

SUMMARY

Thus, in accordance with the present disclosure, there is provided a method of detecting a norovirus infection in a subject comprising (a) contacting a sample from the subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from
5 Tables 3 and 4, respectively; and (b) detecting norovirus in the sample by binding of the antibody or antibody fragment to a norovirus antigen in the sample. The sample may be a body fluid, such as blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, tissue scrapings or feces. Detection may comprise ELISA, RIA, lateral flow assay or Western blot. The method may
10 further comprise performing steps (a) and (b) a second time and determining a change in norovirus antigen levels as compared to the first assay.

The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be
15 encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain
20 variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

In another embodiment, there is provided a method of treating a subject infected with norovirus or reducing the likelihood of infection of a subject at risk of contracting norovirus,
25 comprising delivering to the subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy
30 chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences

from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

The antibody or antibody fragment may be administered prior to infection or after infection. The subject may be a pregnant female, a sexually active female, or a female undergoing fertility treatments. Delivering may comprise antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.

In yet another embodiment, there is provided a monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as

enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

5 In yet another embodiment, there is provided a hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90%
10 identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired
15 sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

 The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase
20 half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide
25 and/or is an intrabody.

 In still yet another embodiment, there is provided a vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and
30 heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences

according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain
5 fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as
10 enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

Also provided is vaccine formulation comprising one or more expression vectors
15 encoding a first antibody or antibody fragment as defined above. The expression vector(s) may be Sindbis virus or VEE vector(s). The vaccine formulation may be formulated for delivery by needle injection, jet injection, or electroporation. The vaccine formulation may further comprise one or more expression vectors encoding for a second antibody or antibody fragment as defined above.

20 An additional embodiment includes a method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with norovirus comprising delivering to the subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1,
25 may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and
30 heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.

The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern. The antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

The antibody or antibody fragment may be administered prior to infection or after infection. The subject may be a pregnant female, a sexually active female, or a female undergoing fertility treatments. Delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment. The antibody or antibody fragment may increase the size of the placenta as compared to an untreated control and/or may reduce viral load and/or pathology of the fetus as compared to an untreated control.

Yet another embodiment involves a method of determining the antigenic integrity, correct conformation and/or correct sequence of a norovirus antigen comprising (a) contacting a sample comprising the antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (b) determining antigenic integrity, correct conformation and/or correct sequence of the antigen by detectable binding of the first antibody or antibody fragment to the antigen. The sample may comprise recombinantly produced antigen. The sample may comprise a vaccine formulation or vaccine production batch. Detection may comprise ELISA, RIA, western blot, a biosensor using surface plasmon resonance or biolayer interferometry, or flow cytometric staining.

The first antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the first antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The first antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab

fragment, F(ab')₂ fragment, or Fv fragment. The method may further comprise performing steps (a) and (b) a second time to determine the antigenic stability of the antigen over time.

The method may further comprise (c) contacting a sample comprising the antigen with a second antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (d) determining antigenic integrity of the antigen by detectable binding of the second antibody or antibody fragment to the antigen. The second antibody or antibody fragment may be encoded by clone-paired variable sequences as set forth in Table 1, may be encoded light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. Alternatively, the second antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The second antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The method may further comprise performing steps (c) and (d) a second time to determine the antigenic stability of the antigen over time.

Also provided is a human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein the antibody or antibody fragment binds to norovirus capsid protein P domain and/or S domain. The human monoclonal antibody or antibody fragment may bind to norovirus capsid protein P domain P1 or P2 subdomain. The antibody or antibody fragment may cross-react with multiple norovirus GI and/or GII strains.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The word “about” means plus or minus 5% of the stated number.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of

illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGS. 1A-C. Neutralization of GII.4 Sydney 2012 using virus-like particles (VLPs) or live virus. (FIG. 1A) Half-maximal effective concentrations (EC_{50}) for all isolated IgGs and IgAs using a VLP blockade assay, and the antibody concentration at which hemagglutination was inhibited when using VLPs and O+ red blood cells. > symbols indicate blockade EC_{50} values >100 $\mu\text{g/mL}$ for IgGs or >113 $\mu\text{g/mL}$ for IgAs, or HAI titers > 15 $\mu\text{g/mL}$. (FIG. 1B) Plotted are the absorbance values when plates were read at optical density (O.D.) 450nm for selected IgGs and IgAs when antibodies were diluted serially, combined with GII.4 Sydney 2012 VLPs and added to porcine gastric mucin (PGM). (FIG. 1C) Inhibition of replication of GII.4 Sydney 2012 virus using selected IgGs, IgAs and a non-specific human monoclonal antibody (mAb) were tested in a human intestinal enteroid system. An additional control for each experiment was virus incubated without a mAb. The half-maximal inhibitory concentration (IC_{50}) for each mAb is indicated in each individual graph. The data presented is an average of two independent experiments for NORO-263, -250B, -320, -273A, -318 and a dengue virus-specific control antibody 2D22. The number of genome equivalents for each concentration tested for each mAb including the no antibody control was the average of 6 replicates tested.

FIG. 2. Competition binding of GII.4 specific mAbs on GII.4 Sydney 2012 P domain with the Octet® Red96 system. Epitope binning was performed using biolayer interferometry. GST-tagged GII.4 Sydney 2012 P domain was loaded onto anti-GST tips, then the first antibody was loaded followed by loading of the second antibody. The numerical data indicate percent binding of the second antibody in the presence of the first antibody. Yellow, green, and magenta boxes indicate potential binding groups.

FIG. 3. Characterization of human mAb binding to GII.4 Sydney VLPs. Reactivity of serially diluted mAbs or a no-antibody control for GII.4 Sydney 2012 test by ELISA.

FIG. 4. Characterization of blockade activity of GII.4 Sydney VLPs using isolated mAbs. Blockade potential of serially diluted mAbs or a no-antibody control for GII.4 Sydney 2012 VLP binding to porcine gastric mucin was measured.

FIGS. 5A-B. Half-maximal binding concentrations (EC_{50}) of purified mAbs to GII.4 Sydney 2012 protruding (P) or shell (S) domain. (FIG. 5A) EC_{50} value indicates the concentration at which half-maximal binding was obtained when tested by ELISA using either recombinantly expressed P domain or shell domain as antigen. mAbs are organized by isotype, IgG or IgA, and arranged in order from lowest to highest EC_{50} value when binding to GII.4 VLPs. > symbol for IgGs indicates EC_{50} binding value > 150 $\mu\text{g/ml}$ for IgAs. Grey boxes indicate mAbs that exclusively bind to the GII.4 Sydney 2012 shell domain. (FIG. 5B) Binding of mAbs to P or S domain. Graphs are separated by antigen and mAb isotype.

FIG. 6. Competition binding of GII.4 specific mAbs on GST-GII.4 P domain using OctetR HTX System. Epitope binning was performed used biolayer interferometry. GST-tagged GII.4 Sydney 2012 P domain was loaded onto anti-GST tips, then the first antibody was loaded followed by loading of the second antibody. The numerical data has been normalized to a no-antibody buffer control and indicates percent binding of the second antibody in the presence of the first antibody. Yellow, green and magenta boxes indicate potential competition binding groups.

FIG. 7. Predicted blockade epitopes on amino acid sequence alignment of GII.4 Houston 2002 and Sydney 2012. Amino acid sequence alignment of the major capsid protein of GII.4 Houston 2002 (SEQ ID NO: 205) and Sydney 2012 (SEQ ID NO: 206) noroviruses. Boxes indicate predicted blockade epitopes described in (Lindesmith *et al.*, 2012a). Epitope A is amino acids 294, 296-298, 368 and 372. Epitope B is amino acids 333 and 382. Epitope C is amino acids 340 and 376. Epitope D is amino acids 393 and 395. Epitope E is amino acids 407, 412 and 413.

FIGS. 8A-B. Half-maximal binding and blockade concentrations (EC_{50}) of purified mAbs to GII.4 Houston 2002 VLPs. (FIG. 8A) Listed are the EC_{50} of each mAb for binding or blockade activity using GII.4 Houston VLPs. > symbols indicate blockade EC_{50} values >100 $\mu\text{g/mL}$. (FIG. 8B) Serial dilutions of each mAb and a no-antibody control were used to measure blockade activity.

FIGS. 9A-B. Binding activity of cross-reactive human mAbs to GI and GII VLPs. An indirect ELISA was used to assess binding activity of 12 human mAbs to GI.1, GI.2, GI.3, GII.3, GII.4, GII.6, GII.13 or GII.17 VLPs. (FIG. 9A) Half-maximal effective concentration (EC_{50}) for binding to VLPs of indicated genotype. Listed are the isotype, light chain and EC_{50} value to GI.1, GI.2, GI.3, GII.3, GII.4, GII.6, GII.13 or GII.17 VLPs. The > symbol indicates binding was not detected at the highest concentration tested, 500 nm. Greater EC_{50} values are

in the lightest shade of orange and lowest EC₅₀ values are in the darkest shade of orange. (FIG. 9B) Representative ELISA binding curves are shown for indicated genotype.

FIGS. 10A-B. Blockade activity of cross-reactive human mAbs for GI or GII VLPs.

Blocking of VLP binding to PGM was used as a surrogate system to test neutralization of GI.3, GII.4, GII.6, or GII.17 VLPs using the indicated human mAbs. (FIG. 10A) Half-maximal effective concentrations (EC₅₀) for cross-reactive mAbs when blocking GI or GII VLPs from binding to PGM. The > symbol indicates the blocking EC₅₀ value was greater than 1,000 nm. (FIG. 10B) Blockade activity was tested using serial dilutions of each mAb.

FIG. 11. Half-maximal effective concentrations (EC₅₀) for binding of 12 cross-reactive human mAbs to protruding or shell domain. GI.3, GII.4, GII.6 or GII.17 NoV strain protruding or shell domain proteins were used as antigen in an indirect ELISA. The > symbol indicates binding was not detected at the highest concentration tested, 500 nm. ND, not determined; P, protruding domain; S, shell domain; P/S, protruding and shell domain.

FIGS. 12A-D. NORO-320 Fab in complex with GII.4 P domain. (FIG. 12A) X-ray crystal structure of NORO-320-GII.4 P domain complex. The two P domain subunits in the dimer are colored in blue and green. NORO-320 Fab (yellow-heavy chain and red-light chain) along with two molecules of H-type 1 pentasaccharide (stick model) modeled to indicate the glycan binding sites for reference. Depicted are side and top views of the complex. (FIG. 12B) Side view of NORO-320 in complex with GII.4 P domain showing the interacting Fab residues (stick model) with the light and heavy chain residues shown in red and yellow, respectively, interacting. (FIG. 12C) Close-up view of the Fab binding site (black box in B) showing the P domain residues (stick models) which interact with the Fab. Residues in the two-fold related subunit (green) is hyphenated. (FIG. 12D) Close up view of the Fab residues (light chain-red, heavy chain yellow) that interact with the GII.4 P domain (shown in surface representation).

FIGS. 13A-C. Molecular interactions between NORO-320 and GII.4 P domain. (FIG. 13A) Antibody plot analysis using the program LigPlot+ v.2.1 (Laskowski et al., 2011). The P2 subdomain residues in the 2-fold related subunit are hyphenated. The hydrogen bonds are shown as green dashed lines, and the hydrophobic contacts are short spokes radiating from each atom or residue. (FIGS. 13B-C) The interactions of P1 or P2 subdomains with NORO-320. The side chains of mAb and P domain are represented with ball-and-stick and stick models, respectively, and colored as in Figure 4. The hydrogen bonds are shown as black dashed lines.

FIG. 14. Amino acid sequence alignment of the protruding domain of GI.1, GII.3, GII.4, GII.13 and GII.17 strains of human NoV. The protruding domain amino acid sequences of GII.3, GII.4, GII.13 and GII.17, the GII strains tested for which NORO-320

exhibited reactivity, and GI.1 are aligned. Boxed in red are the 18 residues identified on GII.4 that interact with the highly cross-reactive mAb NORO-320. Boxed in gold and blue are the residues previously reported to be involved in GII.4 HBGA binding (Shanker *et al.*, 2011).

FIG. 15. Blockade of GII.4 VLPs by NORO-320 is a result of steric hindrance.

5 NORO-320 was expressed recombinantly as Fab or IgG forms and purified. GII.4 VLPs were pre-incubated with either NORO-320 Fab, IgG or the original hybridoma-secreted dimeric IgA and added to wells that had been coated previously with PGM. Half-maximal concentrations (EC₅₀) for the three antibodies tested are listed. The > symbol indicates blockade EC₅₀ value was greater than 1,000 nm.

FIG. 16. Verification of molecular assembly of recombinantly expressed NORO-

10 **320 variants.** NORO-320 was expressed recombinantly and purified in Fab or IgG forms. Fab, IgG and hybridoma dimeric IgA variants were resolved on a SDS-PAGE gel under non-reducing conditions. Recombinant dimeric IgA, IgG and Fab 4C10 was used as a control.

FIGS. 17A-D. Modeling of NORO-320 Fab bound to GII.4 particle. (FIG. 17A)

15 Superimposition of GII.4 P domain/ NORO-320 Fab complex onto Norwalk virus capsid (PDB ID: 1IHM). P domain and S domain are colored in cyan and gray, respectively. NORO-320 Fab is shown in surface representation with light chain and heavy chain in orange and yellow, respectively. (FIG. 17B-C) Close-up views of a VP1 dimer (green and blue chains) with two molecules of NORO-320 Fab. (FIG. 17D) A schematic of proposed model for the neutralization
20 of GII strains by NORO-320 IgA.

Table A. Isotype, light chain and ELISA binding characterization of GII.4 P VLP-specific human mAbs.

Isotype	mAb clone, NORO-	Light chain	EC ₅₀ (µg/mL)
IgG	115	K	0.1
	313.1	K	0.1
	246A	λ	0.2
	250B	λ	0.2
	279A	λ	0.2
	329A	λ	0.2
	118	λ	0.3
	316	λ	0.3
	202A.1	λ	0.4
	312A	λ	0.4
	317	λ	0.4
	303	λ	0.5
	263	λ	0.6
	296A	λ	0.6
	327A	λ	0.6
	315B	λ	0.7
	251A	λ	0.9
	256A	λ	0.9
	278	λ	4.0
	123	λ	5.4
	310A	K	6.2
IgA	318	K	0.1
	320	K	0.1
	273A	K	0.2
	232A.2	K	0.3

Isotype, light chain and ELISA binding characterization of GII.4 P VLP-specific human mAbs. Listed are the half-maximal concentrations (EC₅₀) at which half-maximal binding was obtained when tested by ELISA using VLPs as the antigen. MAbs are organized by isotype (IgG or IgA) and arranged in order from lowest to highest EC₅₀ value when binding to GII.4 VLPs.

Supplementary Table 1. Antibody variable gene usage for GII.4 Sydney 2012 VLP-binding mAbs.

Heavy Chain			Light Chain													
Donor	NORO mAb clone	Isotype/ Light chain	V gene	V gene % Identity	J gene	J gene % Identity	D gene	Junction (SEQ ID NO. :)	HCDR length	V gene	V gene length	J gene	J gene % Identity	Junction	SEQ ID NO. :)	LCDR length
HD329	115	IgG/K	V3-11*01 F	98.2	J5*02 F	94.1	D8-13*01 F	CARDRLPASGSRHPRHW (171)	8.8.15	V4-1*01 F	95.8	J4*01 F	97.2	DGGVYNSPLAF (168)		12.3.9
HD331	313.1	IgG/K	V3-11*08 F	92.7	J6*02 F	80.7	D1-1*01 F	CARMGRLELERPHYYRLDWW (172)	8.8.20	V2-28*01 F	97.6	J2*02 F	97.2	CMQALQTRTF (185)		11.3.8
HD334	250B	IgG/A	V3-11*08 F	95.5	J4*02 F	97.9	D1-28*01 F	CARATSGGATSYVFDSSW (173)	8.8.15	V1-42*01 F	85.8	J2*01 F	89.5	COSSYDRSYGSAVF (190)		8.3.12
HD337	279A	IgG/A	V3-30*03 F	91.7	J6*02 F	79.0	D2-8*01 F	CARKYEHYYSKLLGMDWW (174)	8.8.17	V1-47*01 F	92.6	J2*02 F	94.6	CATLDMMTWVF (191)		8.3.10
HD331	329A	IgG/A	V3-11*08 F	96.2	J4*02 F	91.7	D3-15*01 F	CARYNYGSGSFVFDYW (175)	8.8.15	V1-44*01 F	96.5	J3*02 F	100	DAAWDDSLNGWVF (192)		8.3.11
980	202A.1	IgG/A	V1-2*02 F	98.3	J3*01 F	92.0	D7-27*01 F	CARDLLRWSDHDAFDWW (176)	8.7.16	V1-47*01 F	97.4	J2*01 F	100	CSAWDDSLSGPVF (193)		1.8.11
HD331	312A	IgG/A	V3-11*08 F	94.1	J4*02 F	89.6	D2-15*01 F	CARDAQYCSGSRCLVFDYW (177)	8.8.15	V1-42*01 F	96.2	J2*01 F	97.1	COSSYDRSLRVF (194)		8.3.9
HD331	317	IgG/A	V4-24*01 F	91.2	J5*02 F	90.0	D3-16*02 F	CARGQMRTRGALFRFDFW (178)	8.7.17	V3-25*03 F	93.6	J3*02 F	89.5	COSSYCTRTGYVVF (195)		8.3.11
HD333	303	IgG/K	V3-30*01 F	97.8	J6*02 F	80.7	D3-15*01 F	CARDCKRWGVFTYGMDFW (179)	8.8.16	V2-8*01 F	96.3	J2*01 F	100	COSSYAGTYNCVVF (196)		9.3.11
HD333	286A	IgG/A	V3-11*08 F	95.1	J1*01 F	96.3	D3-15*01 F	CARYGAEYGSRSFYFLDW (180)	8.8.16	V1-42*01 F	96.9	J2*01 F	92.1	COSSYDRSLSGVVF (197)		8.3.12
HD331	315B	IgG/A	V3-11*08 F	90.3	J4*02 F	89.6	D2-15*01 F	CAREDCHEGTSYSGSDW (181)	8.8.14	V1-42*01 F	97.2	J2*02 F	92.1	COSSYDRSLSGVVF (198)		9.3.12
HD334	251A	IgG/A	V3-30*08 F	97.2	J4*02 F	91.7	D6-19*01 F	CARKYRLTSYSGWFSFDYW (182)	8.8.17	V1-51*01 F	97.9	J3*02 F	91.9	CGTRMDTSLRACLF (199)		8.3.11
HD334	256A	IgG/A	V3-30*03 F	96.2	J3*02 F	92.0	D5-18*01 F	CARKPLRYYSYSGWHSFDWW (183)	8.8.17	V1-51*01 F	96.6	J2*02 F	91.1	CGTMDLSLTAGWVF (200)		8.3.12
HD337	278	IgG/A	V3-30*03 F	100	J4*02 F	93.8	D8-13*01 F	CAKVTIAAADLLDWW (184)	8.8.14	V1-42*01 F	100	J2*01 F	97.4	COSSYSSLSGSAVF (201)		9.3.12
HD335	123	IgG/A	V3-30*04 F	94.1	J6*02 F	92.6	D2-15*01 F	CARVTGCTSNKCSYWAYYYGLDWW (185)	8.8.24	V2-8*01 F	97.6	J1*01 F	94.7	COSSYAGSTTSGVVF (202)		9.3.12
HD331	310A	IgG/K	V3-60*01 F	94.4	J4*02 F	98.8	D3-22*01 F	CTEDPSQVYDSRGHYQZTFPSFCSW (186)	8.7.24	V1-17*03 F	97.5	J4*01 F	100	CLOHDTYPLTF (203)		8.3.9
HD331	320	IgA/K	V1-60*01 F	95.5	J6*02 F	77.4	D3-15*01 F	CARDRVPSYSPSRFSTKGAWGKYGMDFW (187)	8.8.28	V2-28*01 F	98.3	J1*01 F	100	CMQALQTRTF (204)		11.3.8

5 Heavy and light chain variable gene regions were sequenced. All of the mAbs had unique heavy and light complementarity-determining region 3 (CDR3) sequences.

Supplemental Table 1. Antibody sequence analysis for 13 cross-reactive human mAbs. Heavy and variable gene regions were sequenced. All of the mAbs had unique heavy and light chain complementary-determining region 3 (CDR3) sequences.

		Heavy Chain							Light Chain						
NORO mAb clone	Isotype	V gene	V gene % identity	J gene	J gene % identity	D gene	JUNCTION	HCDR length	V gene	V gene % identity	J gene	J gene % identity	AA JUNCTION	LCDR length	
155.5	IgM	V4-34*01 F	100	J6*02 F	96.8	D3-10*01 F	CARGLNTMVRGVLNYYYYGMDVW	8.7.22	V1-6*01 F	100	J1*01 F	97.3	CLQDYNYPRTF	6.3.9	
158.3	IgM	V4-34*01 F	100	J6*02 F	89.1	D3-16*01 F	CARGLMDVW	8.7.7	V1-8*01 F	100	J2*02 F	100	CQQYYSPRTF	6.3.9	
161.2	IgM	V3-74*01 F	100	J3*02 F	92.0	D1-1*01 F	CRATGTSRASKDAFDW	8.8.16	V3-27*01 F	100	J2*01 F	100	CYSAADNIVF	6.3.9	
168.2	IgM	V3-30*01 F	100	J6*02 F	90.3	D3-10*01 F	CARSVIGYYYYGMDVW	8.8.14	V9-49*01 F	100	J2*01 F	89.5	CGADHSGSLF	7.8.9	
170.5	IgM	V1-18*01 F	100	J1*01 F	90.4	D4-23*01 ORF	CARDQYGGNNYFQHW	8.8.13	V1-8*01 F	100	J5*01 F	100	CQQYYSPITF	6.3.9	
167.3	IgG	V5-51*01 F	100	J4*02 F	87.5	D2-15*01 F	CARSGRHRGSGPDYW	8.8.14	V3-11*01 F	100	J3*01 F	97.4	CQQRSNWPFTF	6.3.9	
176.6	IgG	V3-30*03 F	100	J4*02 F	85.2	D6-19*01 F	CAKDLYLAMAGAAFDW	8.8.15	V3-19*01 F	96.1	J2*01 F	81.6	CNSRDSSGKPSF	6.3.10	
202A.2	IgG	V1-2*02 F	93.3	J3*01 F	92.0	D7-27*01 F	CARDLLRNWGDHDAFDW	8.7.16	V1-47*01 F	97.5	J2*01 F	100	CSAWDDSLSGPV	1.3.11	
279A	IgG	V3-30*03 F	91.7	J6*02 F	79.0	D2-8*01 F	CAKVEIHYYSNLLGMDVW	8.8.17	V1-47*01 F	92.6	J3*02 F	94.6	CATLDINMTWVF	8.3.10	
310A	IgG	V3-66*01 F	94.4	J4*02 F	93.8	D3-22*01 F	CTRDPSQYYDSRGHYVQTFTPSFDSW	8.7.24	V1-17*03 F	98.0	J4*01 F	100	CLOHDTYPLTF	6.3.9	
323A	IgG	V3-30*03 F	95.8	J4*02 F	93.8	D1-14*01 ORF	CAKPVLSFDYW	8.8.10	V2-14*01 F	97.2	J2*01 F	94.6	CCSYTSSSTEVF	9.3.10	
232A.2	IgA	V4-34*02 F	83.5	J6*02 F	79.0	D3-10*01 F	CARGRPHDYSRSGYSRPRRYGGLDVW	8.7.24	V3-20*01 F	91.8	J2*01 F	89.5	CQQYSSSPYTF	7.3.9	
320	IgA	V1-69*01 F	95.5	J6*02 F	77.4	D3-10*01 F	CARDRVPYSRSPRSTKGMWVKYGMVDVW	8.8.28	V2-28*01 F	99.3	J1*01 F	100	CNCALQTPRTF	11.3.9	

Table B. Data processing and refinement statistics for GII.4 P domain–Fab 320 complex.

Data Collection	
Beamline	ALS Beamline 5.0.1
Wavelength, Å	0.97741
Space group	P21 21 2
Cell dimensions, Å	119.25, 186.27, 73.44
$\alpha, \beta, \gamma, ^\circ$	90, 90, 90
Resolution, Å	50-2.25 (2.29-2.25) ^a
Total Reflections	1716311
Unique Reflections	78053 (3854) ^a
Redundancy	6.5 (6.2) ^a
Completeness (%)	99.82
$\langle I/\sigma \rangle$	15.6875 (2.375) ^a
R_{meas}^b	0.129 (0.846) ^a
R_{pim}^b	0.050 (0.340) ^a
Refinement Statistics	
Resolution, Å	50-2.25 (2.29-2.25) ^a
Reflections (work)	73965
Reflections (Test)	3926
$R_{\text{work}}^c / R_{\text{free}}^d$ (%)	18.08/22.55
No. of Atoms	
Protein	
P-domain Dimer	4798
Noro-320 Fab	6674
Water	1059
Average B Value (Å²)	
P-domain Dimer	34.2505
Noro-320 Fab	31.67
Water	36.085
RMSD from Ideal Geometry	
Bond length (Å)	0.003
Bond angle (°)	0.614
Ramachandran Statistics^e	
Favored	98.38%
Outliers	0.20%

5 ^a Numbers in parentheses refer to the highest resolution shell

^b $R_{\text{meas}} = \sum_{hkl} \{N(hkl)/[N(hkl)-1]\}^{1/2} \times \sum_i |I_i(hkl) - \{I(hkl)\}| / \sum_{hkl} \sum_i I_i(hkl)$ and $R_{\text{pim}} = \sum_{hkl} (1/(n-1))^{1/2} \sum_i |I_{hkl,i} - \{I(hkl)\}| / \sum_{hkl} \sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection h, k, l , $\{I(hkl)\}$ is the average intensity for that reflection, and n is the redundancy.

10 ^c $R_{\text{work}} = \sum_{hkl} |F_o - F_c| / \sum_{hkl} |F_o| \times 100$, where F_o and F_c are the observed and calculated structure factors, respectively.

^d R_{free} was calculated as for R_{work} , but on a test set comprising 5% of the data excluded from refinement.

^e Calculated with MolProbity (Chen et al., 2010).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

As discussed above, development of norovirus vaccines has proven challenging. In this study, the inventors describe the isolation and characterization of a panel of human monoclonal antibodies (mAbs) that bind to GII.4 Sydney 2012 VLPs. The majority of these antibodies also
5 block receptor binding, as inferred by their ability to inhibit hemagglutination of human O+ red blood cells (RBCs) or the interaction between GII.4 Sydney 2012 VLPs and porcine gastric mucin (PGM). Both of these assays are surrogate systems for testing HuNoV neutralization (Czako *et al.*, 2012; Reeck *et al.*, 2010). For over 40 years there have been numerous documented attempts to cultivate HuNoVs *in vitro*, but previously none of them resulted in the
10 establishment of a robust reproducible system of viral growth (Jones *et al.*, 2014; Duizer *et al.*, 2004; Herbst-Kralovetz *et al.*, 2013). Recent breakthroughs in the development of an *in vitro* replication system using human intestinal organoid technology have now made it possible to cultivate HuNoV and to test inhibition of growth, or neutralization, using antibodies (Ettayebi *et al.*, 2016; Constantini *et al.*, 2018).

15 Here, the inventors used a human jejunal monolayer culture system to identify antibodies that neutralize live GII.4 Sydney 2012 HuNoV. They identified the first neutralizing human mAbs against norovirus, as well as a panel of human anti-GII.4 Sydney 2012 VLP binding IgGs and the first anti-GII.4 human IgA molecules. Almost 70% of the mAbs that the inventors isolated exhibited a high level of potency, inhibiting GII.4 Sydney 2012 VLPs from
20 binding to PGM at half-maximal effective concentrations (EC₅₀) below 24 µg/mL. They also used this panel of mAbs to identify major antigenic sites on the GII.4 Sydney 2012 major capsid protein.

The broadly cross-reactive naturally-occurring human monoclonal included IgMs, IgAs and IgGs reactive with NoV genogroup I or II (GI or GII). Among the panel the inventors
25 noticed three different binding patterns and identified monoclonal antibodies (mAbs) that neutralized at least one GI or GII NoV strain when using a receptor blocking assay. X-ray crystallography studies of a GII-specific neutralizing mAb revealed the antibody neutralizes not by directly inhibiting receptor binding, but instead through steric hindrance. These data will be useful when designing and evaluating new vaccine candidates. Some of the human mAbs
30 described here also could be used as biologics for the prevention or treatment of chronic NoV infections or severe NoV disease during outbreaks.

These studies contribute new insights into natural human humoral immunity to HuNoVs and provide mAbs that have the potential to be used for diagnostic and therapeutic purposes. These and other aspects of the disclosure are described in detail below.

5 I. Norovirus Virus

Norovirus is the most common cause of gastroenteritis. Infection is characterized by diarrhea, vomiting, and stomach pain. Blood is not usually present. Fever or headaches may also occur. This usually develops 12 to 48 hours after being exposed. Recovery typically occurs within 1 to 3 days. Complications may include dehydration.

10 The virus is usually spread by the fecal–oral route. This may be by contaminated food or water or person-to-person contact. It may also spread via contaminated surfaces or through the air. Risk factors include unsanitary food preparation. Diagnosis is generally based on symptoms. Confirmatory testing may be done for public health purposes.

Prevention involves proper hand washing and disinfection of contaminated surfaces.
15 Alcohol-based hand sanitizers are less effective. A vaccine does not exist. There is no specific treatment. Efforts involve supportive care such as drinking sufficient fluids or intravenous fluids. Oral rehydration solutions are the preferred fluids to drink.

Norovirus results in about 685 million cases of disease and 200,000 deaths globally a year. It is common both in the developed and developing world. Those under the age of five
20 are most often affected and in this group it results in about 50,000 deaths in the developing world. Disease more commonly occurs in winter months. It often occurs in outbreaks, especially among those living in close quarters. In the United States it is the cause of about half of food-borne disease outbreaks. The disease is named after Norwalk, Ohio, where an outbreak occurred in 1968.

25 Indeed, Norovirus causes about 18% of all cases of acute gastroenteritis worldwide. It is relatively common in developed countries and in low-mortality developing countries (20% and 19% respectively) compared to high-mortality developing countries (14%). Proportionately it causes more illness in people in the community or in hospital outpatients (24% and 20% respectively) as compared with hospital inpatients (17%) in whom other causes are
30 more common.

Norovirus is a common cause of epidemics of gastroenteritis on cruise ships. The US Centers for Disease Control and Prevention through its Vessel Sanitation Program record and investigate outbreaks of gastrointestinal illness—mostly caused by norovirus—on cruise ships with both a U.S. and foreign itinerary; there were 12 in 2015, and 10 from 1 January to 9 May

2016. An outbreak may affect over 25% of passengers, and a smaller proportion of crew members.

Norovirus infection is characterized by nausea, vomiting, watery diarrhea, abdominal pain, and in some cases, loss of taste. A person usually develops symptoms of gastroenteritis 12 to 48 hours after being exposed to norovirus. General lethargy, weakness, muscle aches, headaches, and low-grade fevers may occur. The disease is usually self-limiting, and severe illness is rare. Although having norovirus can be unpleasant, it is not usually dangerous and most who contract it make a full recovery within two to three days.

A. Transmission

Noroviruses are transmitted directly from person to person (62–84% of all reported outbreaks) and indirectly via contaminated water and food. They are extremely contagious, and fewer than twenty virus particles can cause an infection (some research suggests as few as five). Transmission can be aerosolized when those stricken with the illness vomit, and can be aerosolized by a toilet flush when vomit or diarrhea is present; infection can follow eating food or breathing air near an episode of vomiting, even if cleaned up. The viruses continue to be shed after symptoms have subsided and shedding can still be detected many weeks after infection.

Vomiting, in particular, transmits infection effectively, and appears to allow airborne transmission. In one incident, a person who vomited spread infection across a restaurant, suggesting that many unexplained cases of food poisoning may have their source in vomit. In December 1998, 126 people were dining at six tables; one woman vomited onto the floor. Staff quickly cleaned up, and people continued eating. Three days later others started falling ill; 52 people reported a range of symptoms, from fever and nausea to vomiting and diarrhea. The cause was not immediately identified. Researchers plotted the seating arrangement: more than 90% of the people at the same table as the sick woman later reported becoming ill. There was a direct correlation between the risk of infection of people at other tables and how close they were to the sick woman. More than 70% of the diners at an adjacent table fell ill; at a table on the other side of the restaurant, the attack rate was still 25%. The outbreak was attributed to a Norwalk-like virus (norovirus). Other cases of transmission by vomit were later identified.

In one outbreak at an international scout jamboree in the Netherlands, each person with gastroenteritis infected an average of 14 people before increased hygiene measures were put in place. Even after these new measures were enacted, an ill person still infected an average of 2.1 other people. A US CDC study of 11 outbreaks in New York State lists the suspected mode

of transmission as person-to-person in seven outbreaks, foodborne in two, waterborne in one, and one unknown. The source of waterborne outbreaks may include water from municipal supplies, wells, recreational lakes, swimming pools and ice machines.

Shellfish and salad ingredients are the foods most often implicated in norovirus outbreaks. Ingestion of shellfish that have not been sufficiently heated – under 75 °C (167 °F) – poses a high risk for norovirus infection. Foods other than shellfish may be contaminated by infected food handlers. Many norovirus outbreaks have been traced to food that was handled by one infected person.

B. Classification

Noroviruses (NoV) are a genetically diverse group of single-stranded positive-sense RNA, non-enveloped viruses belonging to the family *Caliciviridae*. According to the International Committee on Taxonomy of Viruses, the genus *Norovirus* has one species, which is called Norwalk virus. Serotypes, strains and isolates include Norwalk virus, Hawaii virus, Snow Mountain virus, Mexico virus, Desert Shield virus, Southampton virus, Lordsdale virus and Wilkinson virus.

Noroviruses commonly isolated in cases of acute gastroenteritis belong to two genogroups: genogroup I (GI) includes Norwalk virus, Desert Shield virus and Southampton virus; and II (GII), which includes Bristol virus, Lordsdale virus, Toronto virus, Mexico virus, Hawaii virus and Snow Mountain virus.

Noroviruses can genetically be classified into seven different genogroups (GI, GII, GIII, GIV, GV, GVI, and GVII), which can be further divided into different genetic clusters or genotypes. For example, genogroup II, the most prevalent human genogroup, presently contains 19 genotypes. Genogroups I, II and IV infect humans, whereas genogroup III infects bovine species, and genogroup V has recently been isolated in mice.

Most noroviruses that infect humans belong to genogroups GI and GII. Noroviruses from Genogroup II, genotype 4 (abbreviated as GII.4) account for the majority of adult outbreaks of gastroenteritis and often sweep across the globe. Recent examples include US95/96-US strain, associated with global outbreaks in the mid- to late-1990s; Farmington Hills virus associated with outbreaks in Europe and the United States in 2002 and in 2004; and Hunter virus which was associated with outbreaks in Europe, Japan and Australasia. In 2006, there was another large increase in NoV infection around the globe. GII.17 emerged and became predominant in many areas in Asia and was detected in other countries in 2014-2016. Since then a GII.2 strains has emerged in a number of countries. Reports have shown a link

between the expression of human histo-blood group antigens (HBGAs) and the susceptibility to norovirus infection. Studies have suggested the viral capsid of noroviruses may have evolved from selective pressure of human HBGAs.

One study suggests the protein MDA-5 may be the primary immune sensor that detects the presence of noroviruses in the body. Some people have common variations of the MDA-5 gene that could make them more susceptible to norovirus infection. Another study suggested a specific genetic version of norovirus (which would not be distinguishable from other types of the virus using standard viral antibody tests) interacts with a specific mutation in the ATG16L1 gene to help trigger symptomatic Crohn's disease in mice that have been subjected to a chemical that causes intestinal injury similar to the process in humans. (There are other similar ways for such diseases to happen like this, and this study in itself does not prove norovirus causes Crohn's disease in humans).

C. Structure

Viruses in Norovirus are non-enveloped, with icosahedral geometries. Capsid diameters vary widely, from 23–40 nm in diameter. The larger capsids (38–40 nm) exhibit T=3 symmetry and are composed of 180 VP1 proteins. Small capsids (23 nm) show T=1 symmetry and are composed of 60 VP1 proteins. The virus particles demonstrate an amorphous surface structure when visualized using electron microscopy.

Noroviruses contain a linear, non-segmented, positive-sense RNA genome of approximately 7.5 kbp, encoding a large polyprotein which is cleaved into six smaller non-structural proteins (NS1/2 to NS7) by the viral 3C-like protease (NS6), a major structural protein (VP1) of about 58~60 kDa and a minor capsid protein (VP2).

The most variable region of the viral capsid is the P2 domain, which contains antigen-presenting sites and carbohydrate-receptor binding regions. The estimated mutation rate (1.21×10^{-2} to 1.41×10^{-2} substitutions per site per year) in this virus is high even compared with other RNA viruses. In addition, a recombination hotspot exists at the ORF1 - ORF2 (VP1) junction.

D. Lifecycle and Persistence

Viral replication is cytoplasmic. Entry into the host cell is achieved by attachment to host receptors, which mediates endocytosis. Replication follows the positive stranded RNA virus replication model. Positive stranded RNA virus transcription is the method of replication.

Translation takes place by leaky scanning and RNA termination-reinitiation. Humans and other mammals serve as the natural host. Transmission routes are fecal-oral and contamination.

The norovirus can survive for long periods outside a human host depending on the surface and temperature conditions: it can stay for weeks on hard surfaces, and up to twelve days on contaminated fabrics, and it can survive for months, maybe even years in contaminated still water. A 2006 study found the virus remained on surfaces used for food preparation seven days after contamination.

E. Pathophysiology

When a person becomes infected with norovirus, the virus is replicated within the small intestine. After approximately one to two days, norovirus infection symptoms can appear. The principal symptom is acute gastroenteritis that develops between 12 and 48 hours after exposure, and lasts for 24–72 hours. The disease is usually self-limiting, and characterized by nausea, forceful vomiting, watery diarrhea, and abdominal pain, and in some cases, loss of taste. General lethargy, weakness, muscle aches, headache, coughs, and low-grade fever may occur.

Severe illness is rare; although people are frequently treated at the emergency ward, they are rarely admitted to the hospital. The number of deaths from norovirus in the United States is estimated to be around 300 each year, with most of these occurring in the very young, the elderly, and persons with weakened immune systems. Symptoms may become life-threatening in these groups if dehydration or electrolyte imbalance is ignored or not treated.

F. Diagnosis and Detection

Specific diagnosis of norovirus is routinely made by polymerase chain reaction (PCR) assays or quantitative PCR assays, which give results within a few hours. These assays are very sensitive and can detect as few as 10 virus particles. Tests such as ELISA that use antibodies against a mixture of norovirus strains are available commercially, but lack specificity and sensitivity. Due to a lack of specific therapy, the need for expensive stool diagnostics is being questioned by experts if gastroenteritis by noroviruses has already been detected in the environment.

Routine protocols to detect norovirus in clams and oysters by reverse transcription polymerase chain reaction are being employed by governmental laboratories such as the Food and Drug Administration (FDA) in the USA.

G. Prevention

After infection, immunity to the *same* strain of the virus – the genotype – protects against reinfection for between 6 months to 2 years. This immunity does not fully protect against infection with the other diverse genotypes of the virus.

5 Hand washing with soap and water is an effective method for reducing the transmission of norovirus pathogens. Alcohol rubs ($\geq 62\%$ ethanol) may be used as an adjunct, but are less effective than hand-washing, as norovirus lacks a lipid viral envelope. Surfaces where norovirus particles may be present can be sanitized with a solution of 1.5% to 7.5% of household bleach in water, or other disinfectants effective against norovirus.

10 In health-care environments, the prevention of nosocomial infections involves routine and terminal cleaning. Non-flammable alcohol vapor in CO₂ systems is used in health care environments where medical electronics would be adversely affected by aerosolized chlorine or other caustic compounds.

In 2011, the Centers for Disease Control and Prevention (CDC) published a clinical
15 practice guideline addressing strategies for the prevention and control of norovirus gastroenteritis outbreaks in health-care settings. Based on a systematic review of published scientific studies, the guideline presents 51 specific evidence-based recommendations, which were organized into 12 categories: 1) patient cohorting and isolation precautions, 2) hand hygiene, 3) patient transfer and ward closure, 4) food handlers in healthcare, 5) diagnostics, 6)
20 personal protective equipment, 7) environmental cleaning, 8) staff leave and policy, 9) visitors, 10) education, 11) active case-finding, and 12) communication and notification. The guideline also identifies eight high-priority recommendations, and suggests several areas in need of future research.

Ligocyte announced in 2007 that it was working on a vaccine and had started phase I
25 trials. The company has since been acquired by Takeda Vaccines. As of 2011, a monovalent nasal vaccine had completed phase I/II trials, while bivalent intramuscular and nasal vaccines were at earlier stages of development. The two vaccines rely on using a virus-like particle that is made of the norovirus capsid proteins in order to mimic the external structure of the virus. Since there is no RNA in this particle, it is incapable of reproducing and cannot cause an
30 infection.

H. Treatment

There is no specific medicine to treat people with norovirus illness. Norovirus infection cannot be treated with antibiotics because it is not a bacterial infection. Treatments aim to avoid

complications by measures such as the management of dehydration caused by fluid loss in vomiting and diarrhea, and to mitigate symptoms using antiemetics and antidiarrheals.

II. Monoclonal Antibodies and Production Thereof

5 An "isolated antibody" is one that has been separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In particular embodiments, the antibody is purified: (1) to greater than 95% by weight of antibody
10 as determined by the Lowry method, and most particularly more than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural
15 environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The basic four-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 basic heterotetramer units along with an additional polypeptide called J chain, and therefore
20 contain 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has
25 regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable region (V_H) followed by three constant domains (C_H) for each of the alpha and gamma chains and four C_H domains for mu and isotypes. Each L chain has at the N-terminus, a variable region (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid
30 residues are believed to form an interface between the light chain and heavy chain variable regions. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, *e.g.*, Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, Conn., 1994, page 71, and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda based on the amino acid sequences of their constant domains (C_L). Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha, delta, epsilon, gamma and mu, respectively. They gamma and alpha classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

The term "variable" refers to the fact that certain segments of the V domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable regions of native heavy and light chains each comprise four FRs, largely adopting a beta-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), and antibody-dependent complement deposition (ADCD).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L , and around about 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the V_H when numbered in accordance with the Kabat numbering system; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)); and/or those residues from a "hypervariable loop" (*e.g.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in

the V_L, and 26-32 (H1), 52-56 (H2) and 95-101 (H3) in the V_H when numbered in accordance with the Chothia numbering system; Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)); and/or those residues from a "hypervariable loop"/CDR (*e.g.*, residues 27-38 (L1), 56-65 (L2) and 105-120 (L3) in the V_L, and 27-38 (H1), 56-65 (H2) and 105-120 (H3) in the V_H when numbered in accordance with the IMGT numbering system; Lefranc, M. P. *et al.* Nucl. Acids Res. 27:209-212 (1999), Ruiz, M. *et al.* Nucl. Acids Res. 28:219-221 (2000)). Optionally the antibody has symmetrical insertions at one or more of the following points 28, 36 (L1), 63, 74-75 (L2) and 123 (L3) in the V_L, and 28, 36 (H1), 63, 74-75 (H2) and 123 (H3) in the V_{subH} when numbered in accordance with AHO; Honneger, A. and Plunkthun, A. J. Mol. Biol. 309:657-670 (2001)).

By "germline nucleic acid residue" is meant the nucleic acid residue that naturally occurs in a germline gene encoding a constant or variable region. "Germline gene" is the DNA found in a germ cell (*i.e.*, a cell destined to become an egg or in the sperm). A "germline mutation" refers to a heritable change in a particular DNA that has occurred in a germ cell or the zygote at the single-cell stage, and when transmitted to offspring, such a mutation is incorporated in every cell of the body. A germline mutation is in contrast to a somatic mutation which is acquired in a single body cell. In some cases, nucleotides in a germline DNA sequence encoding for a variable region are mutated (*i.e.*, a somatic mutation) and replaced with a different nucleotide.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations that include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present disclosure may be prepared by the hybridoma methodology first described by Kohler *et al.*, Nature, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, *e.g.*, U.S. Pat. No. 4,816,567) after single cell sorting of an antigen specific B cell, an antigen specific plasmablast responding to an infection or immunization, or capture of linked heavy and light chains from single cells in a bulk sorted

antigen specific collection. The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature, 352:624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222:581-597 (1991), for example.

5 **A. General Methods**

It will be understood that monoclonal antibodies binding to norovirus will have several applications. These include the production of diagnostic kits for use in detecting and diagnosing norovirus infection, as well as for treating the same. In these contexts, one may link such antibodies to diagnostic or therapeutic agents, use them as capture agents or competitors in competitive assays, or use them individually without additional agents being attached thereto. The antibodies may be mutated or modified, as discussed further below. Methods for preparing and characterizing antibodies are well known in the art (see, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; U.S. Patent 4,196,265).

15 The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. The first step for both these methods is immunization of an appropriate host or identification of subjects who are immune due to prior natural infection or vaccination with a licensed or experimental vaccine. As is well known in the art, a given composition for immunization may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine. As also is well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants in animals include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant and in humans include alum, CpG, MFP59 and combinations of immunostimulatory molecules ("Adjuvant Systems", such as AS01 or AS03). Additional experimental forms of inoculation to induce norovirus-specific B cells is possible, including nanoparticle vaccines, or gene-encoded antigens delivered as DNA or RNA genes in a physical delivery system (such as lipid nanoparticle or on a gold biolistic bead), and delivered with needle, gene gun,

transcutaneous electroporation device. The antigen gene also can be carried as encoded by a replication competent or defective viral vector such as adenovirus, adeno-associated virus, poxvirus, herpesvirus, or alphavirus replicon, or alternatively a virus like particle.

In the case of human antibodies against natural pathogens, a suitable approach is to identify subjects that have been exposed to the pathogens, such as those who have been diagnosed as having contracted the disease, or those who have been vaccinated to generate protective immunity against the pathogen or to test the safety or efficacy of an experimental vaccine. Circulating anti-pathogen antibodies can be detected, and antibody encoding or producing B cells from the antibody-positive subject may then be obtained.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization. A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens, lymph nodes, tonsils or adenoids, bone marrow aspirates or biopsies, tissue biopsies from mucosal organs like lung or GI tract, or from circulating blood. The antibody-producing B lymphocytes from the immunized animal or immune human are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized or human or human/mouse chimeric cells. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984). HMM2.5 cells or MFP-2 cells are particularly useful examples of such cells.

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of

an agent or agents (chemical or electrical) that promote the fusion of cell membranes. In some cases, transformation of human B cells with Epstein Barr virus (EBV) as an initial step increases the size of the B cells, enhancing fusion with the relatively large-sized myeloma cells. Transformation efficiency by EBV is enhanced by using CpG and a Chk2 inhibitor drug in the transforming medium. Alternatively, human B cells can be activated by co-culture with transfected cell lines expressing CD40 Ligand (CD154) in medium containing additional soluble factors, such as IL-21 and human B cell Activating Factor (BAFF), a Type II member of the TNF superfamily. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Gefter *et al.* (1977). The use of electrically induced fusion methods also is appropriate (Goding, pp. 71-74, 1986) and there are processes for better efficiency (Yu *et al.*, 2008). Fusion procedures usually produce viable hybrids at low frequencies, about 1×10^{-6} to 1×10^{-8} , but with optimized procedures one can achieve fusion efficiencies close to 1 in 200 (Yu *et al.*, 2008). However, relatively low efficiency of fusion does not pose a problem, as the viable, fused hybrids are differentiated from the parental, infused cells (particularly the infused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture medium. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the medium is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the medium is supplemented with hypoxanthine. Ouabain is added if the B cell source is an EBV-transformed human B cell line, in order to eliminate EBV-transformed lines that have not fused to the myeloma.

The preferred selection medium is HAT or HAT with ouabain. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells. When the source of B cells used for fusion is a line of EBV-transformed B cells, as here, ouabain may also be used for drug selection of hybrids as EBV-transformed B cells are susceptible to drug killing, whereas the myeloma partner used is chosen to be ouabain resistant.

Culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays dot immunobinding assays, and the like. The selected hybridomas are then serially diluted or single-cell sorted by flow cytometric sorting and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into an animal (*e.g.*, a mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. When human hybridomas are used in this way, it is optimal to inject immunocompromised mice, such as SCID mice, to prevent tumor rejection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. Alternatively, human hybridoma cells lines can be used *in vitro* to produce immunoglobulins in cell supernatant. The cell lines can be adapted for growth in serum-free medium to optimize the ability to recover human monoclonal immunoglobulins of high purity.

MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as FPLC or affinity chromatography. Fragments of the monoclonal antibodies of the disclosure can be obtained from the purified monoclonal antibodies by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present disclosure can be synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonal antibodies. Single B cells labelled with the antigen of interest can be sorted physically using paramagnetic bead selection or flow cytometric sorting, then RNA can be isolated from the single cells and antibody genes amplified by RT-PCR. Alternatively, antigen-specific bulk sorted populations of cells can be segregated into microvesicles and the matched heavy and light chain variable genes recovered from single cells using physical linkage of

heavy and light chain amplicons, or common barcoding of heavy and light chain genes from a vesicle. Matched heavy and light chain genes from single cells also can be obtained from populations of antigen specific B cells by treating cells with cell-penetrating nanoparticles bearing RT-PCR primers and barcodes for marking transcripts with one barcode per cell. The antibody variable genes also can be isolated by RNA extraction of a hybridoma line and the antibody genes obtained by RT-PCR and cloned into an immunoglobulin expression vector. Alternatively, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the cell lines and phagemids expressing appropriate antibodies are selected by panning using viral antigens. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present disclosure include U.S. Patent 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Patent 4,816,567 which describes recombinant immunoglobulin preparations; and U.S. Patent 4,867,973 which describes antibody-therapeutic agent conjugates.

B. Antibodies of the Present Disclosure

Antibodies according to the present disclosure may be defined, in the first instance, by their binding specificity. Those of skill in the art, by assessing the binding specificity/affinity of a given antibody using techniques well known to those of skill in the art, can determine whether such antibodies fall within the scope of the instant claims. For example, the epitope to which a given antibody bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) amino acids located within the antigen molecule (*e.g.*, a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within the antigen molecule (*e.g.*, a conformational epitope).

Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody “interacts with one or more amino acids” within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in *Antibodies*, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Cross-blocking can be measured in various binding assays such as ELISA, biolayer interferometry, or surface plasmon resonance. Other methods include alanine scanning

mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis, high-resolution electron microscopy techniques using single particle reconstruction, cryoEM, or tomography, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. See, e.g., Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A. When the antibody neutralizes norovirus, antibody escape mutant variant organisms can be isolated by propagating norovirus *in vitro* or in animal models in the presence of high concentrations of the antibody. Sequence analysis of the norovirus gene encoding the antigen targeted by the antibody reveals the mutation(s) conferring antibody escape, indicating residues in the epitope or that affect the structure of the epitope allosterically.

The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may

reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the disclosure into groups of antibodies binding different epitopes.

The present disclosure includes antibodies that may bind to the same epitope, or a portion of the epitope. Likewise, the present disclosure also includes antibodies that compete for binding to a target or a fragment thereof with any of the specific exemplary antibodies described herein. One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference, the reference antibody is allowed to bind to target under saturating conditions. Next, the ability of a test antibody to bind to the target molecule is assessed. If the test antibody is able to bind to the target molecule following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the reference antibody. On the other hand, if the test antibody is not able to bind to the target molecule following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference antibody.

To determine if an antibody competes for binding with a reference anti-norovirus antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to the norovirus antigen under saturating conditions followed by assessment of binding of the test antibody to the norovirus antigen. In a second orientation, the test antibody is allowed to bind to the norovirus antigen molecule under saturating conditions followed by assessment of binding of the reference antibody to the norovirus molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the norovirus, then it is concluded that the test antibody and the reference antibody compete for binding to the norovirus. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99%

as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. Structural studies with EM or crystallography also can demonstrate whether or not two antibodies that compete for binding recognize the same epitope.

In another aspect, there are provided monoclonal antibodies having clone-paired CDRs from the heavy and light chains as illustrated in Tables 3 and 4, respectively. Such antibodies may be produced by the clones discussed below in the Examples section using methods described herein.

In another aspect, the antibodies may be defined by their variable sequence, which include additional “framework” regions. These are provided in Tables 1 and 2 that encode or represent full variable regions. Furthermore, the antibodies sequences may vary from these sequences, optionally using methods discussed in greater detail below. For example, nucleic acid sequences may vary from those set out above in that (a) the variable regions may be segregated away from the constant domains of the light and heavy chains, (b) the nucleic acids may vary from those set out above while not affecting the residues encoded thereby, (c) the nucleic acids may vary from those set out above by a given percentage, *e.g.*, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, (d) the nucleic acids may vary from those set out above by virtue of the ability to hybridize under high stringency conditions, as exemplified by low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C, (e) the amino acids may vary from those set out above by a given percentage, *e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, or (f) the amino acids may vary from those set out above by permitting conservative substitutions (discussed below).

Each of the foregoing applies to the nucleic acid sequences set forth as Table 1 and the amino acid sequences of Table 2.

When comparing polynucleotide and polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, Wis.), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M. O. (1978) A model of evolutionary change in proteins--Matrices for detecting distant relationships. In Dayhoff, M. O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington D.C. Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogeny pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, Calif.; Higgins, D. G. and Sharp, P. M. (1989) CABIOS 5:151-153; Myers, E. W. and Muller W. (1988) CABIOS 4:11-17; Robinson, E. D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) Mol. Biol. Evol. 4:406-425; Sneath, P. H. A. and Sokal, R. R. (1973) Numerical Taxonomy--the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, Calif.; Wilbur, W. J. and Lipman, D. J. (1983) Proc. Natl. Acad., Sci. USA 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL. Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. USA 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.), or by inspection.

One particular example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) Nucl. Acids Res. 25:3389-3402 and Altschul *et al.* (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with

the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the disclosure. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. The rearranged nature of an antibody sequence and the variable length of each gene requires multiple rounds of BLAST searches for a single antibody sequence. Also, manual assembly of different genes is difficult and error-prone. The sequence analysis tool IgBLAST (world-wide-web at ncbi.nlm.nih.gov/igblast/) identifies matches to the germline V, D and J genes, details at rearrangement junctions, the delineation of Ig V domain framework regions and complementarity determining regions. IgBLAST can analyze nucleotide or protein sequences and can process sequences in batches and allows searches against the germline gene databases and other sequence databases simultaneously to minimize the chance of missing possibly the best matching germline V gene.

In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

In one approach, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or

deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Yet another way of defining an antibody is as a “derivative” of any of the below-described antibodies and their antigen-binding fragments. The term “derivative” refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to an antigen but which comprises, one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to a “parental” (or wild-type) molecule. Such amino acid substitutions or additions may introduce naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. The term “derivative” encompasses, for example, as variants having altered CH1, hinge, CH2, CH3 or CH4 regions, so as to form, for example antibodies, *etc.*, having variant Fc regions that exhibit enhanced or impaired effector or binding characteristics. The term “derivative” additionally encompasses non-amino acid modifications, for example, amino acids that may be glycosylated (*e.g.*, have altered mannose, 2-N-acetylglucosamine, galactose, fucose, glucose, sialic acid, 5-N-acetylneuraminic acid, 5-glycolneuraminic acid, *etc.* content), acetylated, pegylated, phosphorylated, amidated, derivatized by known protecting/blocking groups, proteolytic cleavage, linked to a cellular ligand or other protein, *etc.* In some embodiments, the altered carbohydrate modifications modulate one or more of the following: solubilization of the antibody, facilitation of subcellular transport and secretion of the antibody, promotion of antibody assembly, conformational integrity, and antibody-mediated effector function. In a specific embodiment the altered carbohydrate modifications enhance antibody mediated effector function relative to the antibody lacking the carbohydrate modification. Carbohydrate modifications that lead to altered antibody mediated effector function are well known in the art (for example, see Shields, R. L. *et al.* (2002) “*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human FcγRIII And Antibody-Dependent Cellular Toxicity*,” J. Biol. Chem. 277(30): 26733-26740; Davies J. *et al.* (2001) “*Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC Gamma RIII*,” Biotechnology & Bioengineering 74(4): 288-294). Methods of altering carbohydrate contents are known to those skilled in the art, see, *e.g.*, Wallick, S. C. *et al.* (1988) “*Glycosylation Of A VH Residue Of A Monoclonal Antibody Against Alpha (1----6) Dextran Increases Its Affinity For Antigen*,” J.

Exp. Med. 168(3): 1099-1109; Tao, M. H. *et al.* (1989) “*Studies Of Aglycosylated Chimeric Mouse-Human IgG. Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region*,” J. Immunol. 143(8): 2595-2601; Routledge, E. G. *et al.* (1995) “*The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody*,” Transplantation 60(8):847-53; Elliott, S. *et al.* (2003) “*Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering*,” Nature Biotechnol. 21:414-21; Shields, R. L. *et al.* (2002) “*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcγ₃ RIII And Antibody-Dependent Cellular Toxicity*,” J. Biol. Chem. 277(30): 26733-26740).

A derivative antibody or antibody fragment can be generated with an engineered sequence or glycosylation state to confer preferred levels of activity in antibody dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), or antibody-dependent complement deposition (ADCD) functions as measured by bead-based or cell-based assays or *in vivo* studies in animal models.

A derivative antibody or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* In one embodiment, an antibody derivative will possess a similar or identical function as the parental antibody. In another embodiment, an antibody derivative will exhibit an altered activity relative to the parental antibody. For example, a derivative antibody (or fragment thereof) can bind to its epitope more tightly or be more resistant to proteolysis than the parental antibody.

C. Engineering of Antibody Sequences

In various embodiments, one may choose to engineer sequences of the identified antibodies for a variety of reasons, such as improved expression, improved cross-reactivity or diminished off-target binding. Modified antibodies may be made by any technique known to those of skill in the art, including expression through standard molecular biological techniques, or the chemical synthesis of polypeptides. Methods for recombinant expression are addressed elsewhere in this document. The following is a general discussion of relevant goals techniques for antibody engineering.

Hybridomas may be cultured, then cells lysed, and total RNA extracted. Random hexamers may be used with RT to generate cDNA copies of RNA, and then PCR performed using a multiplex mixture of PCR primers expected to amplify all human variable gene

sequences. PCR product can be cloned into pGEM-T Easy vector, then sequenced by automated DNA sequencing using standard vector primers. Assay of binding and neutralization may be performed using antibodies collected from hybridoma supernatants and purified by FPLC, using Protein G columns.

5 Recombinant full-length IgG antibodies can be generated by subcloning heavy and light chain Fv DNAs from the cloning vector into an IgG plasmid vector, transfected into 293 (*e.g.*, Freestyle) cells or CHO cells, and antibodies can be collected and purified from the 293 or CHO cell supernatant. Other appropriate host cells systems include bacteria, such as *E. coli*, insect cells (S2, Sf9, Sf29, High Five), plant cells (*e.g.*, tobacco, with or without engineering
10 for human-like glycans), algae, or in a variety of non-human transgenic contexts, such as mice, rats, goats or cows.

 Expression of nucleic acids encoding antibodies, both for the purpose of subsequent antibody purification, and for immunization of a host, is also contemplated. Antibody coding sequences can be RNA, such as native RNA or modified RNA. Modified RNA contemplates
15 certain chemical modifications that confer increased stability and low immunogenicity to mRNAs, thereby facilitating expression of therapeutically important proteins. For instance, N1-methyl-pseudouridine (N1m Ψ) outperforms several other nucleoside modifications and their combinations in terms of translation capacity. In addition to turning off the immune/eIF2 α phosphorylation-dependent inhibition of translation, incorporated N1m Ψ nucleotides
20 dramatically alter the dynamics of the translation process by increasing ribosome pausing and density on the mRNA. Increased ribosome loading of modified mRNAs renders them more permissive for initiation by favoring either ribosome recycling on the same mRNA or de novo ribosome recruitment. Such modifications could be used to enhance antibody expression in vivo following inoculation with RNA. The RNA, whether native or modified, may be
25 delivered as naked RNA or in a delivery vehicle, such as a lipid nanoparticle.

 Alternatively, DNA encoding the antibody may be employed for the same purposes. The DNA is included in an expression cassette comprising a promoter active in the host cell for which it is designed. The expression cassette is advantageously included in a replicable vector, such as a conventional plasmid or minivector. Vectors include viral vectors, such as
30 poxviruses, adenoviruses, herpesviruses, adeno-associated viruses, and lentiviruses are contemplated. Replicons encoding antibody genes such as alphavirus replicons based on VEE virus or Sindbis virus are also contemplated. Delivery of such vectors can be performed by needle through intramuscular, subcutaneous, or intradermal routes, or by transcutaneous electroporation when *in vivo* expression is desired.

The rapid availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce the duration of process development programs. Lonza has developed a generic method using pooled transfectants grown in CDACF medium, for the rapid production of small quantities (up to 50 g) of antibodies in CHO cells. Although slightly slower than a true transient system, the advantages include a higher product concentration and use of the same host and process as the production cell line. Example of growth and productivity of GS-CHO pools, expressing a model antibody, in a disposable bioreactor: in a disposable bag bioreactor culture (5 L working volume) operated in fed-batch mode, a harvest antibody concentration of 2 g/L was achieved within 9 weeks of transfection.

Antibody molecules will comprise fragments (such as F(ab'), F(ab')₂) that are produced, for example, by the proteolytic cleavage of the mAbs, or single-chain immunoglobulins producible, for example, via recombinant means. F(ab') antibody derivatives are monovalent, while F(ab')₂ antibody derivatives are bivalent. In one embodiment, such fragments can be combined with one another, or with other antibody fragments or receptor ligands to form "chimeric" binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule.

In related embodiments, the antibody is a derivative of the disclosed antibodies, *e.g.*, an antibody comprising the CDR sequences identical to those in the disclosed antibodies (*e.g.*, a chimeric, or CDR-grafted antibody). Alternatively, one may wish to make modifications, such as introducing conservative changes into an antibody molecule. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: basic amino acids: arginine (+3.0), lysine (+3.0), and histidine (-0.5);

acidic amino acids: aspartate ($+3.0 \pm 1$), glutamate ($+3.0 \pm 1$), asparagine ($+0.2$), and glutamine ($+0.2$); hydrophilic, nonionic amino acids: serine ($+0.3$), asparagine ($+0.2$), glutamine ($+0.2$), and threonine (-0.4), sulfur containing amino acids: cysteine (-1.0) and methionine (-1.3); hydrophobic, nonaromatic amino acids: valine (-1.5), leucine (-1.8), isoleucine (-1.8), proline (-0.5 ± 1), alanine (-0.5), and glycine (0); hydrophobic, aromatic amino acids: tryptophan (-3.4), phenylalanine (-2.5), and tyrosine (-2.3).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity and produce a biologically or immunologically modified protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The present disclosure also contemplates isotype modification. By modifying the Fc region to have a different isotype, different functionalities can be achieved. For example, changing to IgG₁ can increase antibody dependent cell cytotoxicity, switching to class A can improve tissue distribution, and switching to class M can improve valency.

Alternatively or additionally, it may be useful to combine amino acid modifications with one or more further amino acid modifications that alter C1q binding and/or the complement dependent cytotoxicity (CDC) function of the Fc region of an IL-23p19 binding molecule. The binding polypeptide of particular interest may be one that binds to C1q and displays complement dependent cytotoxicity. Polypeptides with pre-existing C1q binding activity, optionally further having the ability to mediate CDC may be modified such that one or both of these activities are enhanced. Amino acid modifications that alter C1q and/or modify its complement dependent cytotoxicity function are described, for example, in WO/0042072, which is hereby incorporated by reference.

One can design an Fc region of an antibody with altered effector function, *e.g.*, by modifying C1q binding and/or FcγR binding and thereby changing CDC activity and/or ADCC activity. "Effector functions" are responsible for activating or diminishing a biological activity (*e.g.*, in a subject). Examples of effector functions include, but are not limited to: C1q binding;

complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), *etc.* Such effector functions may require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain) and can be assessed using various assays (*e.g.*, Fc binding assays, ADCC assays, CDC assays, *etc.*).

For example, one can generate a variant Fc region of an antibody with improved C1q binding and improved FcγRIII binding (*e.g.*, having both improved ADCC activity and improved CDC activity). Alternatively, if it is desired that effector function be reduced or ablated, a variant Fc region can be engineered with reduced CDC activity and/or reduced ADCC activity. In other embodiments, only one of these activities may be increased, and, optionally, also the other activity reduced (*e.g.*, to generate an Fc region variant with improved ADCC activity, but reduced CDC activity and *vice versa*).

FcRn binding. Fc mutations can also be introduced and engineered to alter their interaction with the neonatal Fc receptor (FcRn) and improve their pharmacokinetic properties. A collection of human Fc variants with improved binding to the FcRn have been described (Shields *et al.*, (2001). High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR, (J. Biol. Chem. 276:6591-6604). A number of methods are known that can result in increased half-life (Kuo and Aveson, (2011)), including amino acid modifications may be generated through techniques including alanine scanning mutagenesis, random mutagenesis and screening to assess the binding to the neonatal Fc receptor (FcRn) and/or the *in vivo* behavior. Computational strategies followed by mutagenesis may also be used to select one of amino acid mutations to mutate.

The present disclosure therefore provides a variant of an antigen binding protein with optimized binding to FcRn. In a particular embodiment, the said variant of an antigen binding protein comprises at least one amino acid modification in the Fc region of said antigen binding protein, wherein said modification is selected from the group consisting of 226, 227, 228, 230, 231, 233, 234, 239, 241, 243, 246, 250, 252, 256, 259, 264, 265, 267, 269, 270, 276, 284, 285, 288, 289, 290, 291, 292, 294, 297, 298, 299, 301, 302, 303, 305, 307, 308, 309, 311, 315, 317, 320, 322, 325, 327, 330, 332, 334, 335, 338, 340, 342, 343, 345, 347, 350, 352, 354, 355, 356, 359, 360, 361, 362, 369, 370, 371, 375, 378, 380, 382, 384, 385, 386, 387, 389, 390, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 403, 404, 408, 411, 412, 414, 415, 416, 418, 419, 420, 421, 422, 424, 426, 428, 433, 434, 438, 439, 440, 443, 444, 445, 446 and 447 of the Fc region as compared to said parent polypeptide, wherein the numbering of the amino acids in the Fc

region is that of the EU index in Kabat. In a further aspect of the disclosure the modifications are M252Y/S254T/T256E.

Additionally, various publications describe methods for obtaining physiologically active molecules whose half-lives are modified, see for example Kontermann (2009) either by
5 introducing an FcRn-binding polypeptide into the molecules or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved but affinities for other Fc receptors have been greatly reduced or fusing with FcRn binding domains of antibodies.

Derivatized antibodies may be used to alter the half-lives (*e.g.*, serum half-lives) of parental antibodies in a mammal, particularly a human. Such alterations may result in a half-
10 life of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the antibodies of the present disclosure or fragments thereof in a mammal, preferably a human, results in a higher serum titer of said antibodies or antibody fragments in the mammal,
15 and thus reduces the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or fragments thereof with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or
20 adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor.

Beltramello *et al.* (2010) previously reported the modification of neutralizing mAbs, due to their tendency to enhance dengue virus infection, by generating in which leucine residues at positions 1.3 and 1.2 of CH2 domain (according to the IMGT unique numbering for
25 C-domain) were substituted with alanine residues. This modification, also known as “LALA” mutation, abolishes antibody binding to FcγRI, FcγRII and FcγRIIIa, as described by Hessel *et al.* (2007). The variant and unmodified recombinant mAbs were compared for their capacity to neutralize and enhance infection by the four dengue virus serotypes. LALA variants retained the same neutralizing activity as unmodified mAb, but were completely devoid of enhancing
30 activity. LALA mutations of this nature are therefore contemplated in the context of the presently disclosed antibodies.

Altered Glycosylation. A particular embodiment of the present disclosure is an isolated monoclonal antibody, or antigen binding fragment thereof, containing a substantially homogeneous glycan without sialic acid, galactose, or fucose. The monoclonal antibody

comprises a heavy chain variable region and a light chain variable region, both of which may be attached to heavy chain or light chain constant regions respectively. The aforementioned substantially homogeneous glycan may be covalently attached to the heavy chain constant region.

5 Another embodiment of the present disclosure comprises a mAb with a novel Fc glycosylation pattern. The isolated monoclonal antibody, or antigen binding fragment thereof, is present in a substantially homogenous composition represented by the GNGN or G1/G2 glycoform. Fc glycosylation plays a significant role in anti-viral and anti-cancer properties of therapeutic mAbs. The disclosure is in line with a recent study that shows increased anti-
10 lentivirus cell-mediated viral inhibition of a fucose free anti-HIV mAb *in vitro*. This embodiment of the present disclosure with homogenous glycans lacking a core fucose, showed increased protection against specific viruses by a factor greater than two-fold. Elimination of core fucose dramatically improves the ADCC activity of mAbs mediated by natural killer (NK) cells but appears to have the opposite effect on the ADCC activity of polymorphonuclear cells
15 (PMNs).

The isolated monoclonal antibody, or antigen binding fragment thereof, comprising a substantially homogenous composition represented by the GNGN or G1/G2 glycoform exhibits increased binding affinity for Fc gamma RI and Fc gamma RIII compared to the same antibody without the substantially homogeneous GNGN glycoform and with G0, G1F, G2F, GNF,
20 GNGNF or GNGNFX containing glycoforms. In one embodiment of the present disclosure, the antibody dissociates from Fc gamma RI with a K_d of 1×10^{-8} M or less and from Fc gamma RIII with a K_d of 1×10^{-7} M or less.

Glycosylation of an Fc region is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. O-
25 linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. The recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain peptide sequences are asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline.
30 Thus, the presence of either of these peptide sequences in a polypeptide creates a potential glycosylation site.

The glycosylation pattern may be altered, for example, by deleting one or more glycosylation site(s) found in the polypeptide, and/or adding one or more glycosylation site(s) that are not present in the polypeptide. Addition of glycosylation sites to the Fc region of an

antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). An exemplary glycosylation variant has an amino acid substitution of residue Asn 297 of the heavy chain. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original polypeptide (for O-linked glycosylation sites). Additionally, a change of Asn 297 to Ala can remove one of the glycosylation sites.

In certain embodiments, the antibody is expressed in cells that express beta (1,4)-N-acetylglucosaminyltransferase III (GnT III), such that GnT III adds GlcNAc to the IL-23p19 antibody. Methods for producing antibodies in such a fashion are provided in WO/9954342, WO/03011878, patent publication 20030003097A1, and Umana *et al.*, Nature Biotechnology, 17:176-180, February 1999. Cell lines can be altered to enhance or reduce or eliminate certain post-translational modifications, such as glycosylation, using genome editing technology such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). For example, CRISPR technology can be used to eliminate genes encoding glycosylating enzymes in 293 or CHO cells used to express recombinant monoclonal antibodies.

Elimination of monoclonal antibody protein sequence liabilities. It is possible to engineer the antibody variable gene sequences obtained from human B cells to enhance their manufacturability and safety. Potential protein sequence liabilities can be identified by searching for sequence motifs associated with sites containing:

- 1) Unpaired Cys residues,
- 2) N-linked glycosylation,
- 3) Asn deamidation,
- 4) Asp isomerization,
- 5) SYE truncation,
- 6) Met oxidation,
- 7) Trp oxidation,
- 8) N-terminal glutamate,
- 9) Integrin binding,
- 10) CD11c/CD18 binding, or
- 11) Fragmentation

Such motifs can be eliminated by altering the synthetic gene for the cDNA encoding recombinant antibodies.

Protein engineering efforts in the field of development of therapeutic antibodies clearly reveal that certain sequences or residues are associated with solubility differences (Fernandez-

Escamilla *et al.*, *Nature Biotech.*, 22 (10), 1302-1306, 2004; Chennamsetty *et al.*, *PNAS*, 106 (29), 11937-11942, 2009; Voynov *et al.*, *Biocon. Chem.*, 21 (2), 385-392, 2010) Evidence from solubility-altering mutations in the literature indicate that some hydrophilic residues such as aspartic acid, glutamic acid, and serine contribute significantly more favorably to protein solubility than other hydrophilic residues, such as asparagine, glutamine, threonine, lysine, and arginine.

Stability. Antibodies can be engineered for enhanced biophysical properties. One can use elevated temperature to unfold antibodies to determine relative stability, using average apparent melting temperatures. Differential Scanning Calorimetry (DSC) measures the heat capacity, C_p , of a molecule (the heat required to warm it, per degree) as a function of temperature. One can use DSC to study the thermal stability of antibodies. DSC data for mAbs is particularly interesting because it sometimes resolves the unfolding of individual domains within the mAb structure, producing up to three peaks in the thermogram (from unfolding of the Fab, CH_2 , and CH_3 domains). Typically unfolding of the Fab domain produces the strongest peak. The DSC profiles and relative stability of the Fc portion show characteristic differences for the human IgG1, IgG2, IgG3, and IgG4 subclasses (Garber and Demarest, *Biochem. Biophys. Res. Commun.* 355, 751-757, 2007). One also can determine average apparent melting temperature using circular dichroism (CD), performed with a CD spectrometer. Far-UV CD spectra will be measured for antibodies in the range of 200 to 260 nm at increments of 0.5 nm. The final spectra can be determined as averages of 20 accumulations. Residue ellipticity values can be calculated after background subtraction. Thermal unfolding of antibodies (0.1 mg/mL) can be monitored at 235 nm from 25-95 °C and a heating rate of 1 °C/min. One can use dynamic light scattering (DLS) to assess for propensity for aggregation. DLS is used to characterize size of various particles including proteins. If the system is not disperse in size, the mean effective diameter of the particles can be determined. This measurement depends on the size of the particle core, the size of surface structures, and particle concentration. Since DLS essentially measures fluctuations in scattered light intensity due to particles, the diffusion coefficient of the particles can be determined. DLS software in commercial DLA instruments displays the particle population at different diameters. Stability studies can be done conveniently using DLS. DLS measurements of a sample can show whether the particles aggregate over time or with temperature variation by determining whether the hydrodynamic radius of the particle increases. If particles aggregate, one can see a larger population of particles with a larger radius. Stability depending on temperature can be analyzed by controlling the temperature *in situ*. Capillary electrophoresis (CE) techniques include proven methodologies for determining

features of antibody stability. One can use an iCE approach to resolve antibody protein charge variants due to deamidation, C-terminal lysines, sialylation, oxidation, glycosylation, and any other change to the protein that can result in a change in pI of the protein. Each of the expressed antibody proteins can be evaluated by high throughput, free solution isoelectric focusing (IEF) in a capillary column (cIEF), using a Protein Simple Maurice instrument. Whole-column UV absorption detection can be performed every 30 seconds for real time monitoring of molecules focusing at the isoelectric points (pIs). This approach combines the high resolution of traditional gel IEF with the advantages of quantitation and automation found in column-based separations while eliminating the need for a mobilization step. The technique yields reproducible, quantitative analysis of identity, purity, and heterogeneity profiles for the expressed antibodies. The results identify charge heterogeneity and molecular sizing on the antibodies, with both absorbance and native fluorescence detection modes and with sensitivity of detection down to 0.7 µg/mL.

Solubility. One can determine the intrinsic solubility score of antibody sequences. The intrinsic solubility scores can be calculated using CamSol Intrinsic (Sormanni *et al.*, *J Mol Biol* 427, 478-490, 2015). The amino acid sequences for residues 95-102 (Kabat numbering) in HCDR3 of each antibody fragment such as a scFv can be evaluated via the online program to calculate the solubility scores. One also can determine solubility using laboratory techniques. Various techniques exist, including addition of lyophilized protein to a solution until the solution becomes saturated and the solubility limit is reached, or concentration by ultrafiltration in a microconcentrator with a suitable molecular weight cut-off. The most straightforward method is induction of amorphous precipitation, which measures protein solubility using a method involving protein precipitation using ammonium sulfate (Trevino *et al.*, *J Mol Biol*, 366: 449-460, 2007). Ammonium sulfate precipitation gives quick and accurate information on relative solubility values. Ammonium sulfate precipitation produces precipitated solutions with well-defined aqueous and solid phases and requires relatively small amounts of protein. Solubility measurements performed using induction of amorphous precipitation by ammonium sulfate also can be done easily at different pH values. Protein solubility is highly pH dependent, and pH is considered the most important extrinsic factor that affects solubility.

Autoreactivity. Generally, it is thought that autoreactive clones should be eliminated during ontogeny by negative selection; however it has become clear that many human naturally occurring antibodies with autoreactive properties persist in adult mature repertoires, and the autoreactivity may enhance the antiviral function of many antibodies to pathogens. It has been

noted that HCDR3 loops in antibodies during early B cell development are often rich in positive charge and exhibit autoreactive patterns (Wardemann *et al.*, *Science* 301, 1374-1377, 2003). One can test a given antibody for autoreactivity by assessing the level of binding to human origin cells in microscopy (using adherent HeLa or HEp-2 epithelial cells) and flow cytometric cell surface staining (using suspension Jurkat T cells and 293S human embryonic kidney cells). Autoreactivity also can be surveyed using assessment of binding to tissues in tissue arrays.

Preferred residues (“Human Likeness”). B cell repertoire deep sequencing of human B cells from blood donors is being performed on a wide scale in many recent studies. Sequence information about a significant portion of the human antibody repertoire facilitates statistical assessment of antibody sequence features common in healthy humans. With knowledge about the antibody sequence features in a human recombined antibody variable gene reference database, the position specific degree of “Human Likeness” (HL) of an antibody sequence can be estimated. HL has been shown to be useful for the development of antibodies in clinical use, like therapeutic antibodies or antibodies as vaccines. The goal is to increase the human likeness of antibodies to reduce potential adverse effects and anti-antibody immune responses that will lead to significantly decreased efficacy of the antibody drug or can induce serious health implications. One can assess antibody characteristics of the combined antibody repertoire of three healthy human blood donors of about 400 million sequences in total and created a novel “relative Human Likeness” (rHL) score that focuses on the hypervariable region of the antibody. The rHL score allows one to easily distinguish between human (positive score) and non-human sequences (negative score). Antibodies can be engineered to eliminate residues that are not common in human repertoires.

D. Single Chain Antibodies

A single chain variable fragment (scFv) is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short (usually serine, glycine) linker. This chimeric molecule retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide. This modification usually leaves the specificity unaltered. These molecules were created historically to facilitate phage display where it is highly convenient to express the antigen binding domain as a single peptide. Alternatively, scFv can be created directly from subcloned heavy and light chains derived from a hybridoma or B cell. Single chain variable fragments lack the constant Fc region found in complete antibody molecules, and thus, the common binding sites (*e.g.*, protein A/G)

used to purify antibodies. These fragments can often be purified/immobilized using Protein L since Protein L interacts with the variable region of kappa light chains.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alanine, serine and glycine. However, other residues can function as well. Tang *et al.* (1996) used phage display as a means of rapidly selecting tailored linkers for single-chain antibodies (scFvs) from protein linker libraries. A random linker library was constructed in which the genes for the heavy and light chain variable domains were linked by a segment encoding an 18-amino acid polypeptide of variable composition. The scFv repertoire (approx. 5×10^6 different members) was displayed on filamentous phage and subjected to affinity selection with hapten. The population of selected variants exhibited significant increases in binding activity but retained considerable sequence diversity. Screening 1054 individual variants subsequently yielded a catalytically active scFv that was produced efficiently in soluble form. Sequence analysis revealed a conserved proline in the linker two residues after the V_H C terminus and an abundance of arginines and prolines at other positions as the only common features of the selected tethers.

The recombinant antibodies of the present disclosure may also involve sequences or moieties that permit dimerization or multimerization of the receptors. Such sequences include those derived from IgA, which permit formation of multimers in conjunction with the J-chain. Another multimerization domain is the Gal4 dimerization domain. In other embodiments, the chains may be modified with agents such as biotin/avidin, which permit the combination of two antibodies.

In a separate embodiment, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus of the light chain via an appropriate chemical bridge).

Cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules, *e.g.*, a stabilizing and coagulating agent. However, it is contemplated that dimers or multimers of the same analog or heteromeric complexes comprised of different analogs can be created. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (*e.g.*, N-hydroxy succinimide) and the other reacting with a thiol group (*e.g.*, pyridyl disulfide, maleimides, halogens, *etc.*). Through the primary amine

reactive group, the cross-linker may react with the lysine residue(s) of one protein (*e.g.*, the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (*e.g.*, the selective agent).

5 It is preferred that a cross-linker having reasonable stability in blood will be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*, preventing release of the targeting peptide prior to reaching the site of action. These linkers are thus one
10 group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is “sterically hindered” by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood,
15 and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (*e.g.*, the epsilon amino group of lysine). Another possible type of cross-linker includes the
20 hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in
25 accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates
30 of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent

of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent. Particular uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

E. Multispecific Antibodies

In certain embodiments, antibodies of the present disclosure are bispecific or multispecific. Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of a single antigen. Other such antibodies may combine a first antigen binding site with a binding site for a second antigen. Alternatively, an anti-pathogen arm may be combined with an arm that binds to a triggering molecule on a leukocyte, such as a T-cell receptor molecule (*e.g.*, CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and Fc gamma RIII (CD16), so as to focus and localize cellular defense mechanisms to the infected cell. Bispecific antibodies may also be used to localize cytotoxic agents to infected cells. These antibodies possess a pathogen-binding arm and an arm that binds the cytotoxic agent (*e.g.*, saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies). WO 96/16673 describes a bispecific anti-ErbB2/anti-Fc gamma RIII antibody and U.S. Pat. No. 5,837,234 discloses a bispecific anti-ErbB2/anti-Fc gamma RI antibody. A bispecific anti-ErbB2/Fc alpha antibody is shown in WO98/02463. U.S. Pat. No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather

cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, EMBO J., 10:3655-3659 (1991).

According to a different approach, antibody variable regions with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_{H2}, and C_{H3} regions. It is preferred to have the first heavy-chain constant region (C_{H1}) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant effect on the yield of the desired chain combination.

In a particular embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 121:210 (1986).

According to another approach described in U.S. Pat. No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_{H3} domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a

mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Techniques exist that facilitate the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, J. Exp. Med., 175: 217-225 (1992) describe the production of a humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described (Merchant *et al.*, Nat. Biotechnol., 16, 677-681 (1998). doi:10.1038/nbt0798-677pmid.9661204). For example, bispecific antibodies have been produced using leucine zippers (Kostelny *et al.*, J. Immunol., 148(5):1547-1553, 1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form

the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a V_H connected to a V_L by a linker that is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, J. Immunol., 152:5368 (1994).

In a particular embodiment, a bispecific or multispecific antibody may be formed as a DOCK-AND-LOCK™ (DNL™) complex (see, *e.g.*, U.S. Pat. Nos. 7,521,056; 7,527,787; 7,534,866; 7,550,143 and 7,666,400, the Examples section of each of which is incorporated herein by reference.) Generally, the technique takes advantage of the specific and high-affinity binding interactions that occur between a dimerization and docking domain (DDD) sequence of the regulatory (R) subunits of cAMP-dependent protein kinase (PKA) and an anchor domain (AD) sequence derived from any of a variety of AKAP proteins (Baillie *et al.*, *FEBS Letters*, 2005; 579: 3264; Wong and Scott, *Nat. Rev. Mol. Cell Biol.* 2004; 5: 959). The DDD and AD peptides may be attached to any protein, peptide or other molecule. Because the DDD sequences spontaneously dimerize and bind to the AD sequence, the technique allows the formation of complexes between any selected molecules that may be attached to DDD or AD sequences.

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared (Tutt *et al.*, J. Immunol. 147: 60, 1991; Xu *et al.*, *Science*, 358(6359):85-90, 2017). A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present disclosure can be multivalent antibodies with three or more antigen binding sites (*e.g.*, tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide

chains), wherein the polypeptide chain(s) comprise two or more variable regions. For instance, the polypeptide chain(s) may comprise $VD1-(X1)_{sub.n}-VD2-(X2)_n-Fc$, wherein VD1 is a first variable region, VD2 is a second variable region, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable region polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable region polypeptides. The light chain variable region polypeptides contemplated here comprise a light chain variable region and, optionally, further comprise a C_L domain.

Charge modifications are particularly useful in the context of a multispecific antibody, where amino acid substitutions in Fab molecules result in reducing the mispairing of light chains with non-matching heavy chains (Bence-Jones-type side products), which can occur in the production of Fab-based bi-/multispecific antigen binding molecules with a VH/VL exchange in one (or more, in case of molecules comprising more than two antigen-binding Fab molecules) of their binding arms (see also PCT publication no. WO 2015/150447, particularly the examples therein, incorporated herein by reference in its entirety).

Accordingly, in particular embodiments, an antibody comprised in the therapeutic agent comprises

- (a) a first Fab molecule which specifically binds to a first antigen
 - (b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other,
- wherein the first antigen is an activating T cell antigen and the second antigen is a target cell antigen, or the first antigen is a target cell antigen and the second antigen is an activating T cell antigen; and
- wherein
- i) in the constant domain C_L of the first Fab molecule under a) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index); or
 - ii) in the constant domain C_L of the second Fab molecule under b) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to

Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index).

The antibody may not comprise both modifications mentioned under i) and ii). The constant domains CL and CH1 of the second Fab molecule are not replaced by each other (*i.e.*, remain unexchanged).

In another embodiment of the antibody, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering

according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In an even more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

F. Chimeric Antigen Receptors

Artificial T cell receptors (also known as chimeric T cell receptors, chimeric immunoreceptors, chimeric antigen receptors (CARs)) are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell, with transfer of their coding sequence facilitated by retroviral vectors. In this way, a large number of target-specific T cells can be generated for adoptive cell transfer. Phase I clinical studies of this approach show efficacy.

The most common form of these molecules are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, fused to CD3-zeta transmembrane and endodomain. Such molecules result in the transmission of a zeta signal in response to recognition by the scFv of its target. An example of such a construct is 14g2a-Zeta, which is a fusion of a scFv derived from hybridoma 14g2a (which recognizes disialoganglioside GD2). When T cells express this molecule (usually achieved by oncoretroviral vector transduction), they recognize and kill target cells that express GD2 (*e.g.*, neuroblastoma cells). To target malignant B cells, investigators have redirected the specificity of T cells using a chimeric immunoreceptor specific for the B-lineage molecule, CD19.

The variable portions of an immunoglobulin heavy and light chain are fused by a flexible linker to form a scFv. This scFv is preceded by a signal peptide to direct the nascent protein to the endoplasmic reticulum and subsequent surface expression (this is cleaved). A flexible spacer allows to the scFv to orient in different directions to enable antigen binding. The transmembrane domain is a typical hydrophobic alpha helix usually derived from the original molecule of the signaling endodomain which protrudes into the cell and transmits the desired signal.

Type I proteins are in fact two protein domains linked by a transmembrane alpha helix in between. The cell membrane lipid bilayer, through which the transmembrane domain passes, acts to isolate the inside portion (endodomain) from the external portion (ectodomain). It is not so surprising that attaching an ectodomain from one protein to an endodomain of another protein results in a molecule that combines the recognition of the former to the signal of the latter.

Ectodomain. A signal peptide directs the nascent protein into the endoplasmic reticulum. This is essential if the receptor is to be glycosylated and anchored in the cell membrane. Any eukaryotic signal peptide sequence usually works fine. Generally, the signal peptide natively attached to the amino-terminal most component is used (*e.g.*, in a scFv with orientation light chain - linker - heavy chain, the native signal of the light-chain is used

The antigen recognition domain is usually an scFv. There are however many alternatives. An antigen recognition domain from native T-cell receptor (TCR) alpha and beta single chains have been described, as have simple ectodomains (*e.g.*, CD4 ectodomain to recognize HIV infected cells) and more exotic recognition components such as a linked cytokine (which leads to recognition of cells bearing the cytokine receptor). In fact, almost anything that binds a given target with high affinity can be used as an antigen recognition region.

A spacer region links the antigen binding domain to the transmembrane domain. It should be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. The simplest form is the hinge region from IgG1. Alternatives include the CH₂CH₃ region of immunoglobulin and portions of CD3. For most scFv based constructs, the IgG1 hinge suffices. However, the best spacer often has to be determined empirically.

Transmembrane domain. The transmembrane domain is a hydrophobic alpha helix that spans the membrane. Generally, the transmembrane domain from the most membrane proximal component of the endodomain is used. Interestingly, using the CD3-zeta transmembrane domain may result in incorporation of the artificial TCR into the native TCR a factor that is dependent on the presence of the native CD3-zeta transmembrane charged aspartic acid residue. Different transmembrane domains result in different receptor stability. The CD28 transmembrane domain results in a brightly expressed, stable receptor.

Endodomain. This is the "business-end" of the receptor. After antigen recognition, receptors cluster and a signal is transmitted to the cell. The most commonly used endodomain component is CD3-zeta which contains 3 ITAMs. This transmits an activation signal to the T

cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling is needed.

"First-generation" CARs typically had the intracellular domain from the CD3 ζ - chain, which is the primary transmitter of signals from endogenous TCRs. "Second-generation" CARs add intracellular signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. Preclinical studies have indicated that the second generation of CAR designs improves the antitumor activity of T cells. More recent, "third-generation" CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, to further augment potency.

G. ADCs

Antibody Drug Conjugates or ADCs are a new class of highly potent biopharmaceutical drugs designed as a targeted therapy for the treatment of people with infectious disease. ADCs are complex molecules composed of an antibody (a whole mAb or an antibody fragment such as a single-chain variable fragment, or scFv) linked, via a stable chemical linker with labile bonds, to a biological active cytotoxic/anti-viral payload or drug. Antibody Drug Conjugates are examples of bioconjugates and immunoconjugates.

By combining the unique targeting capabilities of monoclonal antibodies with the cancer-killing ability of cytotoxic drugs, antibody-drug conjugates allow sensitive discrimination between healthy and diseased tissue. This means that, in contrast to traditional systemic approaches, antibody-drug conjugates target and attack the infected cell so that healthy cells are less severely affected.

In the development ADC-based anti-tumor therapies, an anticancer drug (e.g., a cell toxin or cytotoxin) is coupled to an antibody that specifically targets a certain cell marker (e.g., a protein that, ideally, is only to be found in or on infected cells). Antibodies track these proteins down in the body and attach themselves to the surface of cancer cells. The biochemical reaction between the antibody and the target protein (antigen) triggers a signal in the tumor cell, which then absorbs or internalizes the antibody together with the cytotoxin. After the ADC is internalized, the cytotoxic drug is released and kills the cell or impairs viral replication. Due to this targeting, ideally the drug has lower side effects and gives a wider therapeutic window than other agents.

A stable link between the antibody and cytotoxic/anti-viral agent is a crucial aspect of an ADC. Linkers are based on chemical motifs including disulfides, hydrazones or peptides (cleavable), or thioethers (noncleavable) and control the distribution and delivery of the

cytotoxic agent to the target cell. Cleavable and noncleavable types of linkers have been proven to be safe in preclinical and clinical trials. Brentuximab vedotin includes an enzyme-sensitive cleavable linker that delivers the potent and highly toxic antimicrotubule agent Monomethyl auristatin E or MMAE, a synthetic antineoplastic agent, to human specific CD30-positive malignant cells. Because of its high toxicity MMAE, which inhibits cell division by blocking the polymerization of tubulin, cannot be used as a single-agent chemotherapeutic drug. However, the combination of MMAE linked to an anti-CD30 monoclonal antibody (cAC10, a cell membrane protein of the tumor necrosis factor or TNF receptor) proved to be stable in extracellular fluid, cleavable by cathepsin and safe for therapy. Trastuzumab emtansine, the other approved ADC, is a combination of the microtubule-formation inhibitor mertansine (DM-1), a derivative of the Maytansine, and antibody trastuzumab (Herceptin®/Genentech/Roche) attached by a stable, non-cleavable linker.

The availability of better and more stable linkers has changed the function of the chemical bond. The type of linker, cleavable or noncleavable, lends specific properties to the cytotoxic (anti-cancer) drug. For example, a non-cleavable linker keeps the drug within the cell. As a result, the entire antibody, linker and cytotoxic agent enter the targeted cancer cell where the antibody is degraded to the level of an amino acid. The resulting complex – amino acid, linker and cytotoxic agent – now becomes the active drug. In contrast, cleavable linkers are catalyzed by enzymes in the host cell where it releases the cytotoxic agent.

Another type of cleavable linker, currently in development, adds an extra molecule between the cytotoxic/anti-viral drug and the cleavage site. This linker technology allows researchers to create ADCs with more flexibility without worrying about changing cleavage kinetics. Researchers are also developing a new method of peptide cleavage based on Edman degradation, a method of sequencing amino acids in a peptide. Future direction in the development of ADCs also include the development of site-specific conjugation (TDCs) to further improve stability and therapeutic index and α emitting immunoconjugates and antibody-conjugated nanoparticles.

H. BiTES

Bi-specific T-cell engagers (BiTEs) are a class of artificial bispecific monoclonal antibodies that are investigated for the use as anti-cancer drugs. They direct a host's immune

system, more specifically the T cells' cytotoxic activity, against infected cells. *BiTE* is a registered trademark of Micromet AG.

BiTEs are fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies, or amino acid sequences from four different genes, on a single peptide chain of about 55 kilodaltons. One of the scFvs binds to T cells via the CD3 receptor, and the other to an infected cell via a specific molecule.

Like other bispecific antibodies, and unlike ordinary monoclonal antibodies, BiTEs form a link between T cells and target cells. This causes T cells to exert cytotoxic/anti-viral activity on infected cells by producing proteins like perforin and granzymes, independently of the presence of MHC I or co-stimulatory molecules. These proteins enter infected cells and initiate the cell's apoptosis. This action mimics physiological processes observed during T cell attacks against infected cells.

I. Intrabodies

In a particular embodiment, the antibody is a recombinant antibody that is suitable for action inside of a cell – such antibodies are known as “intrabodies.” These antibodies may interfere with target function by a variety of mechanism, such as by altering intracellular protein trafficking, interfering with enzymatic function, and blocking protein-protein or protein-DNA interactions. In many ways, their structures mimic or parallel those of single chain and single domain antibodies, discussed above. Indeed, single-transcript/single-chain is an important feature that permits intracellular expression in a target cell, and also makes protein transit across cell membranes more feasible. However, additional features are required.

The two major issues impacting the implementation of intrabody therapeutic are delivery, including cell/tissue targeting, and stability. With respect to delivery, a variety of approaches have been employed, such as tissue-directed delivery, use of cell-type specific promoters, viral-based delivery and use of cell-permeability/membrane translocating peptides. With respect to the stability, the approach is generally to either screen by brute force, including methods that involve phage display and may include sequence maturation or development of consensus sequences, or more directed modifications such as insertion stabilizing sequences (*e.g.*, Fc regions, chaperone protein sequences, leucine zippers) and disulfide replacement/modification.

An additional feature that intrabodies may require is a signal for intracellular targeting. Vectors that can target intrabodies (or other proteins) to subcellular regions such as the

cytoplasm, nucleus, mitochondria and ER have been designed and are commercially available (Invitrogen Corp.; Persic *et al.*, 1997).

By virtue of their ability to enter cells, intrabodies have additional uses that other types of antibodies may not achieve. In the case of the present antibodies, the ability to interact with the MUC1 cytoplasmic domain in a living cell may interfere with functions associated with the MUC1 CD, such as signaling functions (binding to other molecules) or oligomer formation. In particular, it is contemplated that such antibodies can be used to inhibit MUC1 dimer formation.

J. Purification

In certain embodiments, the antibodies of the present disclosure may be purified. The term “purified,” as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein is purified to any degree relative to its naturally-obtainable state. A purified protein therefore also refers to a protein, free from the environment in which it may naturally occur. Where the term “substantially purified” is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Other methods for protein purification include, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; gel filtration, reverse phase, hydroxylapatite and affinity chromatography; and combinations of such and other techniques.

In purifying an antibody of the present disclosure, it may be desirable to express the polypeptide in a prokaryotic or eukaryotic expression system and extract the protein using denaturing conditions. The polypeptide may be purified from other cellular components using an affinity column, which binds to a tagged portion of the polypeptide. As is generally known in the art, it is believed that the order of conducting the various purification steps may be

changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Commonly, complete antibodies are fractionated utilizing agents (*i.e.*, protein A) that bind the Fc portion of the antibody. Alternatively, antigens may be used to simultaneously
5 purify and select appropriate antibodies. Such methods often utilize the selection agent bound to a support, such as a column, filter or bead. The antibodies are bound to a support, contaminants removed (*e.g.*, washed away), and the antibodies released by applying conditions (salt, heat, *etc.*).

Various methods for quantifying the degree of purification of the protein or peptide will
10 be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. Another method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity. The actual units used to represent the
15 amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that
20 under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

III. Active/Passive Immunization and Treatment/Prevention of Norovirus Infection

A. Formulation and Administration

25 The present disclosure provides pharmaceutical compositions comprising anti-norovirus antibodies and antigens for generating the same. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody or a fragment thereof, or a peptide immunogen, and a pharmaceutically acceptable carrier. In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or
30 a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the

like. Water is a particular carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Other suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical agents are described in "Remington's Pharmaceutical Sciences." Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration, which can be oral, intravenous, intraarterial, intrabuccal, intranasal, nebulized, bronchial inhalation, intra-rectal, vaginal, topical or delivered by mechanical ventilation.

Active vaccines are also envisioned where antibodies like those disclosed are produced *in vivo* in a subject at risk of norovirus infection. Such vaccines can be formulated for parenteral administration, *e.g.*, formulated for injection *via* the intradermal, intravenous, intramuscular, subcutaneous, or even intraperitoneal routes. Administration by intradermal and intramuscular routes are contemplated. The vaccine could alternatively be administered by a topical route directly to the mucosa, for example by nasal drops, inhalation, by nebulizer, or via intrarectal or vaginal delivery. Pharmaceutically acceptable salts, include the acid salts and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Passive transfer of antibodies, known as artificially acquired passive immunity, generally will involve the use of intravenous or intramuscular injections. The forms of antibody can be human or animal blood plasma or serum, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, as high-titer human IVIG or IG from immunized

or from donors recovering from disease, and as monoclonal antibodies (MAb). Such immunity generally lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. However, passive immunity provides immediate protection. The antibodies will be formulated in a carrier suitable for injection, *i.e.*, sterile and syringeable.

Generally, the ingredients of compositions of the disclosure are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

B. ADCC

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or fragments thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. By “antibody having increased/reduced antibody dependent cell-mediated cytotoxicity (ADCC)” is meant an antibody having increased/reduced ADCC as determined by any suitable method known to those of ordinary skill in the art.

As used herein, the term “increased/reduced ADCC” is defined as either an increase/reduction in the number of target cells that are lysed in a given time, at a given concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or a reduction/increase in the concentration of antibody, in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The increase/reduction in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard production, purification, formulation and storage methods (which are known

to those skilled in the art), but that has not been engineered. For example the increase in ADCC mediated by an antibody produced by host cells engineered to have an altered pattern of glycosylation (*e.g.*, to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein, is relative to the ADCC mediated by the same antibody
5 produced by the same type of non-engineered host cells.

C. CDC

Complement-dependent cytotoxicity (CDC) is a function of the complement system. It is the processes in the immune system that kill pathogens by damaging their membranes
10 without the involvement of antibodies or cells of the immune system. There are three main processes. All three insert one or more membrane attack complexes (MAC) into the pathogen which cause lethal colloid-osmotic swelling, *i.e.*, CDC. It is one of the mechanisms by which antibodies or antibody fragments have an anti-viral effect.

15 IV. Antibody Conjugates

Antibodies of the present disclosure may be linked to at least one agent to form an antibody conjugate. In order to increase the efficacy of antibody molecules as diagnostic or therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one
20 effector or reporter molecule. Effector molecules comprise molecules having a desired activity, *e.g.*, cytotoxic activity. Non-limiting examples of effector molecules which have been attached to antibodies include toxins, anti-tumor agents, therapeutic enzymes, radionuclides, antiviral agents, chelating agents, cytokines, growth factors, and oligo- or polynucleotides. By contrast, a reporter molecule is defined as any moiety which may be detected using an assay. Non-
25 limiting examples of reporter molecules which have been conjugated to antibodies include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, photoaffinity molecules, colored particles or ligands, such as biotin.

Antibody conjugates are generally preferred for use as diagnostic agents. Antibody
30 diagnostics generally fall within two classes, those for use in *in vitro* diagnostics, such as in a variety of immunoassays, and those for use *in vivo* diagnostic protocols, generally known as "antibody-directed imaging." Many appropriate imaging agents are known in the art, as are methods for their attachment to antibodies (see, for *e.g.*, U.S. Patents 5,021,236, 4,938,948,

and 4,472,509). The imaging moieties used can be paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II),
5 neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one
10 might mention astatine²¹¹, ¹⁴carbon, ⁵¹chromium, ³⁶chlorine, ⁵⁷cobalt, ⁵⁸cobalt, copper⁶⁷, ¹⁵²Eu, gallium⁶⁷, ³hydrogen, iodine¹²³, iodine¹²⁵, iodine¹³¹, indium¹¹¹, ⁵⁹iron, ³²phosphorus, rhenium¹⁸⁶, rhenium¹⁸⁸, ⁷⁵selenium, ³⁵sulphur, technetium^{99m} and/or yttrium⁹⁰. ¹²⁵I is often being preferred for use in certain embodiments, and technetium^{99m} and/or indium¹¹¹ are also often preferred due to their low energy and suitability for long range detection. Radioactively labeled
15 monoclonal antibodies of the present disclosure may be produced according to well-known methods in the art. For instance, monoclonal antibodies can be iodinated by contact with sodium and/or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Monoclonal antibodies according to the disclosure may be labeled with technetium^{99m} by ligand exchange process, for example, by
20 reducing pertechnetate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column. Alternatively, direct labeling techniques may be used, *e.g.*, by incubating pertechnetate, a reducing agent such as SnCl_2 , a buffer solution such as sodium-potassium phthalate solution, and the antibody. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to antibody are
25 diethylenetriaminepentaacetic acid (DTPA) or ethylene diaminetetracetic acid (EDTA).

Among the fluorescent labels contemplated for use as conjugates include Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG,
30 Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

Additional types of antibodies contemplated in the present disclosure are those intended primarily for use *in vitro*, where the antibody is linked to a secondary binding ligand and/or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a

chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase or glucose oxidase. Preferred secondary binding ligands are biotin and avidin and streptavidin compounds. The use of such labels is well known to those of skill in the art and are described, for example, in U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241.

Yet another known method of site-specific attachment of molecules to antibodies comprises the reaction of antibodies with hapten-based affinity labels. Essentially, hapten-based affinity labels react with amino acids in the antigen binding site, thereby destroying this site and blocking specific antigen reaction. However, this may not be advantageous since it results in loss of antigen binding by the antibody conjugate.

Molecules containing azido groups may also be used to form covalent bonds to proteins through reactive nitrene intermediates that are generated by low intensity ultraviolet light (Potter and Haley, 1983). In particular, 2- and 8-azido analogues of purine nucleotides have been used as site-directed photoprobes to identify nucleotide binding proteins in crude cell extracts (Owens & Haley, 1987; Atherton *et al.*, 1985). The 2- and 8-azido nucleotides have also been used to map nucleotide binding domains of purified proteins (Khatoon *et al.*, 1989; King *et al.*, 1989; Dholakia *et al.*, 1989) and may be used as antibody binding agents.

Several methods are known in the art for the attachment or conjugation of an antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3 α -6 α -diphenylglycouril-3 attached to the antibody (U.S. Patents 4,472,509 and 4,938,948). Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate. In U.S. Patent 4,938,948, imaging of breast tumors is achieved using monoclonal antibodies and the detectable imaging moieties are bound to the antibody using linkers such as methyl-p-hydroxybenzimidate or N-succinimidyl-3-(4-hydroxyphenyl)propionate.

In other embodiments, derivatization of immunoglobulins by selectively introducing sulfhydryl groups in the Fc region of an immunoglobulin, using reaction conditions that do not alter the antibody combining site are contemplated. Antibody conjugates produced according to this methodology are disclosed to exhibit improved longevity, specificity and sensitivity (U.S. Patent 5,196,066, incorporated herein by reference). Site-specific attachment of effector

or reporter molecules, wherein the reporter or effector molecule is conjugated to a carbohydrate residue in the Fc region have also been disclosed in the literature (O'Shannessy *et al.*, 1987). This approach has been reported to produce diagnostically and therapeutically promising antibodies which are currently in clinical evaluation.

5

V. Immunodetection Methods

In still further embodiments, the present disclosure concerns immunodetection methods for binding, purifying, removing, quantifying and otherwise generally detecting norovirus and its associated antigens. While such methods can be applied in a traditional sense, another use will be in quality control and monitoring of vaccine and other virus stocks, where antibodies according to the present disclosure can be used to assess the amount or integrity (*i.e.*, long term stability) of antigens in viruses. Alternatively, the methods may be used to screen various antibodies for appropriate/desired reactivity profiles.

Other immunodetection methods include specific assays for determining the presence of norovirus in a subject. A wide variety of assay formats are contemplated, but specifically those that would be used to detect norovirus in a fluid obtained from a subject, such as saliva, blood, plasma, sputum, semen or urine. In particular, semen has been demonstrated as a viable sample for detecting norovirus (Purpura *et al.*, 2016; Mansuy *et al.*, 2016; Barzon *et al.*, 2016; Gornet *et al.*, 2016; Duffy *et al.*, 2009; CDC, 2016; Halfon *et al.*, 2010; Elder *et al.* 2005). The assays may be advantageously formatted for non-healthcare (home) use, including lateral flow assays (see below) analogous to home pregnancy tests. These assays may be packaged in the form of a kit with appropriate reagents and instructions to permit use by the subject of a family member.

Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. In particular, a competitive assay for the detection and quantitation of norovirus antibodies directed to specific parasite epitopes in samples also is provided. The steps of various useful immunodetection methods have been described in the scientific literature, such as, *e.g.*, Doolittle and Ben-Zeev (1999), Gulbis and Galand (1993), De Jager *et al.* (1993), and Nakamura *et al.* (1987). In general, the immunobinding methods include obtaining a sample suspected of containing norovirus, and contacting the sample with a first antibody in accordance with the present disclosure, as the case may be, under conditions effective to allow the formation of immunocomplexes.

These methods include methods for purifying norovirus or related antigens from a sample. The antibody will preferably be linked to a solid support, such as in the form of a column matrix, and the sample suspected of containing the norovirus or antigenic component will be applied to the immobilized antibody. The unwanted components will be washed from the column, leaving the norovirus antigen immunocomplexed to the immobilized antibody, which is then collected by removing the organism or antigen from the column.

The immunobinding methods also include methods for detecting and quantifying the amount of norovirus or related components in a sample and the detection and quantification of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing norovirus or its antigens and contact the sample with an antibody that binds norovirus or components thereof, followed by detecting and quantifying the amount of immune complexes formed under the specific conditions. In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing norovirus or norovirus antigen, such as a tissue section or specimen, a homogenized tissue extract, a biological fluid, including blood and serum, or a secretion, such as feces or urine.

Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the antibody composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, *i.e.*, to bind to norovirus or antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. Patents concerning the use of such labels include U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second

binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an antibody, which may thus be termed a “secondary” antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

Further methods include the detection of primary immune complexes by a two-step approach. A second binding ligand, such as an antibody that has binding affinity for the antibody, is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if this is desired.

One method of immunodetection uses two different antibodies. A first biotinylated antibody is used to detect the target antigen, and a second antibody is then used to detect the biotin attached to the complexed biotin. In that method, the sample to be tested is first incubated in a solution containing the first step antibody. If the target antigen is present, some of the antibody binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin), biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution containing the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced which is macroscopically visible.

Another known method of immunodetection takes advantage of the immuno-PCR (Polymerase Chain Reaction) methodology. The PCR method is similar to the Cantor method up to the incubation with biotinylated DNA, however, instead of using multiple rounds of streptavidin and biotinylated DNA incubation, the DNA/biotin/streptavidin/antibody complex

is washed out with a low pH or high salt buffer that releases the antibody. The resulting wash solution is then used to carry out a PCR reaction with suitable primers with appropriate controls. At least in theory, the enormous amplification capability and specificity of PCR can be utilized to detect a single antigen molecule.

5

A. ELISAs

Immunoassays, in their most simple and direct sense, are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. However, it will be readily appreciated that detection is not limited to such techniques, and western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the antibodies of the disclosure are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the norovirus or norovirus antigen is added to the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection may be achieved by the addition of another anti-norovirus antibody that is linked to a detectable label. This type of ELISA is a simple “sandwich ELISA.” Detection may also be achieved by the addition of a second anti-norovirus antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing the norovirus or norovirus antigen are immobilized onto the well surface and then contacted with the anti-norovirus antibodies of the disclosure. After binding and washing to remove non-specifically bound immune complexes, the bound anti-norovirus antibodies are detected. Where the initial anti-norovirus antibodies are linked to a detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has binding affinity for the first anti-norovirus antibody, with the second antibody being linked to a detectable label.

Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating and binding, washing to remove non-specifically bound species, and detecting the bound immune complexes. These are described below.

In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period

of hours. The wells of the plate will then be washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then “coated” with a nonspecific protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein or solutions of milk powder. The coating allows for blocking of nonspecific
5 adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

In ELISAs, it is probably more customary to use a secondary or tertiary detection means rather than a direct procedure. Thus, after binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material,
10 the immobilizing surface is contacted with the biological sample to be tested under conditions effective to allow immune complex (antigen/antibody) formation. Detection of the immune complex then requires a labeled secondary binding ligand or antibody, and a secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or a third binding ligand.

“Under conditions effective to allow immune complex (antigen/antibody) formation”
15 means that the conditions preferably include diluting the antigens and/or antibodies with solutions such as BSA, bovine gamma globulin (BGG) or phosphate buffered saline (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background.

The “suitable” conditions also mean that the incubation is at a temperature or for a
20 period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours or so, at temperatures preferably on the order of 25°C to 27°C, or may be overnight at about 4°C or so.

Following all incubation steps in an ELISA, the contacted surface is washed so as to remove non-complexed material. A preferred washing procedure includes washing with a
25 solution such as PBS/Tween, or borate buffer. Following the formation of specific immune complexes between the test sample and the originally bound material, and subsequent washing, the occurrence of even minute amounts of immune complexes may be determined.

To provide a detecting means, the second or third antibody will have an associated label to allow detection. Preferably, this will be an enzyme that will generate color development
30 upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact or incubate the first and second immune complex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immune complex formation (*e.g.*, incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, *e.g.*, by incubation with a chromogenic substrate such as urea, or bromocresol purple, or 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS), or H₂O₂, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generated, *e.g.*, using a visible spectra spectrophotometer.

In another embodiment, the present disclosure contemplates the use of competitive formats. This is particularly useful in the detection of norovirus antibodies in sample. In competition-based assays, an unknown amount of analyte or antibody is determined by its ability to displace a known amount of labeled antibody or analyte. Thus, the quantifiable loss of a signal is an indication of the amount of unknown antibody or analyte in a sample.

Here, the inventor proposes the use of labeled norovirus monoclonal antibodies to determine the amount of norovirus antibodies in a sample. The basic format would include contacting a known amount of norovirus monoclonal antibody (linked to a detectable label) with norovirus antigen or particle. The norovirus antigen or organism is preferably attached to a support. After binding of the labeled monoclonal antibody to the support, the sample is added and incubated under conditions permitting any unlabeled antibody in the sample to compete with, and hence displace, the labeled monoclonal antibody. By measuring either the lost label or the label remaining (and subtracting that from the original amount of bound label), one can determine how much non-labeled antibody is bound to the support, and thus how much antibody was present in the sample.

B. Western Blot

The Western blot (alternatively, protein immunoblot) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.

Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication. Cells may also be broken open by one of the above mechanical methods. However, it should be noted that bacteria, virus or environmental samples can be the source of protein and thus Western blotting is not restricted

to cellular studies only. Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturing.

5 The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to determine a protein. It is also possible to use a two-dimensional (2-D) gel which spreads the proteins from a single sample out in two
10 dimensions. Proteins are separated according to isoelectric point (pH at which they have neutral net charge) in the first dimension, and according to their molecular weight in the second dimension.

 In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or polyvinylidene difluoride (PVDF).

15 The membrane is placed on top of the gel, and a stack of filter papers placed on top of that. The entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it. Another method for transferring the proteins is called electroblotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization
20 they had within the gel. As a result of this blotting process, the proteins are exposed on a thin surface layer for detection (see below). Both varieties of membrane are chosen for their non-specific protein binding properties (*i.e.*, binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF but are far more fragile and do
25 not stand up well to repeated probings. The uniformity and overall effectiveness of transfer of protein from the gel to the membrane can be checked by staining the membrane with Coomassie Brilliant Blue or Ponceau S dyes. Once transferred, proteins are detected using labeled primary antibodies, or unlabeled primary antibodies followed by indirect detection using labeled protein A or secondary labeled antibodies binding to the Fc region of the primary
30 antibodies.

C. Lateral Flow Assays

 Lateral flow assays, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence (or absence) of a target analyte in sample (matrix)

without the need for specialized and costly equipment, though many laboratory-based applications exist that are supported by reading equipment. Typically, these tests are used as low resources medical diagnostics, either for home testing, point of care testing, or laboratory use. A widely spread and well-known application is the home pregnancy test.

5 The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid (*e.g.*, urine) spontaneously. The first element (the sample pad) acts as a sponge and holds an excess of sample fluid. Once soaked, the fluid migrates to the second element (conjugate pad) in which the manufacturer has stored the so-called conjugate, a dried format of bio-active particles (see
10 below) in a salt-sugar matrix that contains everything to guarantee an optimized chemical reaction between the target molecule (*e.g.*, an antigen) and its chemical partner (*e.g.*, antibody) that has been immobilized on the particle's surface. While the sample fluid dissolves the salt-sugar matrix, it also dissolves the particles and in one combined transport action the sample and conjugate mix while flowing through the porous structure. In this way, the analyte binds
15 to the particles while migrating further through the third capillary bed. This material has one or more areas (often called stripes) where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third 'capture' molecule binds the complex. After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes
20 color. Typically there are at least two stripes: one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine, the second contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized. After passing these reaction zones, the fluid enters the final porous material – the wick – that simply acts as a waste container. Lateral Flow Tests can operate as either
25 competitive or sandwich assays. Lateral flow assays are disclosed in U.S. Patent 6,485,982.

D. Immunohistochemistry

The antibodies of the present disclosure may also be used in conjunction with both fresh-frozen and/or formalin-fixed, paraffin-embedded tissue blocks prepared for study by
30 immunohistochemistry (IHC). The method of preparing tissue blocks from these particulate specimens has been successfully used in previous IHC studies of various prognostic factors

and is well known to those of skill in the art (Brown *et al.*, 1990; Abbondanzo *et al.*, 1990; Allred *et al.*, 1990).

Briefly, frozen-sections may be prepared by rehydrating 50 ng of frozen “pulverized” tissue at room temperature in phosphate buffered saline (PBS) in small plastic capsules; pelleting the particles by centrifugation; resuspending them in a viscous embedding medium (OCT); inverting the capsule and/or pelleting again by centrifugation; snap-freezing in -70°C isopentane; cutting the plastic capsule and/or removing the frozen cylinder of tissue; securing the tissue cylinder on a cryostat microtome chuck; and/or cutting 25-50 serial sections from the capsule. Alternatively, whole frozen tissue samples may be used for serial section cuttings.

Permanent-sections may be prepared by a similar method involving rehydration of the 50 mg sample in a plastic microfuge tube; pelleting; resuspending in 10% formalin for 4 hours fixation; washing/pelleting; resuspending in warm 2.5% agar; pelleting; cooling in ice water to harden the agar; removing the tissue/agar block from the tube; infiltrating and/or embedding the block in paraffin; and/or cutting up to 50 serial permanent sections. Again, whole tissue samples may be substituted.

E. Immunodetection Kits

In still further embodiments, the present disclosure concerns immunodetection kits for use with the immunodetection methods described above. As the antibodies may be used to detect norovirus or norovirus antigens, the antibodies may be included in the kit. The immunodetection kits will thus comprise, in suitable container means, a first antibody that binds to norovirus or norovirus antigen, and optionally an immunodetection reagent.

In certain embodiments, the norovirus antibody may be pre-bound to a solid support, such as a column matrix and/or well of a microtiter plate. The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated with or linked to the given antibody. Detectable labels that are associated with or attached to a secondary binding ligand are also contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody.

Further suitable immunodetection reagents for use in the present kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first antibody, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label. As noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present disclosure.

The kits may further comprise a suitably aliquoted composition of the norovirus or norovirus antigens, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay. The kits may contain antibody-label conjugates either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit. The components of the kits may be packaged either in aqueous media or in lyophilized form.

The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the antibody may be placed, or preferably, suitably aliquoted. The kits of the present disclosure will also typically include a means for containing the antibody, antigen, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

F. Vaccine and Antigen Quality Control Assays

The present disclosure also contemplates the use of antibodies and antibody fragments as described herein for use in assessing the antigenic integrity of a viral antigen in a sample. Biological medicinal products like vaccines differ from chemical drugs in that they cannot normally be characterized molecularly; antibodies are large molecules of significant complexity and have the capacity to vary widely from preparation to preparation. They are also administered to healthy individuals, including children at the start of their lives, and thus a strong emphasis must be placed on their quality to ensure, to the greatest extent possible, that they are efficacious in preventing or treating life-threatening disease, without themselves causing harm.

The increasing globalization in the production and distribution of vaccines has opened new possibilities to better manage public health concerns but has also raised questions about the equivalence and interchangeability of vaccines procured across a variety of sources. International standardization of starting materials, of production and quality control testing, and the setting of high expectations for regulatory oversight on the way these products are manufactured and used, have thus been the cornerstone for continued success. But it remains a field in constant change, and continuous technical advances in the field offer a promise of developing potent new weapons against the oldest public health threats, as well as new ones - malaria, pandemic influenza, and HIV, to name a few - but also put a great pressure on manufacturers, regulatory authorities, and the wider medical community to ensure that products continue to meet the highest standards of quality attainable.

Thus, one may obtain an antigen or vaccine from any source or at any point during a manufacturing process. The quality control processes may therefore begin with preparing a sample for an immunoassay that identifies binding of an antibody or fragment disclosed herein to a viral antigen. Such immunoassays are disclosed elsewhere in this document, and any of
5 these may be used to assess the structural/antigenic integrity of the antigen. Standards for finding the sample to contain acceptable amounts of antigenically correct and intact antigen may be established by regulatory agencies.

Another important embodiment where antigen integrity is assessed is in determining shelf-life and storage stability. Most medicines, including vaccines, can deteriorate over time.
10 Therefore, it is critical to determine whether, over time, the degree to which an antigen, such as in a vaccine, degrades or destabilizes such that it is no longer antigenic and/or capable of generating an immune response when administered to a subject. Again, standards for finding the sample to contain acceptable amounts of antigenically intact antigen may be established by regulatory agencies.

15 In certain embodiments, viral antigens may contain more than one protective epitope. In these cases, it may prove useful to employ assays that look at the binding of more than one antibody, such as 2, 3, 4, 5 or even more antibodies. These antibodies bind to closely related epitopes, such that they are adjacent or even overlap each other. On the other hand, they may represent distinct epitopes from disparate parts of the antigen. By examining the integrity of
20 multiple epitopes, a more complete picture of the antigen's overall integrity, and hence ability to generate a protective immune response, may be determined.

Antibodies and fragments thereof as described in the present disclosure may also be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective norovirus antibodies. Antibodies, antibody fragment, or variants and derivatives
25 thereof, as described in the present disclosure may also be used in a kit for monitoring vaccine manufacture with the desired immunogenicity.

VI. Examples

The following examples are included to demonstrate preferred embodiments. It should
30 be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of embodiments, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many

changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 - Materials and Methods

5 **Virus-like particles.** GII.4 Sydney 2012 virus-like particles (VLPs), based on strain AFV08795.1, were produced and purified, as previously described (Sapparapu *et al.*, 2016). Briefly, VP1 and VP2 capsid protein sequences were cloned into the transfer vector pVL1392 (Epoch Life Sciences, Inc.). The vector was co-transfected with a bacmid vector into Sf9 insect cells. Recombinant virus then was used to inoculate Sf9 cells. VLPs were purified from the culture supernatant using a cesium chloride cushion gradient. GII.4 VLP assembly was verified
10 visually using electron microscopy, and antigenicity was tested by western blot.

VLP binding assay. Antibody reactivity to GII.4 VLPs was tested using an indirect enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 1 µg/mL of GII.4 VLPs in PBS at 4°C overnight. Wells then were blocked with 5% nonfat dry milk in PBS
15 with 0.05% Tween-20 for 1 hour at room temperature. Purified antibodies were diluted serially in PBS and added to VLP-coated plates for 1 hour at room temperature. Microtiter plates were washed 3 times with PBS-0.05% Tween-20 in between each step. Antibodies that bound to VLPs were detected using horseradish peroxidase tagged anti-κ or -λ chain secondary antibodies (Southern Biotech) for 1 hour at room temperature. Plates were developed using the
20 ultra-TMB reagent (Pierce ThermoFisher) and stopped using sulfuric acid. Absorbance was measured at 450 nm using a BioTek Synergy HT Microplate Reader.

VLP-carbohydrate binding antibody blockade assay. Microtiter plates were coated with 10 µg/mL of pig gastric mucin (PGM) Type III (Sigma) in PBS for 4 hours at room temperature, and then were blocked overnight at 4°C in 5% nonfat dry milk in PBS with 0.05%
25 Tween-20. GII.4 Sydney 2012 VLPs (0.5 µg/mL) were pretreated with each mAb applied in serial 3-fold dilutions with decreasing concentrations. Complexes then were applied to PGM-coated plates for 1 hour at room temperature. Microtiter plates were washed 3 times with PBS-0.05% Tween-20 in between each step. Bound VLPs were detected using guinea pig serum containing anti-GII.4 Sydney 2012 polyclonal antibodies, followed by an alkaline phosphatase-conjugated anti-guinea pig IgG. Optical density was measured at 405 nm using a Synergy HT
30 Microplate Reader (BioTek).

Hemagglutination inhibition assay. Human type O+ red blood cells were purchased from Rockland Immunochemicals, Inc. Cells were pelleted at 500 x g for 10 minutes at 4°C

and washed twice with PBS without Ca^{2+} or Mg^{2+} . GII.4 Sydney 2012 VLPs (3.5 $\mu\text{g/mL}$) were pretreated with decreasing concentrations of each mAb, from 15 to 0.007 $\mu\text{g/mL}$, in PBS pH 5.5 and incubated at room temperature for 30 minutes. VLP-mAb complexes were added to an equal volume of 0.5% washed red blood cells in PBS pH 6.2 and incubated for 2 hours at 4°C in a 96-well V-bottom microtiter plate. The HAI titer was determined as the lowest concentration of antibody that completely inhibited hemagglutination.

Human subjects. The inventors studied otherwise healthy adult subjects with a history of acute gastroenteritis contracted during a HuNoV outbreak in North Carolina between February 27 and March 1, 2013. The cause of the outbreak was determined by the Orange County, NC health department to be a GII.4 Sydney 2012 norovirus strain. Subjects were recruited after recovery to donate a one-time peripheral blood sample. The research study was approved by the Vanderbilt University Medical Center Institutional Review Board; all subjects provided written informed consent prior to participation.

Peripheral blood mononuclear cell (PBMC) isolation and hybridoma generation. The inventors obtained PBMCs from heparinized blood by density gradient centrifugation using Ficoll-Histopaque from 7 donors who had recovered recently from natural infection with HuNoV. B cells were transformed with Epstein Barr virus substrain B95.8 in the presence of 2.5 $\mu\text{g/mL}$ of CpG10103, 10 $\mu\text{g/mL}$ of cyclosporine A, and 10 μM Chk2 inhibitor. Approximately 10^7 PBMCs were plated into a 384-well plate in transformation medium, and a week later were expanded into four 96-well plates containing irradiated human PBMCs as a feeder layer. After an additional 7 days of culture, the supernatants were screened by indirect ELISA for the presence of antibodies that bound to GII.4 Sydney 2012 VLPs. Antibodies that bound to GII.4 Sydney 2012 VLPs were detected using horseradish peroxidase tagged anti-human IgA or IgG secondary antibodies (Southern Biotech). Wells containing transformed B cells secreting anti-GII.4 Sydney 2012 VLP antibodies were fused with HMM2.5 myeloma cells using a CytoPulse Sciences Generator. After fusion, hybridomas were plated in selection medium containing 100 μM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine and 7 $\mu\text{g/mL}$ ouabain. After two weeks, hybridomas were screened for production of human antibodies reacting with GII.4 Sydney 2012 VLPs and then cloned biologically using single cell sorting on a FACS Aria III flow cytometer in the Vanderbilt Flow Cytometry Shared Resource.

Competition-binding assay. To identify groups of antibodies binding to similar antigenic sites on norovirus GII.4 Sydney 2012, the inventors performed biolayer interferometry using an Octet® Red96 or Octet® HTX biosensor system (FortéBio). The Octet®

HTX is a high-throughput biosensor system that was used to validate results obtained from the Octet® Red96 system. With both biosensor systems, antibodies and antigen were diluted in 1X kinetic buffer (FortéBio 18-5032). Glutathione S-transferase (GST)-tagged GII.4 Sydney 2012 P domain dimers were immobilized onto anti-GST biosensor tips (FortéBio 18-5096). The P domain dimers were coated onto the biosensor tip by immersing the tip in a solution containing dimers at a concentration of 5 µg/mL. The biosensor tip with the bound P domains was washed and then submerged into a well containing 50 µg/mL of the first antibody and then dipped into another well containing 50 µg/mL of the second antibody. If binding of the first antibody still resulted in greater than 66% of binding of the second antibody, the result was interpreted to be no competition. If binding of the second antibody was between 34 and 66% in the presence of the first antibody, there is believed to be partial competition. If 33% or less binding of the second antibody was noted in the presence of the first, both antibodies are believed to be in competition with each other. Antibodies then were clustered based on their binding patterns.

Stool filtrates. To prepare 10% stool filtrates, 4.5 mL of sterile PBS was added to 0.5 g of GII.4 Sydney 2012 positive stool sample. The stool suspension was sonicated using a cup horn sonicator and centrifuged at 1,500 x g for 10 minutes at 4°C. Supernatant was collected and transferred to a new tube and centrifuged once again at 1,500 x g, for 10 minutes at 4°C. The resulting supernatant then was passed serially through 5 µm, 1.2 µm, 0.8 µm, 0.45 µm and 0.22 µm filters, and aliquoted and frozen at -80°C.

Expression and purification of GST-GII.4 Sydney 2012 P domain. P1 and P2 domain sequences of GII.4 Sydney 2012, AFV08795.1, VP1 were cloned into the pGEX-4T-1 expression vector with a glutathione S-transferase (GST) tag and thrombin cleavage site. The P domain was expressed in *Escherichia coli* BL-21 cells and purified using standard column chromatography techniques with a prepacked Glutathione Sepharose Fast Flow column (GE Healthcare). GST-tagged proteins were eluted using 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0 and stored at 4°C.

GII.4 Sydney 2012 virus neutralization assay. Human intestinal enteroids (HIEs) were generated and cultured as described previously (Ettayebi *et al.*, 2016). Briefly, HIEs were grown as three-dimensional cultures in Matrigel (Corning) for 5 days and then plated as cell monolayer cultures in 96-well plates. Before plating, 96-well plates were pre-coated with collagen IV (Sigma) at 33 µg/mL in sterile cold water for 1.5 hours at 37°C. Three-dimensional HIEs were collected in 0.5 mM ethylenediaminetetraacetic acid diluted in ice-cold Dulbecco's PBS, no calcium, no magnesium (Life Technologies, Cat# 14190-144) and spun down at 200

x g for 5 minutes at 4°C in a swinging bucket rotor. The pellet then was suspended in 0.05% trypsin/0.5 mL ethylenediaminetetraacetic acid and incubated at 37°C for 4 minutes. Trypsin then was inactivated with complete medium without growth factors [CMGF(-)] supplemented with 10% fetal bovine serum (FBS). The resulting pellet was suspended and passed through a
 5 0.4 µm cell strainer and spun down at 400 x g at room temperature for 5 minutes. The pellet then was suspended in complete medium with growth factors [CMGF(+)] containing 10 µM Y-27632 (Sigma-Aldrich; Y0503) and seeded into a 96-well plate. After 24 hours, the culture medium was removed and replaced with differentiation medium. Cells were differentiated for 5 days. HuNoV GII.4 Sydney 2012 (TCH12-580) (Ettayebi *et al.*, 2016) stool filtrate (2×10^7
 10 genome equivalents/µL) was used to test neutralization. Serial dilutions of the mAbs were prepared in CMGF(-) medium and each dilution was pre-incubated with 2.5×10^5 genome equivalents of GII.4 Sydney 2012 at 37°C for 1 hour. Samples were diluted with equal volume of CMGF(-) medium supplemented with 1000 µM sodium glycochenodeoxycholate. Monolayers then were inoculated with pre-incubated samples. At 1-hour post-infection (HPI),
 15 monolayers were washed twice and incubated with differentiation medium supplemented with 500 µM glycochenodeoxycholate. After 1 and 24 HPI, cells and medium were collected and RNA was extracted using KingFisher Flex Purification System and MagMax Viral RNA Isolation kit. For RT-qPCR, a primer pair (COG2R /QNIF2d) and probe (QNIFS) (Loisy *et al.*, 2005; Kageyama *et al.*, 2003) were used with qScript XLT One-Step RT-qPCR ToughMix
 20 reagent with ROX (Quanta Biosciences). Reactions were performed on an Applied Biosystem StepOne Plus thermocycler. A recombinant HuNoV RNA transcript was used to create a standard curve to quantitate viral genome equivalents in new RNA samples.

Example 2 – Results

25 **Isolation of GII.4 VLP-reactive human mAbs.** The first step here was to isolate naturally occurring human mAbs to GII.4 Sydney 2012 virus capsid protein from human subjects with prior GII.4 Sydney 2012 virus infection. The inventors used PBMCs collected from subjects with previous history of laboratory-confirmed GII.4 Sydney 2012 virus infection to generate human hybridoma cell lines secreting GII.4 VLP-reactive human mAbs. PBMCs
 30 were transformed with EBV and supernatants then were collected from lymphoblastoid cell lines and screened by ELISA for binding to GII.4 Sydney 2012 VLPs. Recombinant expression of norovirus genome ORF2 and ORF3 in a baculovirus expression system were used to generate VLPs that are antigenically and morphologically indistinguishable from native virions (Jiang *et al.*, 1992). Antibodies that bound to VLPs were detected using horseradish peroxidase

conjugated anti- λ or - κ light chain secondary antibodies. The inventors used an anti-light chain secondary antibody for detection in order to isolate antibodies of varying Ig heavy chain isotypes. Previous studies have noted the presence of diverse isotypes in the human polyclonal antibody response to infection, including an increase in IgA, IgG, and IgM antibodies in serum (Iritani *et al.*, 2007; Gray *et al.*, 1994), and the inventors have shown previously that human IgAs can be more potent than IgGs in blocking GII.1 VLPs from binding to histo-blood group antigens (HBGAs) (Sapparapu *et al.*, 2016). Transformed B cell lines corresponding to supernatants that contained antibodies that bound to GII.4 VLPs were fused with myeloma cells to create human mAb-secreting hybridoma cells. They isolated a panel of 25 hybridomas secreting VLP-reactive antibodies (21 IgGs and 4 IgAs) from 7 different donors (Table A and Supplementary Table 1).

MAB binding and blockade of GII.4 VLPs. Next, the inventors sought to determine how well the antibodies bound to GII.4 Sydney 2012 antigens, and to identify if any blocked attachment of VLPs to surrogate receptor molecules. Half-maximal effective concentrations (EC_{50}) were determined for the panel of GII.4 Sydney 2012 VLPs reactive mAbs using indirect ELISA. For the 21 isolated IgGs, EC_{50} values ranged from 0.1 to 6.2 $\mu\text{g/mL}$ (Table A and Supplementary FIGS. 1A-C). For the 4 isolated IgAs, EC_{50} values ranged from 0.1 to 0.4 $\mu\text{g/mL}$. They initially used a surrogate system to assess the neutralizing capacity of all 25 mAbs. The presence of antibodies that block VLPs from binding to HBGAs *in vitro* correlates with protection against clinical NoV gastroenteritis (Atmar *et al.*, 2015; Czako *et al.*, 2015). To test blockade potential, serial dilutions of isolated mAbs were pre-incubated with GII.4 VLPs, and then VLP-antibody complexes were added to microtiter plates that had been coated previously with porcine gastric mucin type III (PGM) (Reeck *et al.*, 2012). Previous studies have validated PGM as a reliable substrate to be used in VLP blockade assays (Lindesmith *et al.*, 2012a). The inventors then determined EC_{50} values for the 4 IgAs and 13 IgGs, which ranged between 2.4 to 23.9 $\mu\text{g/mL}$ (FIGS. 1A-B, and FIG. 4). Blockade activity was not detected for 8 of the IgGs when using antibody concentrations as high as 100 $\mu\text{g/mL}$. The inventors determined the antibody variable gene heavy and light chain sequences for 17 of the 25 isolated mAbs (Supplementary Table 1). All 17 mAbs, both mAbs that did and did not inhibit GII.4 VLP binding to PGM, had distinct variable gene sequences, suggesting that blockade response is not restricted to a specific genetic sequence motif in antibody repertoires.

Hemagglutination inhibition (HAI) assay confirms mAb blockade activity. The inventors used a second functional assay to confirm the activity they observed in the blockade

assay above. Previous studies have shown that an additional surrogate system to determine mAb neutralization is HAI, and that HAI serum antibody levels correlate with protection from symptomatic infection (Czako *et al.*, 2012; Lindesmith *et al.*, 2012a). The inventors used serial dilutions of the isolated mAbs and pre-incubated them with GII.4 Sydney 2012 VLPs. VLP-antibody complexes were then added to human type O+ RBCs. HAI activity was assessed visually, and HAI titers were determined (FIG. 1A). The majority of the mAbs, about 84%, had HAI activity similar to that of the measured blockade activity. Four mAbs differed in these measures, having either a greater than 2-fold difference in activities or exhibiting only HAI activity or only blockade activity.

Neutralization assay using stem-cell derived enteroids. Inhibition of replication of GII.4 Sydney 2012 virus by mAbs NORO-250B, -263, -320, -273A, -318 and a non-GII.4 VLP binding control antibody, 2D22, were tested using an intestinal epithelial stem-cell derived *in vitro* cultivation system. The inventors selected two IgGs, NORO-263 and -250B and three IgAs, NORO-320, -273A and -318. These five mAbs were chosen so that they could test neutralization by representative mAbs belonging to the three major antigenic sites they identified on the GII.4 Sydney 2012 P domain and to test at least two mAbs belonging to each isotype (FIG. 2). Neutralization was measured by comparing the percent reduction of genome equivalents when compared to a no-antibody control within each assay using RT-qPCR (FIG. 1C). A no-antibody control was used in each assay to normalize for any variability between experiments. Variability was noted due to the high sensitivity when using genome equivalents to measure replication. To account for these differences, the inventors used six replicates for each mAb concentration tested within each assay and for the no-antibody control. They then averaged the genome equivalents from two separate assays for each antibody to obtain an IC₅₀ value. To verify that equal amounts of virus were added to each monolayer, each assay was performed in duplicate and RNA was collected from one assay at 1 HPI and from the other at 24 HPI. Four of the five antibodies tested, NORO-250B, -263, -273A and -318, had approximately 5 to 883-fold lower IC₅₀ values compared to blockade EC₅₀ values, or between 17 and 1,227-fold lower than HAI titers (FIGS. 1A and 1C). NORO-320 had a higher IC₅₀ in comparison to its blockade EC₅₀ or HAI titer. The dengue virus mAb 2D22 used as a similarly prepared negative control did not exhibit concentration-dependent inhibit of replication of GII.4 Sydney 2012 virus. Previous studies with polyclonal serum have shown that neutralization IC₅₀ values of GII.4 and GII.3 noroviruses are lower in comparison to blockade EC₅₀ values (Ettayebi *et al.*, 2016). These studies as well as these data suggests that the HIE neutralization assay is likely more sensitive than the HBGA blockade or HAI assays.

Binding studies using GII.4 Sydney 2012 protruding (P) domain dimers and shell

(S) domain. The inventors next sought to determine if the antibodies were binding to the P or S domains of the major capsid protein VP1. Glutathione S-transferase (GST)-tagged recombinant GII.4 Sydney 2012 P domain dimers were expressed and purified using affinity column chromatography, as previously described (Choi *et al.*, 2008). P domain dimers or recombinantly expressed S domains then were used as antigen for an indirect ELISA assay with serial dilutions of each mAb to determine if the 25 isolated mAbs were specific to the P or S domain of the GII.4 major capsid protein VP1. The VP1 region is divided into the S domain, which is not expressed on the recombinant GST-GII.4 P domain dimers, and the P1 and P2 subdomains that are expressed in the dimers. The P domain is the surface-exposed protruding region of the norovirus virion and also believed to include determinants for host cell attachment and antibody binding epitopes (Debbink *et al.*, 2012; (Lindesmith *et al.*, 2012b; Lochridge *et al.*, 2005). The S domain is connected to the P domain by a flexible hinge region and forms the interior core of the viral capsid. The S domain has the highest degree of genetic sequence conservation of any protein domain in diverse norovirus strains (Parra *et al.*, 2013). When binding was tested by ELISA, 20 of the 25 isolated mAbs bound to the GST-GII.4 P domain (FIGS. 5A-B). The five mAbs that did not bind to the P domain did bind to the S domain. NORO- 329A, -312A, -296A and -232A.2, bound to both the P and S domain proteins, indicating that these antibodies likely bind to a quaternary epitope on the GII.4 Sydney 2012 major capsid protein. Isolated mAbs and GII.4 P domain dimers also were used for competition-binding studies. The inventors used a real-time biolayer interferometry biosensor system to identify potential major antigenic sites recognized by the GII.4 Sydney 2012 P domain binding mAbs. Neutralizing mAbs and mAbs that did or did not block GII.4 Sydney 2012 VLPs from binding to PGM were classified into three major competition-binding groups, with some overlap between two groups (FIG. 2). Despite multiple attempts, they were not able to detect binding using biolayer interferometry for 2 of the 20 mAbs that bound to the GII.4 Sydney 2012 P domain by ELISA. Competition-binding studies were performed using the Octet® RED96 and Octet® HTX systems, which are both instruments that can measure biomolecular interactions. The HTX system is a high-throughput system that has the ability to compete all 18 P domain binding mAbs within the same experiment. The RED96 system was only able to compete mAbs in groups of 8. Using data from the RED or HTX experiments, the inventors noted three major competition-binding groups on the GST-GII.4 Sydney 2012 P domain (FIG. 2 and FIG. 6). By using both instruments, in two independent laboratories, they were able to validate the reproducibility of the results.

The emergence of new GII.4 strains has been associated with the evolution of the GII.4 major capsid protein and antigenic variation (Karst *et al.*, 2015). To measure the reactivity of the inventors' panel of mAbs for another GII.4 strain, the inventors tested binding reactivity and blockade activity to a GII.4 Houston 2002 (ABY27560.1) variant. About 93% of the amino acid sequence of the major capsid protein is conserved between GII.4 Houston 2002 and Sydney 2012, but there are remarkable differences among predicted GII.4 blockade epitopes (FIG. 7). All 25 mAbs exhibited binding reactivity to GII.4 Houston 2002 antigen (FIGS. 8A-B). Only 3 mAbs, NORO-115, -329A, and -318 had a greater than 10-fold higher binding EC₅₀ value when compared to GII.4 Sydney 2012 VLPs (Table A). The 18 mAbs that either blocked with EC₅₀ values <100 µg/mL or had HAI titers < 15 µg/mL all blocked GII.4 Houston 2002 VLPs from binding to PGM. GII.4 Houston 2002 and Sydney 2012 had different amino acid sequences in four of the five predicted blockade epitopes, these results suggest the potential existence of additional blockade epitopes or the use of Epitope B, which was conserved among both strains (FIG. 7).

Example 3 - Discussion

Here the inventors report the first instance of neutralization of HuNoV by mAbs and describe a large panel of human mAbs that neutralize the pandemic GII.4 Sydney 2012 strain. Previously it was not possible to test norovirus neutralization directly because of the lack of a reliable *in vitro* culture system for norovirus replication. A surrogate system to predict neutralization was devised and used to study inhibition of the interaction between VLPs and HBGAs. The presence of blocking antibodies in serum correlates with protection from clinical gastroenteritis induced by HuNoV infections, and therefore the HBGA blocking assay has been considered a surrogate system for HuNoV neutralization (Reeck *et al.*, 2010). Recently, the inventors developed an *in vitro* system using human enteroids to replicate multiple HuNoV strains¹³. Here, they used that system to identify the first norovirus human mAbs with demonstrated virus neutralizing activity. Of the 25 human mAbs isolated in this study, 17 of them blocked GII.4 Sydney 2012 VLPs from binding to PGM at concentrations as low as 2.4 µg/mL. The 18 mAbs that blocked GII.4 Houston 2002 from binding to PGM at concentrations as low as 2 µg/mL, also either blocked GII.4 Sydney 2012 VLPs from binding to PGM or inhibited hemagglutination at the concentrations tested. Interestingly, 13 of the 14 IgGs that blocked GII.4 Houston 2002 VLPs from binding to PGM did so at a lower EC₅₀ value in comparison to blockade EC₅₀ values for GII.4 Sydney 2012. This finding could indicate that donors had prior exposure to an earlier norovirus variant similar to GII.4 Houston 2002. This

panel also contains the first reported human IgA mAbs that bind to GII.4 Sydney 2012 VLPs and also inhibit receptor binding. The inventors tested neutralization of live GII.4 Sydney 2012 HuNoV using the mAbs NORO-263, -320, -250B, -273A and -318. These antibodies were selected for testing so that they could investigate differences in neutralizing activity between
5 mAbs of differing isotypes, those which belong to different competition-binding groups, and those with different binding and blockade EC_{50} values. NORO-250B, -263, -273A and -318, had lower neutralization IC_{50} values in comparison to blockade EC_{50} values and HAI titers. Surprisingly, NORO-320 had an IC_{50} value about 2-fold higher than its HAI titer and about 3-fold higher than its blockade EC_{50} value. Previous studies have noted differences in blockade
10 potency of GI.1 VLPs among human IgG and IgA mAbs with blockade potency being enhanced for IgAs (Sapparapu *et al.*, 2016). To draw a similar conclusion for GII.4 Sydney 2012 neutralizing human antibodies, it would be essential to test mAbs binding to different epitopes with identical variable domain sequences and distinct isotypes. Such studies could determine if isotype plays a critical role in neutralization of GII.4 Sydney 2012 by human mAbs.

15 HuNoV-specific antibodies have been described previously, but these were antibody fragments derived from phage display libraries (Huang *et al.*, 2014), murine mAbs from infected mice (Crawford *et al.*, 2015), nanobodies from alpacas immunized with VLPs (Koromyslova and Hansman, 2017), or mAbs isolated from patient PBMCs with an unknown norovirus history of exposure (Lindesmith *et al.*, 2012a). Such antibodies do not provide direct
20 information about the physiologic human humoral immune response to HuNoV infection. The therapeutic potential of mouse mAbs is limited, since they have been shown to induce human anti-mouse antibody responses. There has been little progress in understanding individual HuNoV-specific antibodies in the past because of the difficulty in generating human mAbs with functional activity. Here the inventors used a hybridoma technology (Smith and Crowe,
25 2015) and circulating B cells from convalescent patients to produce human mAbs. This approach generates hybridoma cell lines from circulating B cells that express naturally occurring and matched heavy and light chain genes. An additional benefit of using this approach is that it does not involve the use of any laboratory animals to produce antibodies. Using this method, they isolated 25 GII.4 Sydney 2012 VLP-reactive mAbs. The majority of
30 these antibodies were neutralizing when tested in a surrogate system for neutralization and all five of the mAbs tested also inhibited replication of live GII.4 Sydney 2012 virus by direct neutralization *in vitro*. Neutralizing human mAbs have potential for use in prophylactic, therapeutic or diagnostic applications. The inventors currently do not have any drugs available to treat or prevent HuNoV infection, so the inventors' panel of neutralizing mAbs now have

the high potential to impact the design of improved diagnostic and therapeutic measures for HuNoVs.

Since the mid-1990s, new antigenically diverse GII.4 pandemic viral strains have emerged continuously every 2 to 5 years, and today these strains continue to be the predominant cause of norovirus outbreaks. In 2012, the epidemic GII.4 Sydney variant emerged in Australia and began spreading globally. Even though blockade epitopes among some contemporary GII.4 strains have been predicted or identified, the inventors have limited information about the neutralization determinants on GII.4 Sydney 2012 viruses (Debbink *et al.*, 2013; Lindesmith *et al.*, 2012a). Here, they determined that there are at least three major antigenic and neutralizing sites on the P domain of GII.4 Sydney 2012 viruses. In the future, defining neutralization epitopes in high resolution with neutralizing antibodies could contribute valuable insights for rational structure-based vaccine design efforts.

HuNoV is one of the leading causes of severe acute gastroenteritis, therefore the global burden of norovirus infection is extremely high in both developed and developing countries. Unfortunately, there is currently no licensed vaccine to prevent norovirus infection. Efforts to design a vaccine have been hindered by the lack of a small animal model or tissue culture model to test neutralization or infection, the antigenic heterogeneity among noroviruses, and uncertainty about the durability of protective immunity (Debbink *et al.*, 2014). Vaccine efforts have focused on the use of monovalent GI.1 or bivalent GI.1/GII.4 virus-like particles or P particle subunits (El-Kamary *et al.*, 2010; Bernstein *et al.*, 2015; Tan *et al.*, 2011). Clinical trials have shown that norovirus VLP vaccines are immunogenic and without frequent serious adverse events (Ramirez *et al.*, 2012; Leroux-Roels *et al.*, 2018). The inventors now have developed a reliable *in vitro* system to test the replication or inhibition of replication of live noroviruses. Mapping the neutralization or blockade epitopes using the panel of mAbs they isolated against this circulating pandemic strain of norovirus will provide critical information that can be used for the design of future VLP vaccines that can elicit a protective immune response.

Example 4 – Materials and Methods

Generation of virus-like particles. Virus-like particles (VLPs) based on norovirus strains GI.1 (M87661), GI.2 (AF435807), GI.3 (AF439267), GII.3 (TCH02-104), GII.4 (AFV08795.1), GII.6 (AF414410), GII.13 (JN899242) and GII.17 (AB983218) were expressed recombinantly and purified as previously described (Jiang *et al.*, 1992). The inventors used a baculovirus recombinant protein expression system for VLP production. The

inventors cloned the VP1 and VP2, major and minor, protein capsid sequences from each strain into the transfer vector pVL1392 (Epoch Life Science, Inc). Sf9 insect cells were co-transfected with a transfer vector corresponding to a specific strain and with a bacmid vector. Recombinant baculovirus was isolated and expanded. VLPs were purified from cell culture supernatants using a sucrose and cesium chloride gradient. VLP formation was verified using electron microscopy.

Reactivity to VLPs by ELISA. An ELISA was used to testing binding of human mAbs to VLPs, as was previously described (Alvarado *et al.*, 2018). Each VLP was coated individually at 1 µg/mL on 384-well microtiter plates at 4°C overnight. Plates then were blocked for one hour at room temperature using 5% nonfat dry milk in PBS with 0.05% Tween-20. For screening and EC₅₀ analysis, antibody reactivity to VLPs was detected using horseradish peroxidase (HRP) tagged anti-κ or -λ chain secondary antibodies (Southern Biotech). 1-Step™ Ultra-TMB Substrate Solution (Pierce ThermoFisher) was used to detect HRP activity.

Human subjects. The Vanderbilt University Medical Center Institutional Review Board approved of the participation of the 6 adult subjects used in this study. All participants provided written informed consent before the inventors obtained blood samples. The subjects were healthy with a previous history of acute gastroenteritis.

Human hybridoma generation. Human hybridomas secreting human mAbs were generated as previously described (Alvarado *et al.*, 2018). Briefly, PBMCs were isolated from human subject blood samples using Ficoll-Histopaque and density gradient centrifugation and then cryopreserved. Later, cells were thawed, transformed using Epstein-Barr virus, CpG10103, cyclosporine A and a Chk2 inhibitor and plated in a 384-well plate. Transformed cells were incubated at 37°C for 7 days, and then expanded into 96-well plates containing irradiated human PBMCs. Four days later, cell supernatants were screened by indirect ELISA for the presence of anti-norovirus VLP cross-reactive mAbs. B cells secreting cross-reactive mAbs were electrofused to HMMA2.5 myeloma cells and plated in medium containing hypoxanthine, aminopterin, thymidine and ouabain. Hybridoma cell lines were incubated at 37°C for 14 days, and then supernatants were screened by indirect ELISA for productions of cross-reactive mAbs. Cell lines expressing cross-reactive mAbs then were cloned biologically using single-cell fluorescence-activated cell sorting.

Purification of cross-reactive mAbs. After cloning, hybridoma cell lines producing cross-reactive mAbs were expanded gradually from 48-well plates to 12-well plates, T-25, T-

75 and eventually to four T-225 flasks for each cell line. Supernatant from each cell line also was screened by ELISA to determine the corresponding light chain for each clone. Following 4-weeks of incubation at 37°C, supernatant from the four T-225 flasks was harvested and filtered through a 0.4-µm filter. The supernatant was filtered using column chromatography, specifically HiTrap KappaSelect and Lambda FabSelect affinity resins (GE Healthcare Life Sciences).

VLP-carbohydrate mAb blockade assay. To test the ability of each mAb to inhibit the interaction between the selected VLPs and glycans *in vitro*, the inventors used a blockade assay. As previously described, the inventors coated microtiter plates with 10 µg/mL of pig gastric mucin Type III (Sigma) for 4 hours at room temperature. Plates then were blocked overnight at 4°C in 5% nonfat dry milk. VLPs at 0.5 µg/mL were pretreated with serially diluted concentrations of each mAb for 1 hour at room temperature. VLP-mAb complexes were added to the PGM-coated and blocked microtiter plates. After 1 hour of incubation, the plates were washed 3 times with PBST and the same was done in between each step. Bound VLPs were tested using murine serum containing anti-GI.3, GII.4, GII.6 or GII.17 polyclonal antibodies, followed by an HRP conjugated goat anti-mouse IgG human adsorbed antibody. Optical density was measured at 450 nm using a Synergy HT Microplate Reader (BioTek).

Expression and purification of protruding and shell domain for selected NoV strains to be used in Ab binding studies. In order to map the epitope of cross-reactive mAbs, the inventors first recombinantly expressed P1 and P2 domain sequences or shell domain of GI.3 (AF439267), GII.4 (AFV08795.1), GII.6 (AF414410), GII.13 or GII.17 (AB983218). P domain sequences were cloned into the pGEX-4T-1 expression vector with a GST tag and thrombin cleavage site. The P domain then was expressed in *Escherichia coli* BL-21 cells and purified using a Glutathione Sepharose Fast Flow Column (GE Healthcare) and column chromatography. The S domain sequences were cloned into pVL1392 and co-transfected with a bacmid vector into Sf9 insect cells. Recombinant baculovirus particles then were harvested and used to inoculate Sf9 cells. S domain particles were then purified from the inoculated Sf9 cell culture supernatant using a sucrose and a cesium chloride cushion gradient.

Expression, purification and crystallization of GII.4 P domain and NORO-320 Fab. The sequence for the GII.4 protruding domain was cloned into the expression vector pMal-C2E (New England BioLabs). The expression vector includes a N-terminal His₆-maltose binding protein (MBP) tag and a tobacco etch virus (TEV) protease cleavage site between the MBP and P domain sequence. The P domain was expressed in *E. coli* BL21(DE3) and purified

using an AffiPure Ni-NTA agarose bead column (GenDepot). The His-MBP tag was then removed using TEV protease and separated from the P domain by purifying it once again using His-Trap (GE Healthcare), MBPTrap (GE Healthcare) affinity columns and size exclusion chromatography. Finally, the purified P domain was concentrated and stored in 20 mM Tris-HCl buffer (pH 7.2) containing 150 mM NaCl, and 2.5 mM MgCl₂.

The nucleotide sequences of the variable domain of mAb NORO-320 was optimized for mammalian expression and synthesized (Genscript) for expression and purification of recombinant Fab. The heavy chain fragment was cloned into a vector for expression of recombinant human Fabs (McLean *et al.*, 2000). The light chain was cloned into a vector for light chain. Each vector was transformed independently into *E. coli* cells, and DNA was then purified. Both the heavy and light chain encoding vectors were transfected into CHO cells using an ExpiCHOTM expression system. Cell supernatant was collected, centrifuged and filtered using a 0.45 µm filter. NORO-320 Fab was purified by affinity chromatography using a KappaSelect (GE Healthcare).

Purified GII.4 P domain and NORO-320 Fab were combined in a 1:1.5 molar ration and incubated for 1 hour at 4 °C. The mixture was passed through an S75pg 16/60 gel filtration column, and the peak corresponding to the complex was collected. The size of the complex and presence of both proteins was validated on an SDS-PAGE gel. The peak fractions were then pooled and concentrated to 10 mg/mL for crystallization trials. Crystallization screening using hanging-drop vapor diffusion method at 20 °C was set up using a Mosquito nanoliter handling system (TTP LabTech) with commercially available crystal screens, and crystals were visualized by using a Rock Imager (Formulatrix). The GII.4 P domain–NORO-320 Fab complex crystallized in a buffer containing 0.1 M BIS-TRIS prop 8.5 pH, 0.2 M KSCN, 20% w/v PEG 3350. Crystals diffracted to 2.25 Å resolution.

Diffraction, data collection, and structure determination. X-ray diffraction data for the GII.4 Sydney 2012 P domain–Fab NORO-320 crystals were collected on the beamline 5.0.1 at Advanced Light Source (Berkeley, CA). Diffraction data were processed using HKL2000 (Z. Otwinowski and W. Minor, 1997). The previously published GII.4 (strain TCH05) P domain structure (PDB ID 3SJP) and the neutralizing Fab 5I2 (PDB ID 5KW9) were used as the search models by molecular replacement using program PHASER (McCory *et al.*, 2007). Iterative cycles of refinement and further model building were carried out using PHENIX (Adams *et al.*, 2010) and COOT programs (Emsley, P., and K. Cowtan, 2004). During the course of the refinement, and following the final refinement, the stereochemistry of the

structures was checked using Molprobit (molprobit.biochem.duke.edu/). Data refinement and statistics are given in Table B. The interactions between P domain and the Fab for NORO-320 were analyzed using LigPlot+ v.2.1 (Laskowski *et al.*, 2011). Figures were prepared using Chimera (Pettersen *et al.*, 2004).

5

Example 5 - Results

Isolation of broadly binding anti-NoV human mAbs. To isolate cross-reactive NoV human mAbs, the inventors used EBV and additional B cell stimuli to transform memory B cells in PBMCs obtained from patients who were overall healthy but with a previous history of acute gastroenteritis, as previously described (Sapparapu *et al.*, 2016). A week later, transformed PBMCs supernatants were tested by ELISA to screen for the expression of mAbs that bound to more than one representative strain NoV VLP. The VLPs used to screen were NoV GI.1, GI.2, GI.3, GII.3, GII.4, GII.6, GII.13 or GII.17. Each VLP was coated individually and blocked on a microtiter plate before the screening. The bound antibodies were detected using alkaline phosphatase conjugated goat anti-human κ or λ chain secondary antibodies to capture binding activity by any antibody isotype. Wells that contained transformed B cells expressing mAbs that recognized more than one VLP then were expanded. B cells secreting anti-NoV mAbs were rescued by hybridoma formation. Binding reactivity to pandemic GII.4 Sydney 2012 was previously characterized for 5 of the mAbs included in the inventors' panel (Alvarado *et al.*, 2018). The heavy and light variable gene regions were sequenced for all 12 mAbs and the V, D, J and other variable gene sequence features were analyzed (Supplemental Table 2) (Brochet *et al.*, 2008). Each of the mAbs had unique variable gene sequences, suggesting that cross-reactivity was not limited to one antibody clonotype.

Binding and blockade activity of cross-reactive mAbs to NoV GI and GII VLPs. To assess binding reactivity and blockade function of the 12 mAbs, the inventors used indirect ELISA and a VLP blockade assay. The concentration of each mAb was normalized first for the number of antigen binding sites. The inventors then tested binding starting at a concentration of 500 nM, followed by 11 serial dilutions. Each concentration was tested in duplicate, and the complete experiment was repeated 3 times. They used these data to determine the EC₅₀ value of each mAb when binding to NoV GI.1, GI.2, GI.3, GII.3, GII.4, GII.6, GII.13 or GII.17 VLPs (FIGS. 9A-B). The inventors noticed 3 distinct binding patterns. NORO-168.2, -156.3 and -170.5, all IgMs, each exhibited wide breadth by binding to all VLPs tested. Both IgAs, NORO-232A.2 and -320, as well as NORO-167.3 and -202A.1, both IgGs, exhibited specificity of

binding only for GII variants. The remaining mAbs, NORO-155.5, -178.6, -279A, -310A and -323A reacted with at least one GI and GII strain.

To determine if any of the isolated cross-reactive mAbs had functional activity, the inventors used a surrogate system to analyze neutralization. PGM purified from porcine stomach mucosa contains both H and Lewis antigens, α -1,2- fucose and α -1,4-fucose (Lindesmith *et al.* 2008; Lindesmith *et al.* 2012; Tian *et al.*, 2010). The use of PGM in blockade assays has been validated previously (Lindesmith *et al.*, 2012). They first tested if GI.1, GI.2, GI.3, GII.3, GII.4, GII.6, GII.13 or GII.17 VLPs could bind to the glycans present in PGM. The optimal concentrations of mAbs were normalized before testing blockade ability and tested at concentrations beginning at 1,000 nM and then diluted serially (FIGS. 10A-B). Blockade studies also were repeated three times. Only GI.3, GII.4, GII.6 and GII.17 bound to PGM. NORO-155.5 and -170.5, both IgMs, and -167.3, an IgG, did not inhibit GI.3, GII.4, GII.6 or GII.17 VLPs from binding to PGM *in vitro*. 9 of the 12 mAbs blocked at least 1 of the 4 VLPs tested. None of the 8 mAbs with binding reactivity to GII.17 VLPs had any GII.17 blockade activity. The absence of GII.17 blockade could be due to the broad binding HBGA spectrum of GII.17 NoVs and the glycan heterogeneity present in PGM.

Binding to GI.3, GII.4, GII.6 or GII.17 variant protruding (P) vs shell (S) domains.

The major capsid protein, VP1, which forms the icosahedral capsid, is divided into the protruding (P) and shell (S) domains (Prasad *et al.*, 1996). To map where the cross-reactive mAbs bind, the inventors first recombinantly expressed and purified the S and P domains of GI.3, GII.4, GII.6 and GII.17 NoV strains. Antibody binding to S, P and VLPs, containing both S and P, was tested and compared. His₆-MBP (maltose binding protein)-tagged recombinant P domains were expressed in *E. coli* and purified using affinity column chromatography, as previously described (Choi *et al.*, 2008). S domain sequences were cloned into pVL1392, expressed using a baculovirus expression system and purified using a sucrose and cesium chloride cushion gradient. P and S domain recombinant proteins were coated at equal concentrations of 2 μ g/mL on microtiter plates and blocked with 5% nonfat dry milk in 1X DPBS with 0.05% Tween-20. Before adding the mAbs to plates, each mAb was normalized according to the number of antigen binding sites. The inventors tested binding starting at a concentration of 500 nM followed by 11 serial dilutions of each mAb to obtain the half-maximal binding concentrations. Both IgAs, NORO-232A.2 and -320, appeared to bind specifically to the GII.4, GII.6 and GII.17 P domains (FIG. 11). Cross-reactive murine mAbs also have been mapped to the NoV P domain (Parker *et al.*, 2005). NORO-168.2 bound to both

the P and S domains of GII.3, GII.4, GII.6 and GII.17, but in all instances had a lower EC₅₀ value when bound to the S domain. Some mAbs like NORO-155.5 and -156.3 did not bind to any of the P or S domains tested. Loss of binding may suggest that these mAbs require both the S and P domain to be present for Ab binding.

5 **Structure of NORO-320 Fab in complex with GII.4 P domain.** In previous studies, the inventors determined that NORO-320 not only neutralizes GII.4 Sydney 2012 using a surrogate neutralization assay, but also inhibits replication of infectious GII.4 Sydney 2012 virus when using a human intestinal enteroid culture (Alvarado *et al.*, 2018). Here, the inventors discovered that NORO-320 binds broadly across selected GII strains and also blocks
10 GII.6 from binding to PGM. To determine how NORO-320 binds so broadly and neutralizes diverse GII NoV strains, the inventors used X-ray crystallography to identify the structural basis for molecular recognition. They obtained a 2.3 Å resolution structure of NORO-320 Fab in complex with the GII.4 P domain (FIGS. 12A-B). According to the structure, NORO-320 Fab binds perpendicular to the 2-fold axis of the P domain dimer near a region close to the shell
15 domain. This finding shows that NORO-320 Fab does not bind directly or close to the HBGA binding site to inhibit GII.4 VLP-carbohydrate binding or replication of GII.4 Sydney 2012 virus. The inventors considered the possibility that NORO-320 Fab binding allosterically could inhibit HBGA binding by causing a conformational change in the glycan binding site. However, the structural superposition of the GII.4 P domain in complex with NORO-320 and that with
20 the bound HBGA revealed that the P domain dimer structure remains invariant with an r.m.s.d of ~1.1 Å for the matching Cα atoms. The inventors then hypothesized that neutralization of GII.4 by NORO-320 could be a result of steric hindrance since full-length NORO-320 is originally a dimeric IgA (see below).

Molecular details of recognition of GII.4 P domain by NORO-320. The crystal
25 structure of NORO-320 Fab-GII.4 P domain complex shows that the antibody makes extensive interactions with the P domain. The Fab binding site on the P domain is formed by residues from the P1 subdomain of one subunit and the P2' subdomain of its dimeric partner (FIGS. 12C-D). The paratope in NORO-320 includes residues from the CDRs of both light and heavy chains. The Fab binding is stabilized by both hydrogen bond and hydrophobic interactions (FIG.
30 13A). For instance, the sidechain of N479 in the P1 subdomain hydrogen bonds with I54 from CDRH2 and E74 of a non-CDR loop, whereas residues L486, V508, P510, P511, and N512 are involved in hydrophobic interactions with residues from CDRH2 and CDRH3 (FIG. 13B). Residue D312 of the P2' subdomain interact with K119 and Y120 of CDRH3, involving both direct hydrogen bond and hydrophobic interactions (FIG. 13C). In addition to CDRHs, three

light chain residues Y35 and Y37 of CDRL1 and L55 of CDRL2 form hydrophobic interactions with P313' and T314' of the P1' subdomain. To understand how NORO-320 can bind to VLPs of various GII strains, the inventors aligned the P domain amino acid sequences of GI.1, GII.3, GII.4, GII.6, GII.13 and GII.17 (FIG. 14). Sequence alignment revealed a 78 to 89% conservation at these sites among the GII strains. In contrast, the P domain sequence in the GI genogroup show significant changes in this region. The high level of sequence conservation could explain why NORO-320 binds broadly among GII.3, GII.4, GII.6, GII.13 and GII.17 strains.

Mechanism of neutralization of NoV by broad human mAbs. To determine if NORO-320 neutralizes GII.4 because it sterically hinders NoV binding to glycans, the inventors tested if blockade activity was influenced by the molecular weight or size of NORO-320. In order to obtain varying forms of NORO-320, they recombinantly expressed the sequence of the variable domain in Fab or IgG heavy chain recombinant expression vectors. The light chain variable sequence also was cloned into a kappa light chain recombinant expression vector. Corresponding heavy and light chains were transfected using Chinese hamster ovary (CHO) cells in an ExpiCHO™ expression system. To verify the molecular weight of the original hybridoma-expressed IgG and each of the recombinantly expressed mAbs, 4 µg of each mAb, along with a set of control mAbs of known molecular weight, were resolved on an SDS-PAGE gel under non-reducing conditions (FIGS. 17A-D). All the mAbs were of the expected apparent molecular weight, dIgA ~350 kDa, IgG ~150 kDa and Fab ~50 kDa. The inventors then tested NORO-320 IgA, recombinant IgG, recombinant Fab or a no mAb control for their ability to inhibit GII.4 Sydney 2012 VLPs from binding to PGM *in vitro*. As hypothesized based on size, the large NORO-320 IgA had the lowest EC₅₀ value followed by NORO-320 recombinant IgG (FIG. 15). Recombinant NORO-320 Fab did not block GII.4 Sydney 2012 VLPs from binding to PGM at concentrations as high as 1,000 nM. This finding indicates that the dimeric NORO-320 likely neutralizes GII strains broadly because of the capacity of this large molecule to mediate steric hindrance to receptor binding (FIGS. 17A-D). The IgMs and IgGs that bind to the S domain of VP1 could potentially neutralize NoVs by disrupting the structural integrity of the virus particles. For example, the IgM NORO-168.2 broadly cross-reacts with GI and GII NoVs by binding to the P and S domains of GI.3, GII.4, GII.6 and GII.17 (FIG. 11). Further understanding of the diverse neutralization mechanisms for circulating human NoVs warrants future structural studies of these human mAbs (FIGS. 17A-D).

Example 6 – Discussion

Isolation of naturally occurring broad-spectrum human mAbs to NoV holds great promise for the discovery of new candidate therapeutics, as well as identifying critical epitopes for the rational design of new structure-based broadly protective NoV vaccines. In the past, the genetic and antigenic diversity across circulating strains of NoV has made the generation of a broadly immunogenic vaccine extremely difficult. The primary goal of this study was to define the molecular and structural determinants of cross-reactivity and blockade, or neutralization, using human mAbs to circulating strains of HuNoV. Previous studies have characterized the antigenic landscape of specific HuNoV strains, but with the rapid emergence of new genetically diverse strains, there is a need to map new immunogenic epitopes. This new information also can build upon previous studies to help track the evolution of HuNoVs (Lindesmith *et al.*, 2013). Identification of antigenic epitopes using human mAbs also will provide insight into the immunogenicity of NoVs in humans.

Here, the inventors described the isolation of 12 anti-NoV cross-reactive human mAbs, 4 IgMs, 6 IgGs, and 2 IgAs, from subjects with a previous history of acute gastroenteritis. To determine the functional activity of the isolated mAbs, they used a previously validated surrogate blockade assay that measures the inhibition of VLPs from binding to glycans *in vitro* (Reeck *et al.*, 2010). Not all of the strains tested bind to the same glycan, so the inventors were only able to test the inhibition of binding for NoV VLPs GI.3, GII.4, GII.6, and GII.17. Of the 12 mAbs isolated, 9 mAbs blocked at least 1 of VLPs tested from binding to PGM with EC₅₀ values less than 1 μ M.

Prior to these studies, the inventors expected that cross-reactive and neutralizing human mAbs would bind predominantly to the P subdomain of the circulating strains, similar to the findings for cross-reactive murine Abs (Crawford *et al.*, 2015). The P domain has more surface exposure on a live virion than the S domain and should, therefore, be more accessible than the S domain. Both previously characterized mAbs, the human IgA 5I2 and the mouse IgG 10E9, target regions adjacent or at the HBGA-binding site on the P2 subdomain thereby directly preventing the binding of glycans (Shanker *et al.*, 2016; Koromyslova *et al.*, 2019). Here, the inventors describe a crystal structure of a human derived neutralizing antibody in complex with a GII.4 strain of NoV. The crystal structure of Nano-85, an alpaca derived nanobody, which is much smaller in molecular size when compared to a Fab, in complex with the GII.4 P domain was also recently reported (Koromyslova *et al.*, 2015). Nano-85 binds toward the proximal end of the P domain dimer, which in the context of the capsid would be closer to the S and P domain

interface. The epitope recognized by this nanobody which consists of W520, N522, and T526 is entirely distinct from that recognized by NORO-320. Based on negative-stain images of VLPs in complex with this nanobody it is suggested that the binding disrupts particle assembly.

It is indeed intriguing that even though NORO-320 does not directly bind in close proximity to the receptor binding domain it still inhibits GII.4 VLPs from binding to glycans *in vitro* and inhibits viral replication of GII.4 Sydney 2012 virus (Alvarado *et al.*, 2018). The blockade studies using IgG and Fab recombinant variants, as well as the originally isolated antibody that was a dimeric IgA molecule, show that the molecular size of the mAb influenced the potency of neutralization (FIG. 15). Blocking potential increased with the increase in molecular size of the NORO-320 variant tested. Therefore, blockade potential is a result of NORO-320 sterically blocking GII.4 VLPs from binding to glycans *in vitro*. Modeling of the Fab binding to the P domain in the context of the capsid using the only available X-ray structure of the GI.1 indicates that constant domain of the Fab clashes with the neighboring VP1 subunits potentially affecting the particle assembly. But the inventors' observation that Fab alone does not block the HBGA binding suggest that Fab binding does not affect capsid structure, and it is possible that the orientation of the constant domain in the context of the capsid is slightly altered to avoid clashes. However, the additional Fc region projecting out from the Fab, as in the context of IgA or IgG, may sterically hinder the glycan binding sites in the neighboring VP1 subunits. Thus, this study potentially identifies a novel mechanism of neutralization for GII.4 Sydney 2012 NoV. The amino acids critical for the interaction between the GII.4 P domain and NORO-320 also are conserved among GII.3, GII.6 and GII.17 strains, which suggests that they are neutralized similarly. It is also possible the bound NORO-320 IgG or the IgA affecting the particle integrity leading to disassembly is an additional factor contributing to neutralization.

These results suggest that even though there is a high degree of sequence and antigenic diversity in the capsid protein VP1 among circulating strains of human NoVs, common protective antigenic sites exist among these strains. Recognition of the P2 subdomain and the most conserved S domain of VP1 by human mAbs could be the molecular basis for broad cross-reactive neutralization. The novel neutralizing NoV epitope identified also informs us about a critical antigenic GII site that could later be used in the reformulation of broadly protective NoV vaccine candidates. These human mAbs also could be directly used as a prophylactic, a therapeutic, or a reagent for diagnosis.

TABLE 1 – NUCLEOTIDE SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	Variable Sequence Region	SEQ ID NO:
NORO-115	caggtgcagctggaggagctctggggaggccttggtcaagcctggggggccctgagactctcctgtac agcctctggaatcaccatcagtggtactacatgagttggatccgccaggctccagggaaggactgg aatggattgtatacattaatacaagtggtagaacatatactacgcagactctgtgaaggccggttct Heavy ccgtctccagggacaacccaaggagtcgtgtatttgcaaatggacagcctgacggctgatgacacg ggcatatattattgtgcgagagatcgattaccagcatctggttccactggttccaccctggggccag ggaacctggtcaccgtctcctcag	1
NORO-115	gacatcgtgatgaccagctctccagactccctggctgtgtctctgggcgggagggccaccatcaactgc aagtcagccagagtggtttatacacctccgacaataagaactacttagcctggtagcagcaacc aggacagcctcctaagctgctcatttctgggcttctactcggaatccggggtccctgaccgattcagt light ggcagcgggtctgggacagatttactctcaccatcagcaacctgcaggctgaagatgtggcagttat tactgtcagcagattataatagtcctctcgctttcggcggagggaaccaaggtagagatcaaa	2
NORO-123	caggtgcagctggaggagctctggggaggcgtggccagcctgggagggtccctgagactctcctgttc aacctctggattcacctcagtcataatcctatgcactgggtccgccaggctccaggcaagggtgga Heavy gtgggtggcacttatcctatgatggaatgaataaatactacgcagactccgtgaggggcccattcac catctccagagacaattccgagaacacgcagtatctgcaaatgaacagcctgagaggtgacgacacg gctgtctattattgtgcgagagtcacgggcgattgtactggtaatagatgctcatattgggcatactact actacggtctggacgtctggggccaagggaccctggtcaccgtctcctca	3
NORO-123	cagtctgccctgactcagcctccctccgctccgggtctcctggacagtcagtcaccatctcctgcactg gaaccagtagtgacgttggtggtataagatgtctcctggtaccaacagcagccaggcaaagccccc aaactcatgatttatgaggtcactagggcgccctcaggggtccctgatcgcttctctgggtccaagtctg light gcaacacggccttctgaccgtctctgggtccaggctgaggatgaggctgattattactgcggctcat atgcaggcagcaccacttccgggtatgtcttcggaactgggaccaaggctcatcgtccta	4
NORO-202A.1	caggtgcagttggtgcagtcctggggctgaggtgaagaagcctggggcctcagtgagggtctcctgcaa ggcttctgaatacacctttaccggctactatttgagtggtgacagggccctggacatgggcttga Heavy atggatgggatggatcaatcccagtggtggcacaaagtattcacagaagtttcagggcagggtcacca tgaccagggacacgtccatcactactgtctacatggaactgagcagactgagatctgacgacacggcc gtctattactgtgcgagagaccttcttaggaactgggggtgatcatgatgcttttgatgtctggggccagg ggacaatggtcaccgtctcctcag	5
NORO-202A.1	cagtctgtgctgactcagccaccctcagcgtctgagacccccgggcagggggtcaccatctctgttctg gaagcagctccaacatcggaagtaattttgtatgctggtaccagcaggtcccagggaacggccccc ctcctcatctataagaataatcagcgccctcaggggtccctgaccgattctctggctccaagtctggca light cctcagcctccctggccatcagtggtccgggtccgaggatgaggctgattattactgttcagcatggg atgacagcctgagtggtccggtattcggcggagggaaccaagctgaccgtcctag	6

NORO-250B	Heavy	caggtgcagctggtggagctctgggggaggccttggtcaagcctggagggtccctgacactctcctgtgc agcctctggattcaccttcagtgactactacatgaactggatccgccaggctccagggaagagactgg agtgggtttcatacattagtggtattatgagttccacaaactacgcagactctgtgaagggccgattca ccatctccagagacaccgccaagaagtcagtgtatctgcaaataaacagcctgagagccgaggatac ggctgtgtattactgtgcgagagcaacctcacaggagctacctcttactactttgactcctggggcca gggaacctggtcaccgtctcctca	7
NORO-250B	Light	cagtctgtctgacgcagccgccctcagtgctctggggccccagggcagagggtcaccatctcctgcact gggagcagctccaacatcggggcagattatgatgttactggtaccaacaactccaggagcagcccc cagactcctcatctatgtgacatcaatcgccctcaggggtccctgatcgattctctggctccaagtct ggcacctcagcctcctggccatcactgggctccaggctgaggatgaggctgattattactgccaatcc tatgacagaagcgtgagtggttcggcgggtgttcggcggaggggaccaaggtgacctccta	8
NORO-251A	Heavy	caggtgcagctggtggagctctgggggaggcgtggtccagcctgggagggtccctgagactctcctgtgc agcctctggattcaccttcaggagctatggcattcactgggtccgccaggctccaggcaaggggctgg agtgggtggcacttatatcatatgatggaagtagtaaatactatgcagactcagtgaagggccgattc accatctccagagacaattccaagaacacgttgatctgcaaataaacagcctgagaagtgaggaca cggctgtgtattactgtgcgaaagtgaggttgacaagttatagcattggctggttttcctttgactactgg ggccagggtaccctggtcaccgtctcctca	9
NORO-251A	Light	caggtgcagctggtggagctctgggggaggcgtggtccagcctgggagggtccctgagactctcctgtgc agcctctggattcaccttcaggagctatggcattcactgggtccgccaggctccaggcaaggggctgg agtgggtggcacttatatcatatgatggaagtagtaaatactatgcagactcagtgaagggccgattc accatctccagagacaattccaagaacacgttgatctgcaaataaacagcctgagaagtgaggaca cggctgtgtattactgtgcgaaagtgaggttgacaagttatagcattggctggttttcctttgactactgg ggccagggtaccctggtcaccgtctcctca	10
NORO-256A	Heavy	caggtgcagctggtggagctctgggggaggcgtggtccaggctgggagggtccctgagactctcctgtgc agcctctggattcaccttcagaagttatggcatgcactgggtccgccaggctccaggcaaggggctgg agtgggtggcaattatatcatatgatggaggtagtaaatactatgcagactccgtgaagggccgattca ccatctccagagacaattccaagaacacgttgatctgcaaataaacagcctgagagttgaggacacg gctctatattactgtgcgaaagatttttacgtgtatacagttatggttggcactctttgatatttggggc ctagggaccctggtcaccgtctcctca	11
NORO-256A	Light	cagtctgtgtgacgcagccgccctcagtgctctgcggccccaggacagagggtcaccatctcctgtct ggaagcagctccaacattgggacttattatgtctcctggtaccagcatctccaggaaacagccccaaa ctctcatttatgacaatgataagcgacctcagggattcctgaccgattctctgcctccaagtctggca cgtcagccaccctggacatcgccgactccaaactggggacgaggccgattattactgcggaacatgg gatctcagcctgactgctggctgggtgttcggcggaggggaccaagttgacctccta	12
NORO-278	Heavy	caggtgcagctggtggagctctgggggaggcgtggtccagcctgggagggtccctgagactctcctgtgc agcctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctgg agtgggtggcagttatatcatatgatggaagtaataaatactatgcagactccgtgaagggccgattc accatctccagagacaattccaagaacacgctgtatctgcaaataaacagcctgagagctgaggaca cggctgtgtattactgtgcgaaagttaccattatagcagcagctgatttgcttgactactggggccaggg aacctggtcaccgtctcctca	13

NORO- 278 light	cagtctgtgctgacgcagccgccccagtgctctggggccccagggcagagggtcaccatctcctgcact gggagcagctccaacatcggggcaggttatgatgtacactgggtaccagcagctccaggaacagccc ccaaactcctcatctatggtaacagcaatcgccctcaggggtccctgaccgattctctggctccaagt ctggcacctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagt cctatgacagcagcctgagtgggtccgggtggtattcggcggagggaaccaagctgaccgtccta	14
NORO- 279A Heavy	caggtgcagctgggtggagtctgggggaggcgtgggtccagcctgggaggtccctgagactctcctgcgc agcctctggattcaccttcacaaaatatggcatgcactgggtccgccagggtccaggcaaggggctgg agtgggtggcttttatagcatatgatggaagtgttaaatattatggagactccgcgaagggccgattcg ccatttcagagacaatccaagaatacagtatatctgcaaatgaacagcctgagagcagaagacac ggctgtatattattgtgcgaaagtggagatacactattattcgaatagtctcttaggtatggacgtctgg ggccaagggaccctgggtcaccgtctcctca	15
NORO- 279A Light	cagtctgtgctgactcagccaccctcagcgtctgggacccccgggcagagggtcaccatctcttgttctg gaggcaactccaacatcggaattagttatgtgtactgggtaccagcatctccaggaacggccccaaa ctcctcatatctaaaaataatctcgggccctcaggggtccctgaccgattctctgcctccaagtctggca cttcagcctccctggccatcagtggactccgggtccaggatgaggctgaatattactgtgcaacattgg atatcaatatgacctgggtattcggcggagggaaccaagctgaccgtccta	16
NORO- 296A Heavy	caggtgcagctgggtggagtctgggggaggcgtgggtcaagcctggagggtccctgagactctcctgtgc agcctctggattcaccttcagtgagtactacatgagctgggtccgccagggtccagggaaggcgctgg agtgggtttcatacattactagtagtttcagttccacaagctatgcagactctgtgaagggccgattcgt catctccagagacaacccaagaactcactgtatctacaaatgaacagcctgagagtcgaggacacg gccgtgtattactgtgcgagatatggcgaggatggttcgaggatcttacttcttgactggaggcc agggcacctgggtcaccgtctcctca	17
NORO- 296A Light	cagtctgtgctgacgcagccgccccagtgctctggggccccagagcagagggtcaccatctcctgcact gggagcagctccaacatcggggcaggttatgatgtacactgggtaccaggtgctccaggaatagcccc caactcctcatctacgataacaacaagcgccctcaggggtccctgaccgattctctggctccaagtc tggcacctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagt ctatgacagcaggctgagtagcaatgtggtattcggcggagggaacaaactgaccgtcctg	18
NORO- 303 Heavy	caggtgcagctgggtggagtctgggggaggcgtgggtccagcctgggaggtccctgagactctcctgtgc agcctctggattcatcttcagtagctatgctatgcactgggtccgccagggtccaggcaaggggctaga gtgggtggcagttatgtcatatgatggaataataaataactacgcagactccgtgaagggccgattca ccatctccagagacaattccaagaacacgctgtttctgcaaatgaacagcctgagacctgaggacacg gccgtgtattattgtgcgagagattgtcgagtcgggtgggtattcacctacgggtatggacgtctggggc caagggaccctgggtcaccgtctcctca	19
NORO- 303 Light	cagtctgcctgactcagcctccctccgctccgggtctcctggacagtcagtcaccatctcctgcactg gaaccagcagtgacgttggtggtataactatgtctcctgggtaccaacagcaccaggcaagcccc aaactcatgatgatgagatcagtaagcgccctcaggggtccctattcgcttctctggctccaagtctg gcaacacggcctccctgaccgtctctgggctccaggctgaggatgaggctgattattactgcagctcat atgcaggcacctacaactgtgtggtattcggcggagggaaccaagctgaccgtccta	20

NORO-310A	gaggtgcagctggaggagctctggggaggccttggtccggcctgggagtcctgagactctcctgtgta 21
Heavy	gcctctggattcagcgtcagtgacaactacatgagctgggtccgccaggctccagggaagggaactgca gtgggtctccgttatttatagcgggtggtactacattctacacagactccgtgaagggcagattcaccatc tccagagacaagtccaagaacacgctgtttcttcaaataaacagcctgagagccgaggacacggctg tgtattctgtacgcgagatccatcgcaatactatgatagtcgtggtcattactaccagactttcactccc tcctttgactcctggggccagggaaccctggtcaccgtctcctca
NORO-310A	gacatccagatgaccagctctccatctgtcatgtctgcatctgtaggagacagagtcaccatcacttgtc 22
Light	gggcgagtcaggacattagcgattatttagcctgggttcagcagaagccagggaagtccctaagcgc ctgatctatgtctgcatccagtttgcaaaatggggctcccatcaaggttcagcggcagtggtatgggaca gaattcactctcacaatcagcagcctgcagcctgaagattttgcaacttattactgtctacagcatgata cttaccctctcactttcggcggagggaaccaaggtggagatcaaa
NORO-312A	caggtgcagctggaggagctctggggaggccttggtcaagcctggagagtcctgagactctcctgtgc 23
Heavy	agcctctggattcaccttcagtgactctcatagagctggatccgccaggctccagggaagggaactgg agtgggttgcatattagtggtatcatgagttccaaaactacgcagattctgtgaaaggccgattca ccatctccagagacaacggcaagaactcagtgatctgcaaatgaacaacctgagagccgaggacac ggctgtgtactactgtgcaagagatgcccaatattgcagtggtgctacttgggtatttgactac tggggccagggaaccctggtcaccgtctcctca
NORO-312A	cagtctgtgctgacgcagccgcccctcagtgctctggggccccaggacagagggtcaccatctcttgact 24
Light	gggagcagctccaacatcggggcagggttatgaagtacactggtaccagcaacttcaggagcagccc ccaaactcatctatggttaacaacaatcgccctcaggggtccctgaccgattctctggctccaagt ctggcacctcagcctccctggccatcactgggctccaggctgacgatgaggctgattattactgccagt cttatgacaacaggctaagggtattcggcggggggaccaagctgaccgtccta
NORO-313.1	caggtgcagctggaggagctctggggaggccttggtcaagcctggagggtccctgagactctcctgtgc 25
Heavy	agcctctggattcaccttcagtgactctcatagagctggatccgccaggctccagggaaggggctgga gtgggttgggtacattagctctattgctggtgccaaaattacgcagactctgtgaagggccgattcac catctccagagacagctccaagaggctactgtatctgcaaatgaacagcctgagagtcgaggacacg gctgtatactactgtgcgagaatggggcgcttagagctggagcgacggccccattattactacctttg gacgtctggggccaagggaaccctggtcaccgtctcctca
NORO-313.1	gatattgtgatgaccagctctcactctccctgcccgtcaccctggagagccggcctcatctcctgca 26
Light	ggtctagtgcagcctcctgcatagtaatggatacaactatttgattggtactgcagaagccagggc agtctccacagctcctgatcttttgggttctaactcgggcctccgggtccctgacaggttcagtggcag tggatcaggcacagattttactgaaaatcagcagagtgagggtgaggatgttgggggtttattactg catgcaagctctacaaactcggacttttggccaggggaccaagctggagatcaaa

NORO-315B Heavy	caggtgcagctggtggagctctgggggaggcttggtcaagcctggagggtccctgagactctcctgtgtg 27 gcctctggattcaccttcagcgactactacatgacctggtccgccagactccaggagggggctggag tggctttcatacattagtgctatcgaggttacacagaatacgagactctgtgaagagccgattcatc atctctagagacaacggcaagaaatcactgtatcttcaatgaacagcctcagagccgaggactccg gtgtctattactgtgcgagagaagattgtcatggtactagtgtctactcgggcgactggggccaggga ccctggtcaccgtctctca
NORO-315B Light	cagtctgtctgacgcagccgacctcagtgctctggggcccagggcagagggtcaccatctcctgcact 28 gggagcagctccaacatcggggcaggttatgatgtacactggtagcagctccaggaaacagccc ccaaactcctcatctatcataacaccaatcgccctcgggggtccctgaccgattctctggtccaagt tggcacctcagcctacctgaccatcactgggtccaggctgaggatgaggctgattattactgccagtc atatgacagaagcctcagtaaactcgggggtgttcagcggagggaaccaagctgaccgtccta
NORO-317 Heavy	caggtgcagctggtggagctctgggggaggcttggtcaagcctggagggtccctgagactctcctgtgta 29 gcatctggattcaccttcagtgactactacatcagctgggtccgccaggctccagggaaggggctgga gtgggtttcatacattagtgctattaatagtatacaaaactatgcagactctgtgaggggccgattcacc gtctccagagacaacgccaagaagtcaagtcttttgcaaatgaatagcctgagagtcgacgacacggc tgtgtattattgtgcgagagatgtcggcggtcgggttcggtatagcagcaactcgaacaaaaaatccta caactacttcgaccctggggccagggaacccctggtcaccgtctctca
NORO-317 Light	tcctatgagctgacacagccaccctcgggtgtcagtgctcccaggacagacggccacgatcacctgtctc 30 gggagatgtttgtcaaaggagtttgcttattggtagcagagaagccaggccaggccctgtgtgtgt gatataaaagacaatgagaggccctcagggtatctgagcgattctctggtccacctcagggacaa cagtcacgttgacctcagtgaggtctggcagaagacgaggtcactattattgtcaatcagtagac accaggggcacttataaagtgttcggcgagggaaccaagctgaccgtccta
NORO-320 Heavy	caggtgcagctggtgcagctctgggcctgaggtgaagaagcctgggtcctcgggtgaaggctcctgcaa 31 ggcttctggaggcaccgtcagcagctatgctatcagctgggtgcgacaggcccctggacaagggttg agtggatgggagggatcatcccatctttgatacaaaaactacgcacagaaattccagggcagagtt acgattaccgcggacgaatccacgggcacaagcgacatggaactgagcagccttagatctgaggaca cggccgtgtattactgtgcgagagatcgcgtcccgtcttattctccttcgagggtttccactaaggg ggcgtatgggggaaaatacgggtatggagctctggggccaagggaacctggtcaccgtctctca
NORO-320 Light	gatattgtgatgactcagctccactctccctgcccgtcaccctggagagccggcctcatctcctgna 32 ggtctagtcagagcctcctgcatagtaatggatacaactatttgattggtacctgcagaagccagggc agtctccacagctcctgatctatttgggttctaactcgggcctccgggtccctgacaggttcagtggcag tggatcaggcacagattttactgaaaatcagcaaagtggaggctgaggatgttggggtttattactg catgcaagctctacaaactcctcgacgttcggccaagggaaccaaggtggaaatcaaa

NORO-329A Heavy	caggtgcagctggtggagctctgggggaggcttggtcaagcctggagggtccctgagactctcctgtgc agcctctggattcaccttcagtactactacatgagctggatccgccaggctccagggaaggggctgg agtggatttcataatattagcagtagtttgggtccacagactacgcaggctctatgaaggccgattcac catctccagagacaacccaagaattcactgtatctgcaaatgaacagcctgagagccgaggacacg gctgtgtattactgtgcgagatacaattactatggttcggggagtttctcttgactactggggccagg ggacctggtcaccgtctcctca	33
NORO-329A Light	cagtctgtggcgactcagccaccctcagcgtctgggacccccgggcagagggtcaccatctctgttct gggagcagctccaacatcggaagtaatactgtaagctggtaccagcaactcccaggaacggcccca aactcctcatttatagtaatgatcagcggccctcagggtccctggccgattctctggctccaagtctgg cacctcagcctccctggccatcagtggtccagctgaggatgagggtgattattcctgtgcagcatg ggatgacagcctgaatggttgggtgttcggcgagggaaccaagctgaccgtccta	34
NORO-118 Heavy	caggtgcagctggtggagctctgggggaggcgtggtccagcctgggggggtccctgagactctcctgtac agcgtctggattcaccttcagtgtcatggcatgcactgggtccgccaggctccaggcaaggggctgg aatgggtgacatttatatcatatgatggaagtaataaattctatcgagactcagtgaaggccgattca tcctctccagagacaattccgagaacacgttgttctgcagatgaacagcctgagaccggaagacacg ggagtctattggtgtgcgagagatggttacagaaatttggtcctcgttgggtggtacttcgatctctggg gccgtggcacctggtcaccgtctcctca	207
NORO-118 Light	Not available	208
NORO-232 Heavy	Not available	209
NORO-232 Light	gagattgtgctgacccaaagtcccgtacacttagcctgtctcctggagattctgctacactgagctgta gagcttctcaggctgtgtctaccacatcttgccctggtatcagcagacaagaggacaagcccctaga ctgctgattcacggcacctacacaagagctatcggcattcctgacaagtttctggaaccggtctggc accgattttaccctgacatctctggacttgccctgaggattttgctgtgtactactgccagcagtacag ctctagcccttacaccttggacagggcaccaagatcgagatcacc	210
NORO-246A Heavy	Not available	211
NORO-246A Light	agcaacgaactgacacaagcccccttctgtgtctgtgtctcctggacaaacagccagaatcacctgcttc ggagatgctctggctaaccagtacagctactggtaccagagaaaaccaggacagggtccagtgtg tgatctacaaggacagcgagagacctagcggaattcctcagagattttctgcctccaatagcggcacc acagtgacactgacatcacaggagtgcaggctgaagatgaagccgatttctgtcagagcgccg attctacaggcatctacaaggtgttggcgggcgacaaaactgacgttctg	212
NORO-263 Heavy	caggtgcagctggtggagctctgggggaggcttggtcaagcctggagggtccctgagactctcctgtgc agcctctggattcaccttgagtactactacatgagttggatccgccaggctccagggaaggggctgg agtgggttgcatatcagtagtagtcatacttccatactacagagactctgtgaaggccgattca ccatctccagggaacagccaagaagtcactgtatctgcaaatgaacagcctgagagtcgaggacac ggctatatattactgtgcgagagattgggtagggtgtaggagcgctatagtaccactggggccagg gaacctggtcaccgtctcctca	213

NORO- 263 Light	tcctatgagctgacacagccaccctcggtgtcagtggtccccaggacaaacggccaggatcacctgtctt 214 ggagatgcgttgccaagaagacctgcttttggatcagcagaagtcaggccaggcccctgtgttggtc atgtatgacgacagcaaacgacctccggggtccctgagagattctctggctccagctcaggacagt ggccaccctgactattagtggggcccagggtggaggatgaagctgactaccctgttactcaacagaca gcagtggttaaggaaagagtttccggcggagggaccaagctgaccgtctc
NORO- 273A Heavy	caggctccagctggtgcagctctggggctgaggtgaagaagcctggggcctcagtgaataattctgcaa 215 ggcttctggatacgacttcactagtttctatgcattgggtgcgcctgggccccggacaaaggcttgag tggatgggatggatcagcgctgccaatggtaacacaaaatattcacagaagttccaggacagattcac cattaccagggacacatccgcgagtatagtcacatggagttgagtagcctgagatctgaagacacgg ctgtatactactgtgcgaggggggggtatagtagtgggctgtacagatattccaactactactacggta tgaccgtctggggccaagggacacctggtcaccgtctcctca
NORO- 273A Light	gatattgtgatgaccagactccactctctgtccgtcaccctggacagccggcctccatctcctgca 216 agtctagttagagcctcctgcataatgatcgaataacatttggtttggtagctgcagaagccaggcc agcctccacaactctgatctttcaagtctcaaccggttctctggagtgcagataggttcagtggcag cggatcagggacagacttcacactgagaatcagccgtgtggaggctgaggatgttggatttattact gcttgcaactacacagcttccgctcactttcggcggagggaccaaggtggagatcaaa
NORO- 316 Heavy	Not available 217
NORO- 316 Light	Not available 218
NORO- 318 Heavy	Not available 219
NORO- 318 Light	gatattgtgatgactcagctctccactctccctgcccgtcaccctggagagccggcctccatctcctgca 220 ggctagttagagcctcctcacagtgtgactcaactatttggattggtacgtgtaagccagggc agcctccacagctcctgatctatttggcttctaatacgggcctccggggcccctgacaggttcagtggcag tggatcaggcacagattttacactgacaatcagcagagtggaggctgaggatgttggatttattactg catggaagctctacaaactcctcccactttcggcggagggaccaaggtggagatcaaa
NORO- 327A Heavy	Not available 221
NORO- 327A Light	cagctgtgtgctgacgcagtcgccctcagtgcttggggcccagggcagagggtcaccatctcctgcact 222 gggaccagctccaacatcggggcaggttatgatgtacactgggtatcagcagtttctggaacagcccc caaaactcctcatctctataacaccaatcgccctcagggtccctgaccgattctctggctccaagtct ggcacctcagctcctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcc tttgacagcagcctcggggttccagggtgttcggcggagggaccaagctgaccgtccta

NORO-156.3	Heavy	caggtgcagctacagcagtggggcgaggactgttgaagccttcggagaccctgtccctcacctgcgc tgtctatgggtgggtccttcagtggttactactggagctggatccgccagccccaggggaaggggctgga gtggattggggaaatcaatcatagtgaagcaccaactacaacccgtccctcaagagtcgagtcacc atatcagtagacacgtccaagaaccagttctccctgaagctgagctctgtgaccgccgaggacacggc tgtgtattactgtgcgagaggcttaatggacgtctggggccaagggaaccctggtcaccgtctcctca gccatccggatgaccagctctcatctctattctctgcatctacaggagacagagtcacatcacttgcgggcga gtcagggtattagcagttatttagcctggtatcagcaaaaaccagggaaagcccctaagctcctgatctatgctg catccactttgcaaagtgggggtcccatcaaggttcagcggcagtggtatctgggacagatttcaactctcaccatca gctgcctgcagctgaagattttgcaacttattactgtcaacagattatagttacccctgcacttttggccagggg accaagctggagatcaaac	288
NORO-156.3	Light	gcatccggatgaccagctctcatctctattctctgcatctacaggagacagagtcacatcacttgcgggcga gtcagggtattagcagttatttagcctggtatcagcaaaaaccagggaaagcccctaagctcctgatctatgctg catccactttgcaaagtgggggtcccatcaaggttcagcggcagtggtatctgggacagatttcaactctcaccatca gctgcctgcagctgaagattttgcaacttattactgtcaacagattatagttacccctgcacttttggccagggg accaagctggagatcaaac	289
NORO-168.2	Heavy	caggtgcagctggaggctctgggggaggcggtgtccagcctgggaggtccctgagactctcctgtgcagcctct ggattcaccttcagtagctatgctatgcaactgggtccgccaggctccaggcaaggggctagagtggtggcagtt atatcatatgatggaagtaataaatactacgcagactccgtgaaggccgattcacatctccagagacaattcc aagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggctgtgtattactgtgcgaggtcag tattgggtactactactacggtatggacgtctggggccaagggaaccctggtcaccgtctcctca cagcctgtgctgactcagccaccttctgcatcagcctccctgggagcctcggtcacactcacctgcacccctgagca gcggctacagtaattataaagtggactggtagcagcagagaccaggggaagggcccccggtttgtgatgcgagtg ggcactggtgggattgtgggatccaagggggatggcatccctgatcgcttctcagctcttgggctcaggcctgaat cggtaactgacatcaagaacatccaggaagaggatgagagtgactaccactgtggggcagaccatggcagtg ggatcctattcggcggagggaaccaagctgaccgtcctag	290
NORO-168.2	Light	cagcctgtgctgactcagccaccttctgcatcagcctccctgggagcctcggtcacactcacctgcacccctgagca gcggctacagtaattataaagtggactggtagcagcagagaccaggggaagggcccccggtttgtgatgcgagtg ggcactggtgggattgtgggatccaagggggatggcatccctgatcgcttctcagctcttgggctcaggcctgaat cggtaactgacatcaagaacatccaggaagaggatgagagtgactaccactgtggggcagaccatggcagtg ggatcctattcggcggagggaaccaagctgaccgtcctag	291
NORO-178.6	Heavy	caggtgcagctggaggctctgggggaggcggtgtccagcctgggaggtccctgagactctcctgtgcagcctct ggattcaccttcagtagctacggatgaactgggtccgccaggctccaggcaggggactggagtggtggcaga tatatcatatgatggaagtaataagtcgctatgcagactccgtgaaggccgattcacatctccagagacaattc caagaacacgctatctctgcagatgaacggcctgagaactgaggacacggctgtgtattattgtgcgaagatt ggatattggcaatggctgtgcagcttttgactcctggggccccgggaaccctggtcaccgtctcctcag tcttctgagctgactcaggaccctgttgtgtctgtggccttgggacagacagtcaggatcacatgccaaaggagac agcctcacacaccttttatgaagctggttcagcagaagccaggacaggccccctgacttctgtatgtgtaac gacaaccggccctcagggatccagaccgattctctggctccaggtcaggaaacacagcttcttgaccatcact ggggctcaggcgggaagatgaggctgactattactgtaactccgggacagcagtggttaaaccctcattcggcgg agggaccaagctgaccgtcctaa	292
NORO-178.6	Light	tcttctgagctgactcaggaccctgttgtgtctgtggccttgggacagacagtcaggatcacatgccaaaggagac agcctcacacaccttttatgaagctggttcagcagaagccaggacaggccccctgacttctgtatgtgtaac gacaaccggccctcagggatccagaccgattctctggctccaggtcaggaaacacagcttcttgaccatcact ggggctcaggcgggaagatgaggctgactattactgtaactccgggacagcagtggttaaaccctcattcggcgg agggaccaagctgaccgtcctaa	293
NORO-279A	Heavy	caggtgcagctggaggctctgggggaggcggtgtccagcctgggaggtccctgagactctcctgcgcagcctct ggattcaccttcacaaaatatggcatgcactgggtccgccaggctccaggcaaggggctggagtggtggcctttt atagcatatgatggaagtgttaaatattatggagactccgcgaaggccgattcgccatttcagagacaatccc aagaatacagtatatctgcaaatgaacagcctgagagcagaagacacggctgtatattattgtgcgaagtgga gatacactattattcgaatagctcttaggtatggacgtctggggccaagggaaccctggtcaccgtctcctca cagctgtgtgctgactcagccacccctcagcgtctgggacccccgggacaggggtcaccatctctgttctggaggc aactccaacatcggaattagtgtgtactggtaccagcatctccaggaaacggcccccaactcctcatatcta aaaataatctcgccctcagggtccctgaccgattctctgctccaagcttggtcacttcagcctcctggccat cagtgagctccggtccgaggatgaggctgaatattactgtgaacattggatatcaatatgacctgggtattcgg cggagggaaccaagctgaccgtcctaa	294
NORO-279A	Light	cagctgtgtgctgactcagccacccctcagcgtctgggacccccgggacaggggtcaccatctctgttctggaggc aactccaacatcggaattagtgtgtactggtaccagcatctccaggaaacggcccccaactcctcatatcta aaaataatctcgccctcagggtccctgaccgattctctgctccaagcttggtcacttcagcctcctggccat cagtgagctccggtccgaggatgaggctgaatattactgtgaacattggatatcaatatgacctgggtattcgg cggagggaaccaagctgaccgtcctaa	295
NORO-323A	Heavy	caggtgcagctggaggctctgggggaggcggtgtccagcctgggaggtccctgcgactctcctgtgcagcctct ggattcaccttcagtggtcttctactgcactgggtccgccaggctccaggcaaggggctggagtggtggcagct atatcatatgatggaactaatacactatgcagactccgtgaaggccgattcacctctccagagacaattcc aagaacacgctgtatctgcagatgaacagcctgagagctgaggacacggctgtgtattactgtgcgaaccagct cctttccccctttgactactggggccagggaaccctggtcaccgtctcctcag	296

NORO- cagtctgccttgactcagcctgcctccgtgtctgggtctcctggacagtcgatcaccatctcctgcactggaacca 297
 323A gcagtgcgttggtggttataattatgttcttggtaccaacaacacccaggcaagcccccaactcttgattta
 Light tgaggtcagtaatcgccctcaggggttctagacgttctctggctccaagtctggcaacacggcctcctgacc
 atctctgggctccaggctgaggacgaggctgattattactgctgctcatataccagcagcagcactgaggtgttc
 ggaggaggaccagctgaccgtcctag

TABLE 2 – PROTEIN SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	Variable Sequence	SEQ ID NO.
NORO-115 Heavy	QVQLVESGGGLVKPGGSLRLSCTASGITISGYMSWIRQAPGKGLEWIVYINTSG RTIYYADSVKGRFSVSRDNAKESLYLQMDSLTVDDTGIYYCARDRLPASGSHWFH PWGQGTLTVSS	35
NORO-115 light	DIVMTQSPDSLAVSLGGRATINCKSSQSVLYTSDNKNYLAWYQQPGQPPKLLIS WASTRESGVPDRFSGSGSGTDFLTISNLQAEDVAVYYCQYYNSPLAFGGGTK VEIK	36
NORO-123 Heavy	QVQLVESGGGVVQPGRSLRLSCTSGFTFSQYPMHWVRQAPGKGLEWVALISY DGMNKYYADSVRGRFTISRDNSENTQYLQMNSLRGDDTAVYYCARVTGDCTGN RCSYWAYYYYGLDVWGQGTTLTVSS	37
NORO-123 light	QSALTQPPSASGSPGQSVTISCTGTSSDVGGYKYVSWYQQHPGKAPKLMIEVT RRPSGVPDRFSGSKSGNTAFLTVSGLQAEDVAVYYCGSYAGSTTSGYVFGTGKVI VL	38
NORO-202A.1 Heavy	QVQLVQSGAEVKKPGASVKVSCKASEYFTGYLQWVRQAPGHGLEWMGWIN PSGGTKYSQKFQGRVTMTRDTSITTVYMELSRLRSDDTAVYYCARDLLRNWGDH DAFDVWGQGTMTMTVSS	39
NORO-202A.1 light	QSVLTQPPSAETPGQGVTISCSGSSSNIGSNFVCWYQQVPGTAPKLLIYKNNQR PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCSAWDDSLSGPVFGGGTKLTVL	40
NORO-250B Heavy	QVQLVESGGGLVKPGGSLTSCAASGFTFSDYYMNWIRQAPGKRLEWVSYSISGI MSSTNYADSVKGRFTISRDTAKKSVYLQMNSLRAEDTAVYYCARATSQGATSYF DSWGQGTTLTVSS	41
NORO-250B Light	QSVLTQPPSVSGAPGQRTISCTGSSSNIGADYDVHWYQQLPGAAPRLIYADIN RPSGVPDRFSGSKSGTSASLAITGLQAEDVAVYYCQSYDRSVSGSAVFGGGTKVT VL	42
NORO-251A Heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYGIHWVRQAPGKGLEWVALISYD GSSKYYADSVKGRFTISRDNKNTLYLQMNSLRSEDTAVYYCAKVRLLTSYIGWFS FDYWGQGTTLTVSS	43
NORO-251A Light	QSVLTQPPSVSAAPGQKVTISCSGSSSNIANYYVSWYQQLPGRAPKLLIYDNNKR PSGIPDRISGSKSGTSATLGITGLQTGDEADYYCGTWDTLSAWVFGGGTKLTVL	44

NORO- 256A Heavy	QVQLVESGGGVVQAGRSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVAIISY DGGSKYYADSVKGRFTISRDN SKNTLYLQMNSLRVEDTALYYCAKDFLRVYSYGW HSFDIWGLGLTVTVSS	45
NORO- 256A Light	QSVLTQPPSVSAAPGQRTISCSGSSSNIGTYVSWYQHLPGTAPKLLIYDNDKRP SGIPDRFSASKSGTSATLDIAGLQTGDEADYYCGTWDLSTAGWVFGGGTKLTVL	46
NORO- 278 Heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKVTHIAAADLLD YWGQGT LTVTVSS	47
NORO- 278 light	QSVLTQPPSVSGAPGQRTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSN RPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSPVVFSGGGTKLTV L	48
NORO- 279A Heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFTKYGMHWVRQAPGKGLEWVAFIAY DGSVKYYGDSAKGRFAISRDNPKNTVYLQMNSLRAEDTAVYYCAKVEIHYYNSL LGMDVWGQGT LTVTVSS	49
NORO- 279A Light	QSVLTQPPSASGTPGQRTISCSGGNSNIGISYVYQHLPGTAPKLLISKNNLRP SGVPDRFSASKSGTSASLAISGLRSEDEAEYYCATLDINMTWVFGGGTKLTVL	50
NORO- 296A Heavy	QVQLVESGGGLVKPGGSLRLSCAASGFTFSEYYMSWVRQAPGKALEWVSYITSS FSSTSYADSVKGRFVISRDNAKNSLYLQMNSLRVEDTAVYYCARYGAEYGSRSFYF LDWGQGT LTVTVSS	51
NORO- 296A Light	QSVLTQPPSVSGAPEQRTISCTGSSSNIGAGYDVHWYQVLPGIAPKLLIYDNNKR PSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSRLSSNVVFSGGGTKLTVL	52
NORO- 303 Heavy	QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGKGLEWVAVMS YDGN NKYYADSVKGRFTISRDN SKNTLFLQMNSLRPEDTAVYYCARD CRVGVWF TYGMDVWGQGT LTVTVSS	53
NORO- 303 Light	QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMMYEIS KRPSGVPIRFSGSKSGNTASLTVSGLQAEDEADYYCSSYAGTYNCVVFSGGGTKLTV L	54
NORO- 310A Heavy	EVQLVESGGGLVRPGESLRLSCVASGFSVDNYMSWVRQAPGKGLQWVSVIYS GGTTFYTD SVKGRFTISRDKSKNTLFLQMNSLRAEDTAVYFCTRDPSQYYDSRGH YYQTFTPSFDSWGQGT LTVTVSS	55
NORO- 310A Light	DIQMTQSPSVMSASVGDRTITCRASQDISDYLAWFQQKPGKVPKRLIYAASSLQ NGVPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHDTYPLTFGGGTKVEIK	56

NORO- 312A Heavy	QVQLVESGGALVKPGESLRLSAASGFTFSDSYMSWIRQAPGKGLEWVAYISGI MSSTNYADSVKGRFTISRDNKGNSVYLQMNNLRAEDTAVYYCARDAYCSGGR CYLVFDYWGGQGLTVTVSS	57
NORO- 312A Light	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYEVHWYQQLPGAAPKLIYGNNN RPSGVPDRFSGSKSGTSASLAITGLQADDEADYYCQSYDNRLRVFGGGTKLTVL	58
NORO- 313.1 Heavy	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDFYMSWIRQAPGKGLEWVGYISSIA GATNYADSVKGRFTISRDSKRSLYLQMNSLRVEDTAVYYCARMGRLELERPHY YYPLDVWGQGLTVTVSS	59
NORO- 313.1 Light	DIVMTQSPLSLPVTGPGEASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIFLG SNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTRTFGQGTKLEIK	60
NORO- 315B Heavy	QVQLVESGGGLVKPGGSLRLSCVASGFTFSDYYMTWFRQTPGRGLEWLSYISAIG GYTEYADSVKSRFIISRDNKGKSLYLQMNSLRAEDSGVYYCAREDCHGTSCYSGD WGQGLTVTVSS	61
NORO- 315B Light	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYHNTN RPSGVPDRFSGSKSGTSAYLTITGLQAEDEADYYCQSYDRSLSKSRVFSGGTKLTVL	62
NORO- 317 Heavy	QVQLVESGGGLVKPGGSLRLSCVASGFTFSDYYISWVRQAPGKGLEWVSYSISAIN SYTNYADSVRGRFTVSRDNAKKSFLQMNSLRVDDTAVYYCARDVGGSVRYSSN SNKKSYNYFDPWGQGLTVTVSS	63
NORO- 317 Light	SYELTQPPSVSVSPGQTATITCSGDVLSKEFAYWYQQKPGQAPVLVIYKDNERPS GIPERFSGSTSGTTVTLTISGVLAEDEAHYYCQSDTRGTYKVFGGGTKLTVL	64
NORO- 320 Heavy	QVQLVQSGPEVKKPGSSVKVSCKASGGTVSSYAIWVRQAPGQGLEWMGGIPI FDTTNYAQKFQGRVTITADESTGTSDMELSSLRSEDYAVYYCARDRVPSYSPSRRF STKGAMWGKYGMDVWGQGLTVTVSS	65
NORO- 320 Light	DIVMTQSPLSLPVTGPGEASISXRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDFTLKISKVEAEDVGVYYCMQALQTPRTFGQGTKVEI K	66

NORO- 329A Heavy	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDDYMSWIRQAPGKGLEWISYISSSF GSTDYAGSMKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARYNYYGSGSFVF DYWGQGTLVTVSS	67
NORO- 329A Light	QSVATQPPSASGTPGQRVTISCSGSSSNIGSNTVSWYQQLPGTAPKLLIYSNDQR PSGVPGRFSGSGSGTSASLAISGLQSEDEGDYSCAAWDDSLNGWVFGGGTKLTV L	68
NORO- 118 Heavy	Not available	223
NORO- 118 Light	Not available	224
NORO- 232 Heavy	Not available	225
NORO- 232 Light	EIVLTQSPATLSLSPGDSATLSCRASQAVSTTYLAWYQQTRGQAPRLLIHGTYTRAI GIPDKFSGTGSFTDLTISGLAPEDFAVYYCQYSSSPYTFGQGTKEIT	226
NORO- 246A Heavy	Not available	227
NORO- 246A Light	SNELTQAPSVSVSPGQTARITCFGDALANQYSYWYQRKPGQAPVLVIYKDSERPS GIPQRFASNSGTTVTLITGVQAEDEADYFCQSADSTGIYKVFGGGTKLTVL	228
NORO- 263 Heavy	QVQLVESGGGLVKPGGSLRLSCAASGFTLSDDYMSWIRQAPGKGLEWVAYISSS HTSTYYRDSVKGRFTISRDSAKKSLYLQMNSLRVEDTAIYYCARDWVGGRSAYSD HWGQGTLVTVSS	229
NORO- 263 Light	SYELTQPPSVSVSPGQTARITCSGDALPRRPAFWYQQKSGQAPVLVMYDDSKRP SGIPERFSGSSSGTVATLTISGAQVEDEADYHCYSTDSSGKERVFGGGTKLTVL	230
NORO- 273A Heavy	QVQLVQSGAEVKKPGASVKISKASGYDFTSFPMHWVRLGPGQRLEWMGWIS AANGNTKYSQKFQDRFTITRDTSASIVHMESSLRSEDVAVYYCARGGYSSGLYRY SNYYYGMTVWGQGTLVTVSS	231
NORO- 273A Light	DIVMTQTPLSLSVTPGQPASISCKSSESLHNDRKTYLFWYLQKPGQPPQLLIFQV SNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCLQTTQLPLTFGGGKVEIK	232
NORO- 316 Heavy	Not available	233
NORO- 316 Light	Not available	234

NORO-318	Not available	235
Heavy		
NORO-318	DIVMTQSPSLPVTGPGEASISCRSSQSLLHSDGLNYLDWYLLKPGQPPQLLIYLAS	236
Light	NRASGAPDRFSGSGSGTDFTLTISRVEAEDVGIYYCMEALQTPPTFGGGTKVEIK	
NORO-327A	Not available	237
Heavy		
NORO-327A	QSVLTQSPSVSGAPGQRTISCTGTSSNIGAGYDVHWYQQFPGTAPKLLISHNTN	238
Light	RPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSFDSLGRSRVFGGGTKLTVL	
NORO-156.3	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHS	298
Heavy	GSTNYPNPSLKSRTISVDTSKNQFSLKSSVTAADTAVYYCARGLMDVWGQGT	
Light	VTVSS	
NORO-156.3	AIRMTQSPSSFSASTGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASLTQS	299
Light	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYSSYPRTFGQGKLEIK	
NORO-168.2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKLEWVAVISY	300
Heavy	DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSVIGYYYYGM	
Light	DVWGQGT LVT VSS	
NORO-168.2	QPVLTPPPSASASLGASVTLTCTLSSGYSNYKVDWYQQRPKGPRFVMRVGTG	301
Light	GIVGSKGDGIPDRFSVLGSGLNRYLTIKNIQEEDESDYHCGADHGSGILFGGGTKLTVL	
NORO-178.6	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMNWVRQAPGRGLEWVADISY	302
Heavy	DGSNSRYADSVKGRFTISRDN SKNTLSLQMNLRTEDTAVYYCAKDWYLAMAG	
Light	AAFDSWGP GT LVT VSS	
NORO-178.6	SSELTQDPVVSVALGQTVRITCQGDSLTTFYASWQQKPGQAPALVLYGNDNRP	303
Light	SGIPDRFSGSRSGNTASLTITGAQAEDEADYYCNSRDSSGKPSFGGGTKLTVL	
NORO-279A	QVQLVESGGGVVQPGRSLRLSCAASGFTFTKYGMHWVRQAPGKLEWVAFIAY	304
Heavy	DGSVKYYGDSAKGRFAISRDNPKNTVYLQMNSLRAEDTAVYYCAKVEIHYYNSL	
Light	LGMDVWGQGT LVT VSS	
NORO-279A	QSVLTQPPSASGTPGQRTISCSGGNSNIGISYVYQYHLPGTAPKLLISKNNLRP	305
Light	SGVPDRFSASKSGTSASLAISGLRSEDEAEYYCATLDINMTWVFGGGTKLTVL	
NORO-323A	QVQLVESGGGVVQPGRSLRLSCAASGFTFSGSLHWVRQAPGKLEWVAAISY	306
Heavy	DGTNTYYADSVKGRFTLSRDNSKNTLYLQMNSLRAEDTAVYYCAKPVLSFPDYW	
Light	GQGT LVT VSS	
NORO-323A	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLLIYEVSN	307
Light	RPSGVSRRFSGSKSGNTASLTISGLQAEDEADYYCCSYTSSSTEVEFGGGTKLTVL	

TABLE 3 – CDR HEAVY CHAIN SEQUENCES

Antibody	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
NORO-115	GITISGY (69)	INTSGRTI (70)	ARDRLPASGSHWFHP (71)
NORO-123	GFTFSQYP (72)	ISYDGMNK (73)	ARVTGDCTGNRCSYWAYYYYGLDV (74)
NORO-202A.1	EYFTFGYY (75)	INPSGGT (76)	ARDLLRNWGDHDAFDV (77)
NORO-250B	GFTFSDYY (78)	ISGIMSST (79)	ARATSQGATSYYFDS (80)
NORO-251A	GFTFRSYG (81)	ISYDGSSK (82)	AKVRLTSYSIGWFSFDY (83)
NORO-256A	GFTFRSYG (84)	ISYDGGSK (85)	AKDFLRVYSYGWHSFDI (86)
NORO-278	GFTFSSYG (87)	ISYDGSNK (88)	AKVTIIAAADLLDY (89)
NORO-279A	GFTFTKYG (90)	IAYDGSVK (91)	AKVEIHYYSNSLLGMDV (92)
NORO-296A	GFTFSEYY (93)	ITSSFST (94)	ARYGAEYGSRSFYFLD (95)
NORO-303	GFIFSSYA (96)	MSYDGNK (97)	ARDCRVGWVFTYGMVDV (98)
NORO-310A	GFSVSDNY (99)	IYSGGTT (100)	TRDPSQYYDSRGHYYQTFTPSFDS (101)
NORO-312A	GFTFSDSY (102)	ISGIMSST (103)	ARDAQYCSGGRCYLVDY (104)
NORO-313.1	GFTFSDFY (105)	ISSIAGAT (106)	ARMGRLELERRPHYYPPLDV (107)
NORO-315B	GFTFSDYY (108)	ISAIGGYT (109)	AREDCHGTSCYSGD (110)
NORO-317	GFTFSDYY (111)	ISAINSYT (112)	ARDVGGSVRYSSNSNKKSYNYFDP (113)
NORO-320	GGTVSSYA (114)	IPIFDTT (115)	ARDRVPSYSPSRFSTKGAMWGKYGMDV (116)
NORO-329A	GFTFSDYY (117)	ISSFGST (118)	ARYNYYGSGSFVFDY (119)
NORO-118	GFTFSGHG (239)	ISYDGSNK (240)	ARDGYRNLVLVGWYFDL (241)
NORO-232	Not available (242)	Not available (243)	Not available (245)
NORO-246A	Not available (246)	Not available (247)	Not available (248)
NORO-263	GFTLSYY (249)	ISSHTST (250)	ARDWVGGRSAYS DH (251)
NORO-273A	GYDFTSFP (252)	ISAANGNT (253)	ARGGYSSGLYRYSNYYYGMTV (254)
NORO-316	Not available (255)	Not available (256)	Not available (257)

NORO-318	Not available (258)	Not available (259)	Not available (260)
NORO-327A	Not available (261)	Not available (262)	Not available (263)
NORO-156.3	GGSFSGYY (308)	INHSGST (309)	ARGLMDV (310)
NORO-168.2	GFTFSSYA (311)	ISYDGSNK (312)	ARSVIGYYYYGMDV (313)
NORO-178.6	GFTFSTYG (314)	ISYDGSNS (315)	AKDWYLAMAGAAFD (316)
NORO-279A	GFTFTKYG (317)	IAYDGSVK (318)	AKVEIHYYNSLLGMD (319)
NORO-323A	GFTFSGSS (320)	ISYDGTNT (321)	AKPVLSPFDY (322)

TABLE 4 – CDR LIGHT CHAIN SEQUENCES			
Antibody	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
NORO-115	QSVLYTSDNKNY (120)	WAS (121)	QQYYNSPLA (122)
NORO-123	SSDVGGYKY (123)	EVT (124)	GSYAGSTTSGYV (125)
NORO-202A.1	SSNIGSNF (126)	KNN (127)	SAWDDSLSGPV (128)
NORO-250B	SSNIGADYD (129)	ADI (130)	QSYDRSVSGSAV (131)
NORO-251A	SSNIANY (132)	DNN (133)	GTWDTLSAWV (134)
NORO-256A	SSNIGTY (135)	DND (136)	GTWDLSTAGWV (137)
NORO-278	SSNIGAGYD (138)	GNS (139)	QSYDSSLSPV (140)
NORO-279A	NSNIGISY (141)	KNN (142)	ATLDINMTWV (143)
NORO-296A	SSNIGAGYD (144)	DNN (145)	QSYDSRLSSNVV (146)
NORO-303	SSDVGGYNY (147)	EIS (148)	SSYAGTYNCVV (149)

NORO-310A	QDISDY (150)	AAS (151)	LQHDTYPLT (152)
NORO-312A	SSNIGAGYE (153)	GNN (154)	QSYDNRLRV (155)
NORO-313.1	QSLHNSNGYNY (156)	LGS (157)	MQALQTRT (158)
NORO-315B	SSNIGAGYD (159)	HNT (160)	QSYDRSLSKSRV (161)
NORO-317	VLSKEF (162)	KDN (163)	QSVDRGTYKV (164)
NORO-320	QSLHNSNGYNY (165)	LGS (166)	MQALQTPRT (167)
NORO-329A	SSNIGSNT (168)	SND (169)	AAWDDSLNGWV (170)
NORO-118	Not available (264)	Not available (265)	Not available (266)
NORO-232	QAVSTTY (267)	GTY (268)	QQYSSPYT (269)
NORO-246A	ALANQY (270)	KDS (271)	QSADSTGIYKV (272)
NORO-263	ALPRRP (273)	DDS (274)	YSTDSSGKERV (275)
NORO-273A	ESLLHNDKTY (276)	QVS (277)	LQTTQLPLT (278)
NORO-316	Not available (279)	Not available (280)	Not available (281)
NORO-318	QSLHSDGLNY (282)	LAS (283)	MEALQTPPT (284)
NORO-327A	SSNIGAGYD (285)	HNT (286)	QSFSSLRGSRV (287)
NORO-156.3	QGISSY (323)	AAS (324)	QQYSSYPRT (325)
NORO-168.2	SGYSNYK (326)	VGTGGIVG (327)	GADHGSGL (328)

NORO- 178.6	SLTTFY (329)	GND (330)	NSRDSSGKPS (331)
NORO- 279A	NSNIGISY (332)	KNN (333)	ATLDINMTWV (334)
NORO- 323A	SSDVGGYNY (335)	EVS (336)	CSYTSSSTEY (337)

* * * * *

5 All of the compositions and methods disclosed and claimed herein can be made and
executed without undue experimentation in light of the present disclosure. While the
compositions and methods of this disclosure have been described in terms of preferred
embodiments, it will be apparent to those of skill in the art that variations may be applied to
the compositions and methods and in the steps or in the sequence of steps of the method
described herein without departing from the concept, spirit and scope of the disclosure. More
specifically, it will be apparent that certain agents which are both chemically and
physiologically related may be substituted for the agents described herein while the same or
10 similar results would be achieved. All such similar substitutes and modifications apparent to
those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure
as defined by the appended claims.

VII. REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent 3,817,837

U.S. Patent 3,850,752

U.S. Patent 3,939,350

U.S. Patent 3,996,345

U.S. Patent 4,196,265

U.S. Patent 4,275,149

U.S. Patent 4,277,437

U.S. Patent 4,366,241

U.S. Patent 4,472,509

U.S. Patent 4,554,101

U.S. Patent 4,680,338

U.S. Patent 4,816,567

U.S. Patent 4,867,973

U.S. Patent 4,938,948

U.S. Patent 5,021,236

U.S. Patent 5,141,648

U.S. Patent 5,196,066

U.S. Patent 5,563,250

U.S. Patent 5,565,332

U.S. Patent 5,856,456

U.S. Patent 5,880,270

U.S. Patent 6,485,982

Atmar *et al.*, *Curr Opin Infect Dis.*, 2018.

“Antibodies: A Laboratory Manual,” Cold Spring Harbor Press, Cold Spring Harbor, NY, 1988.

Abbondanzo *et al.*, *Am. J. Pediatr. Hematol. Oncol.*, 12(4), 480-489, 1990.

Allred *et al.*, *Arch. Surg.*, 125(1), 107-113, 1990.

Atherton *et al.*, *Biol. of Reproduction*, 32, 155-171, 1985.

Barzon *et al.*, *Euro Surveill.* 2016 Aug 11; 21(32).

- Beltramello *et al.*, *Cell Host Microbe* 8, 271-283, 2010.
- Brown *et al.*, *J. Immunol. Meth.*, 12;130(1), :111-121, 1990.
- Campbell, *In: Monoclonal Antibody Technology, Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 13, Burden and Von Knippenberg, Eds. pp. 75-83, Amsterdam, Elsevier, 1984.
- Capaldi *et al.*, *Biochem. Biophys. Res. Comm.*, 74(2):425-433, 1977.
- De Jager *et al.*, *Semin. Nucl. Med.* 23(2), 165-179, 1993.
- Dholakia *et al.*, *J. Biol. Chem.*, 264, 20638-20642, 1989.
- Diamond *et al.*, *J Virol* 77, 2578-2586, 2003.
- Doolittle and Ben-Zeev, *Methods Mol. Biol.*, 109, :215-237, 1999.
- Duffy *et al.*, *N. Engl. J. Med.* 360, 2536–2543, 2009.
- Elder *et al.* Infections, infertility and assisted reproduction. Part II: Infections in reproductive medicine & Part III: Infections and the assisted reproductive laboratory. Cambridge UK: Cambridge University Press; 2005.
- Gefter *et al.*, *Somatic Cell Genet.*, 3:231-236, 1977.
- Gornet *et al.*, *Semin Reprod Med.* 2016 Sep; 34(5):285-292. Epub 2016 Sep 14.
- Gulbis and Galand, *Hum. Pathol.* 24(12), 1271-1285, 1993.
- Halfon *et al.*, *PLoS ONE* 2010; 5 (5) e10569
- Hessell *et al.*, *Nature* 449, 101–4, 2007.
- Khatoon *et al.*, *Ann. of Neurology*, 26, 210-219, 1989.
- King *et al.*, *J. Biol. Chem.*, 269, 10210-10218, 1989.
- Kohler and Milstein, *Eur. J. Immunol.*, 6, 511-519, 1976.
- Kohler and Milstein, *Nature*, 256, 495-497, 1975.
- Kyte and Doolittle, *J. Mol. Biol.*, 157(1):105-132, 1982.
- Mansuy *et al.*, *Lancet Infect Dis.* 2016 Oct; 16(10):1106-7.
- Nakamura *et al.*, *In: Enzyme Immunoassays: Heterogeneous and Homogeneous Systems*, Chapter 27, 1987.
- O'Shannessy *et al.*, *J. Immun. Meth.*, 99, 153-161, 1987.
- Persic *et al.*, *Gene* 187:1, 1997
- Potter and Haley, *Meth. Enzymol.*, 91, 613-633, 1983.
- Purpura *et al.*, *Lancet Infect Dis.* 2016 Oct; 16(10):1107-8. Epub 2016 Sep 19.
- Remington's Pharmaceutical Sciences, 15th Ed., 3:624-652, 1990.
- Tang *et al.*, *J. Biol. Chem.*, 271:28324-28330, 1996.

- Wawrzynczak & Thorpe, In: *Immunoconjugates, Antibody Conjugates In Radioimaging And Therapy Of Cancer*, Vogel (Ed.), NY, Oxford University Press, 28, 1987.
- Yu *et al.*, *J Immunol Methods* 336, 142-151, doi:10.1016/j.jim.2008.04.008, 2008.
- Glass *et al.*, *N Engl J Med* 361:1776–1785, 2009.
- Fields *et al.*, *Fields Virology*. Lippincott Williams & Wilkins; 2013.
- Bok & Green, *N Engl J Med*. 367:2126–2132, 2012.
- Vinje J., *J Clin Microbiol* 53:373–381, 2015.
- Jiang *et al.*, *J Virol* 66:6527–6532, 1992.
- Richardson *et al.*, *Expert Rev Vaccines* 12:155–167, 2013.
- Erdman *et al.*, *J Clin Microbiol* 27:1417–1418, 1989.
- Czako *et al.*, *Clin Vaccine Immunol* 19:284–287, 2012.
- Reeck *et al.*, *J Infect Dis* 202:1212–1218, 2010.
- Jones *et al.*, *Science* 346:755–759, 2014.
- Duizer *et al.*, *J Gen Virol* 85:79–87, 2004.
- Herbst-Kralovetz *et al.*, *Emerging Infect Dis* 19:431–438, 2013.
- Ettayebi *et al.*, *Science* 353:1387–1393, 2016.
- Costantini *et al.*, *Emerging Infect Dis* 24:1453–1464, 2018.
- Sapparapu *et al.*, *PLoS Pathog* 12:e1005719, 2016.
- Loisy *et al.*, *J Virol Methods* 123:1–7, 2005.
- Kageyama *et al.*, *J Clin Microbiol* 41:1548–1557, 2003.
- Green *et al.*, *J Clin Microbiol* 31:2185–2191, 1993.
- Iritani *et al.*, *J Med Virol* 79:1187–1193, 2007.
- Gray *et al.*, *J Clin Microbiol* 32:3059–3063, 1994.
- Atmar *et al.*, *Clin Vaccine Immunol* 22:923–929, 2015.
- Czako *et al.*, *Clin Vaccine Immunol* 22:221–228, 2015.
- Lindesmith *et al.*, *PLoS Pathog* 8:e1002705, 2012a.
- Choi J-M *et al.*, *Proc Natl Acad Sci U.S.A.* 105:9175–9180, 2008.
- Debbink *et al.*, *J Virol* 86:1214–1226, 2012.
- Lindesmith *et al.*, *J Virol* 86:873–883, 2012b.
- Lochridge *et al.*, *J Gen Virol* 86:2799–2806, 2005.
- Parra *et al.*, *PLoS ONE* 8:e67592, 2013.
- Karst & Baric, *J Virol* 89:5756–5759, 2015.
- Huang *et al.*, *Protein Eng Des Sel* 27:339–349, 2014.
- Crawford *et al.*, *Clin. Vaccine Immunol* 22:168–177, 2015.

- Koromyslova *et al.*, ed. *PLoS Pathog* 13:e1006636, 2017.
- Smith & Crowe, *Microbiol Spectr* 3:AID-0027-2014, 2015.
- Debbink *et al.*, *J Infect Dis* 208:1877-1887, 2013.
- Debbink *et al.*, *Clin Infect Dis* 58:1746-1752, 2014.
- El-Kamary *et al.*, *J Infect Dis* 202:1649-1658, 2010.
- Bernstein *et al.*, *J Infect Dis* 211:870-878, 2015.
- Tan *et al.*, *J Virol* 85:753-764, 2011.
- Ramirez *et al.*, *Clin Immunol* 144:98-108, 2012.
- Leroux-Roels *et al.*, *J Infect Dis* 217:597-607, 2018.
- Alvarado *et al.*, *Gastroenterology* 155(6): 1898-1907, 2018.
- Bartsch *et al.*, *PLoS ONE* 11, e0151219, 2016.
- Brochet *et al.*, *Nucleic Acids Res.* 36, W503-W508, 2008.
- Hale *et al.*, *J. Medical Virol.*, 54(4), pp.305-312, 1998.
- Hall *et al.*, A.J., Wikswo, M.E., Pringle, K., Gould, L.H., Parashar, U.D., Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, CDC; United States, 2009-2012. *MMWR Morb. Mortal. Wkly. Rep.* 63, 491-495, 2014.
- Knipe and Howley, *Fields Virology* (Lippincott Williams & Wilkins), 2013.
- Koromyslova *et al.*, *J. Virol.* 93, 1580, 2019.
- Kou *et al.*, *Clin. Vaccine Immunol.* 22, 160-167, 2015.
- Laskowski and Swindells, *J. Chem. Inf. Model.*, 51, 2778-2786. [PubMed id: 21919503], 2011.
- McCoy *et al.*, *J. Appl. Cryst.* 40, 658-674, 2007.
- McLean *et al.*, *Mol. Immunol.* 37, 837-845, 2000.
- Parker *et al.*, *J. Virol.* 79, 7402-7409, 2005.
- Parra *et al.*, *J. Virol.* 86, 7414-7426, 2012.
- Pettersen *et al.*, *Comput Chem.* 25(13):1605-12, 2004.
- Pires *et al.*, *PLoS ONE* 10, e0142927, 2015.
- Pletneva *et al.*, *Virus Genes* 23, 5-16, 2001.
- Prasad *et al.*, *Science* 286, 287-290, 1999.
- Shanker *et al.*, *Proc Natl Acad Sci USA* 113(40):E5830-E5837 (Epub 2016 Sep 19), 2016.
- Shanker *et al.*, *J. Virol.* 85(17): 8635-8645, 2011.
- Tian *et al.*, *J. Appl. Microbiol.* 109, 1753-1762, 2010.
- Treanor *et al.*, *J. Clin. Microbiol.* 31, 1630-1634, 1993.
- Yoda *et al.*, *J. Clin. Microbiol.* 41, 2367-2371, 2003.
- Zheng *et al.*, *J. Med. Virol.* 90, 671-676, 2018.

WHAT IS CLAIMED IS:

1. A method of detecting a norovirus infection in a subject comprising:
 - (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (b) detecting norovirus in said sample by binding of said antibody or antibody fragment to a norovirus antigen in said sample.
2. The method of claim 1, wherein said sample is a body fluid.
3. The method of claims 1-2, wherein said sample is blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, tissue scrapings or feces.
4. The method of claims 1-3, wherein detection comprises ELISA, RIA, lateral flow assay or Western blot.
5. The method of claims 1-4, further comprising performing steps (a) and (b) a second time and determining a change in norovirus antigen levels as compared to the first assay.
6. The method of claims 1-5, wherein the antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
7. The method of claims 1-5, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.
8. The method of claims 1-5, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.

9. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
10. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
11. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
12. The method of claims 1-11, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
13. A method of treating a subject infected with norovirus, or reducing the likelihood of infection of a subject at risk of contracting norovirus, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
14. The method of claim 13, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.
15. The method of claim 13-14, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identity to as set forth in Table 1.
16. The method of claim 13-14, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
17. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

18. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
19. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
20. The method of claims 13-19, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
21. The method of claims 13-20, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
22. The method of claims 13-19, wherein said antibody is a chimeric antibody or a bispecific antibody.
23. The method of claim 13-22, wherein said antibody or antibody fragment is administered prior to infection or after infection.
24. The method of claim 13-23, wherein said subject is a pregnant female, a sexually active female, or a female undergoing fertility treatments.
25. The method of claim 13-24, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.

26. A monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
27. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
28. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
29. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.
30. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
31. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
32. The monoclonal antibody of claims 26-31, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
33. The monoclonal antibody of claims 26-31, wherein said antibody is a chimeric antibody, or is bispecific antibody.
34. The monoclonal antibody of claim 26-33, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or

glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.

35. The monoclonal antibody of claim 26-34, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.
36. A hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
37. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
38. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 1.
39. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired variable sequences from Table 1.
40. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
41. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 2.
42. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

43. The hybridoma or engineered cell of claims 36-42, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
44. The hybridoma or engineered cell of claim 36-43, wherein said antibody is a chimeric antibody or a bispecific antibody.
45. The hybridoma or engineered cell of claim 36-43, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
46. The hybridoma or engineered cell of claim 36-45, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.
47. A vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
48. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.
49. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

50. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.
51. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
52. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
53. The vaccine formulation of claims 47-52, wherein at least one of said antibody fragments is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
54. The vaccine formulation of claims 47-52, wherein at least one of said antibodies is a chimeric antibody, or is bispecific antibody.
55. The vaccine formulation of claims 47-54, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
56. The vaccine formulation of claims 47-55, wherein at least one of said antibodies or antibody fragments further comprises a cell penetrating peptide and/or is an intrabody.
57. A vaccine formulation comprising one or more expression vectors encoding a first antibody or antibody fragment according to claims 26-34.

58. The vaccine formulation of claim 57, wherein said expression vector(s) is/are Sindbis virus or VEE vector(s).
59. The vaccine formulation of claims 57-58, formulated for delivery by needle injection, jet injection, or electroporation.
60. The vaccine formulation of claim 57, further comprising one or more expression vectors encoding for a second antibody or antibody fragment, such as a distinct antibody or antibody fragment of claims 26-34.
61. A method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with norovirus comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.
62. The method of claim 61, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.
63. The method of claim 61-62, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identity to as set forth in Table 1.
64. The method of claim 61-62, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.
65. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
66. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.

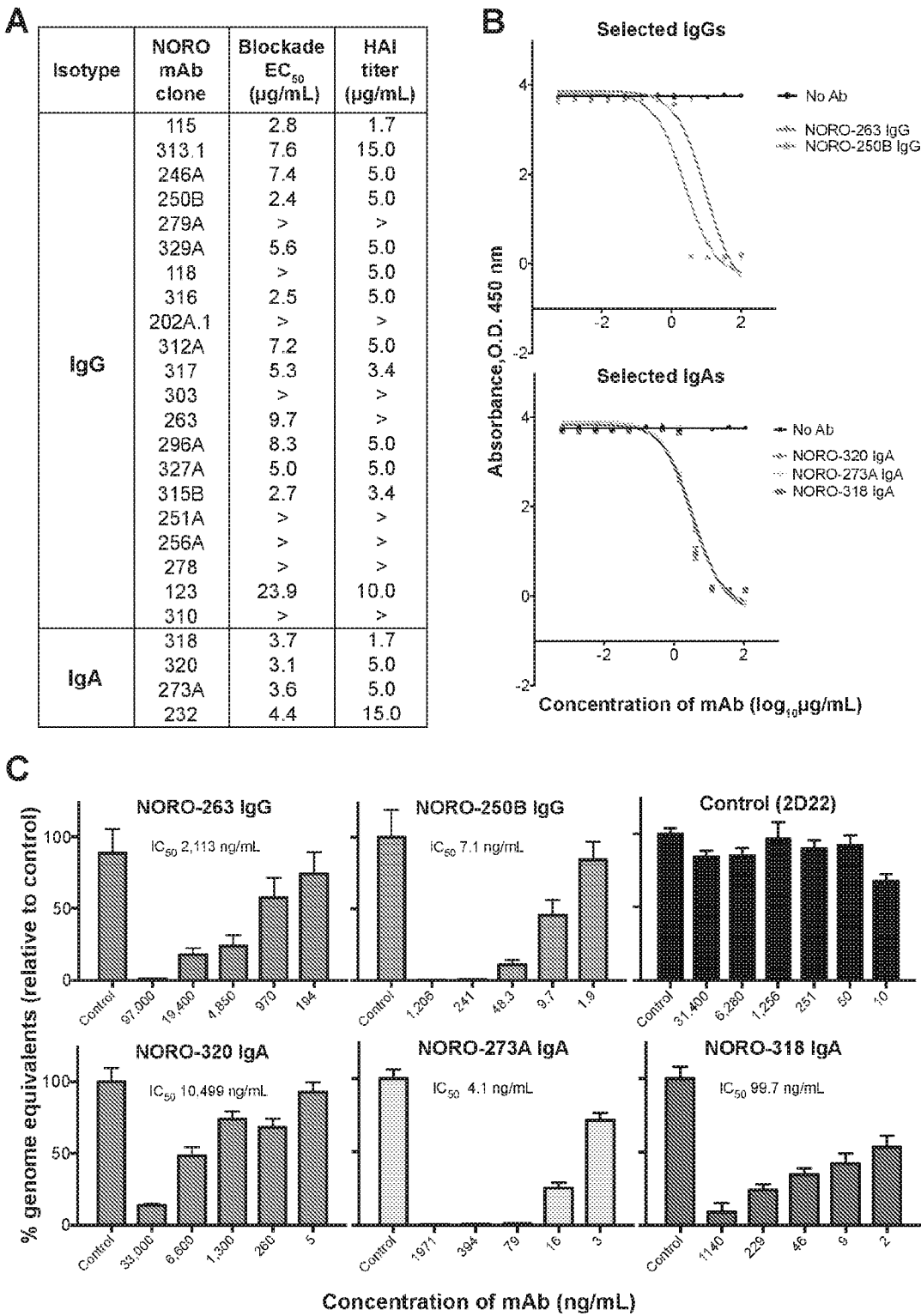
67. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
68. The method of claims 61-67, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
69. The method of claims 61-68, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to alter (eliminate or enhance) FcR interactions, to increase half-life and/or increase therapeutic efficacy, such as a LALA, N297, GASD/ALIE, YTE or LS mutation or glycan modified to alter (eliminate or enhance) FcR interactions such as enzymatic or chemical addition or removal of glycans or expression in a cell line engineered with a defined glycosylating pattern.
70. The method of claims 61-67 wherein said antibody is a chimeric antibody or a bispecific antibody.
71. The method of claim 61-70, wherein said antibody or antibody fragment is administered prior to infection or after infection.
72. The method of claim 61-71, wherein said subject is a pregnant female, a sexually active female, or a female undergoing fertility treatments.
73. The method of claim 61-72, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.
74. The method of claim 61, wherein the antibody or antibody fragment increases the size of the placenta as compared to an untreated control.
75. The method of claim 61, wherein the antibody or antibody fragment reduces viral load and/or pathology of the fetus as compared to an untreated control.

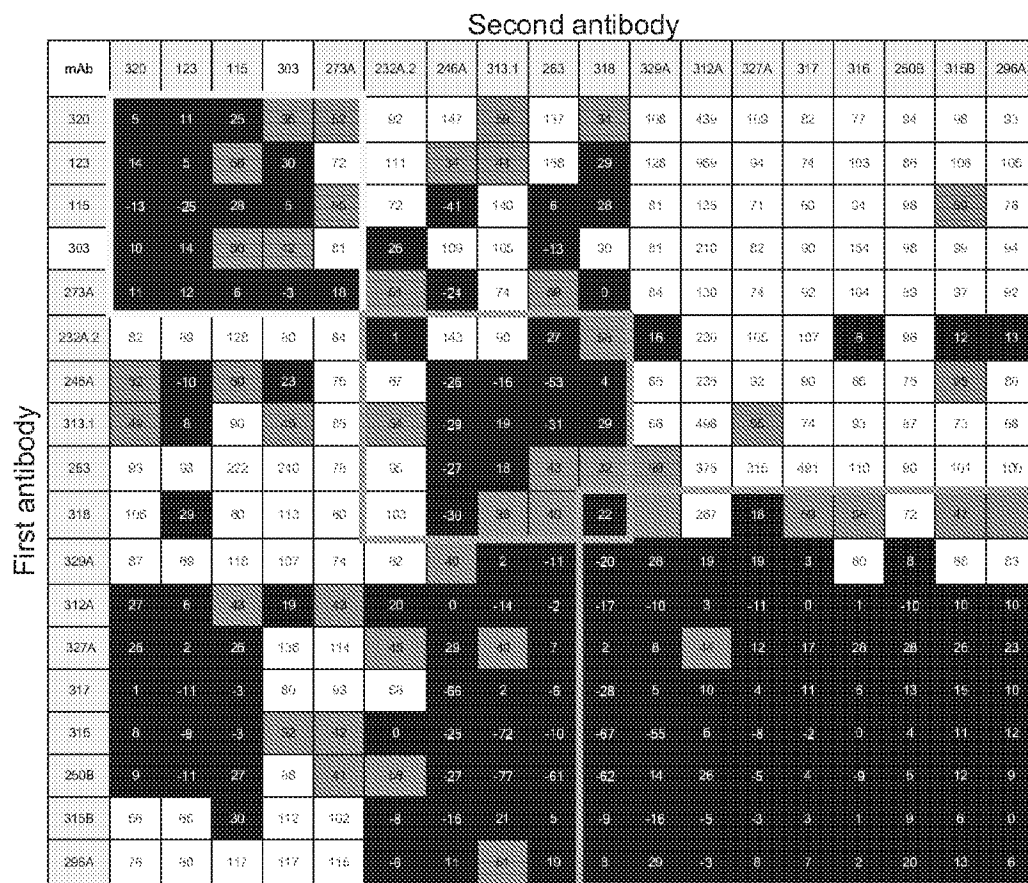
76. A method of determining the antigenic integrity, correct conformation and/or correct sequence of a norovirus antigen comprising:
- (a) contacting a sample comprising said antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (b) determining antigenic integrity, correct conformation and/or correct sequence of said antigen by detectable binding of said first antibody or antibody fragment to said antigen.
77. The method of claim 76, wherein said sample comprises recombinantly produced antigen.
78. The method of claim 76, wherein said sample comprises a vaccine formulation or vaccine production batch.
79. The method of claims 76-78, wherein detection comprises ELISA, RIA, western blot, a biosensor using surface plasmon resonance or biolayer interferometry, or flow cytometric staining.
80. The method of claims 76-79, wherein the first antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
81. The method of claims 76-79, wherein said first antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.
82. The method of claims 76-79 wherein said first antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.
83. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

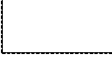
84. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
85. The method of claims 76-79, wherein said first antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
86. The method of claims 76-85, wherein the first antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
87. The method of claims 76-86, further comprising performing steps (a) and (b) a second time to determine the antigenic stability of the antigen over time.
88. The method of claims 76-87, further comprising:
- (c) contacting a sample comprising said antigen with a second antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and
 - (d) determining antigenic integrity of said antigen by detectable binding of said second antibody or antibody fragment to said antigen.
89. The method of claim 88, wherein the second antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.
90. The method of claim 89, wherein said second antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.
91. The method of claim 89, wherein said second antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.

92. The method of claims 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.
93. The method of claim 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.
94. The method of claim 89, wherein said second antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.
95. The method of claim 89, wherein the second antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
96. The method of claim 89, further comprising performing steps (c) and (d) a second time to determine the antigenic stability of the antigen over time.
97. A human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody or antibody fragment binds to norovirus capsid protein P domain and/or S domain.
98. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, of claim 97, wherein said antibody or antibody fragment binds to norovirus capsid protein P domain but not norovirus capsid protein S domain.
99. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, of claim 97, wherein said antibody or antibody fragment binds to norovirus capsid protein S domain but not norovirus capsid protein P domain.

100. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, of claim 97, wherein said antibody or antibody fragment binds to norovirus capsid protein P domain P1 or P2 subdomain.
101. The human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, of claim 97, wherein said antibody or antibody fragment is cross-reactive to multiple norovirus GI and/or GII strains.





Color	Interpretation	% of max uncompleted signal
	First Ab blocks the binding of the second Ab	≤33%
	First Ab partially blocking the binding of second Ab	34-66%
	First Ab does not block the binding of the second Ab	>66%

	Block GII.4 Sydney VLPs from binding to HBGAs <i>in vitro</i>
---	---

FIG. 2

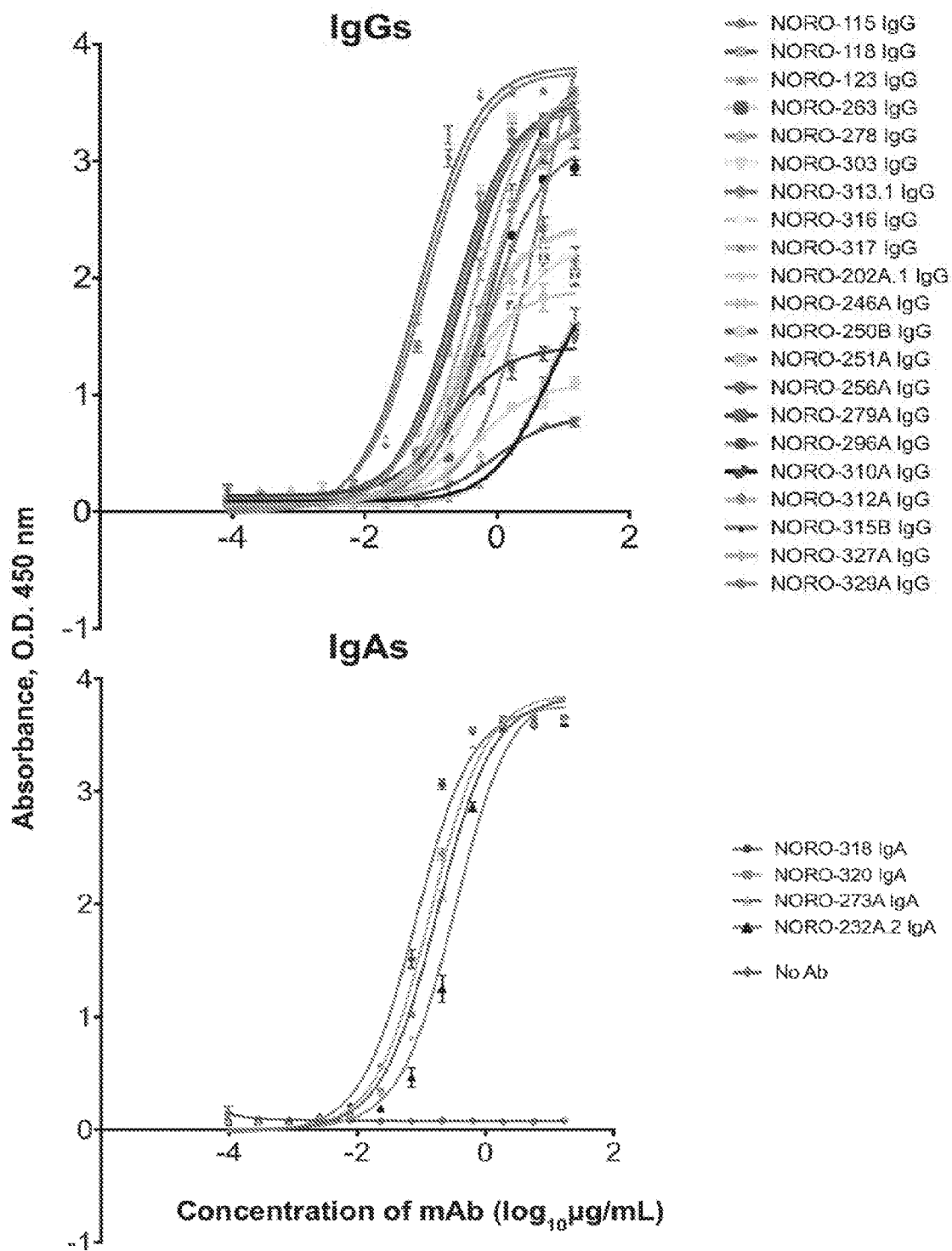


FIG. 3

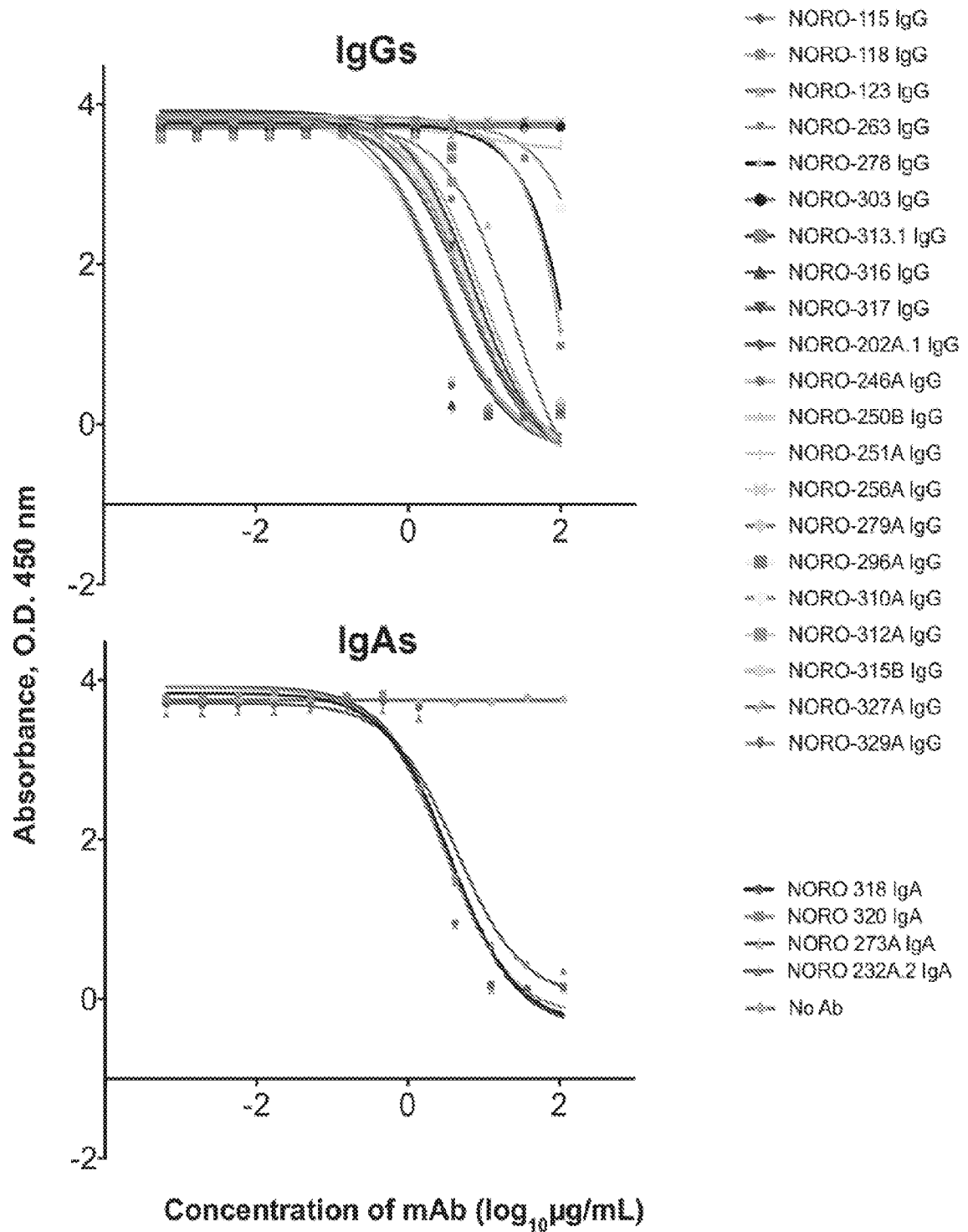
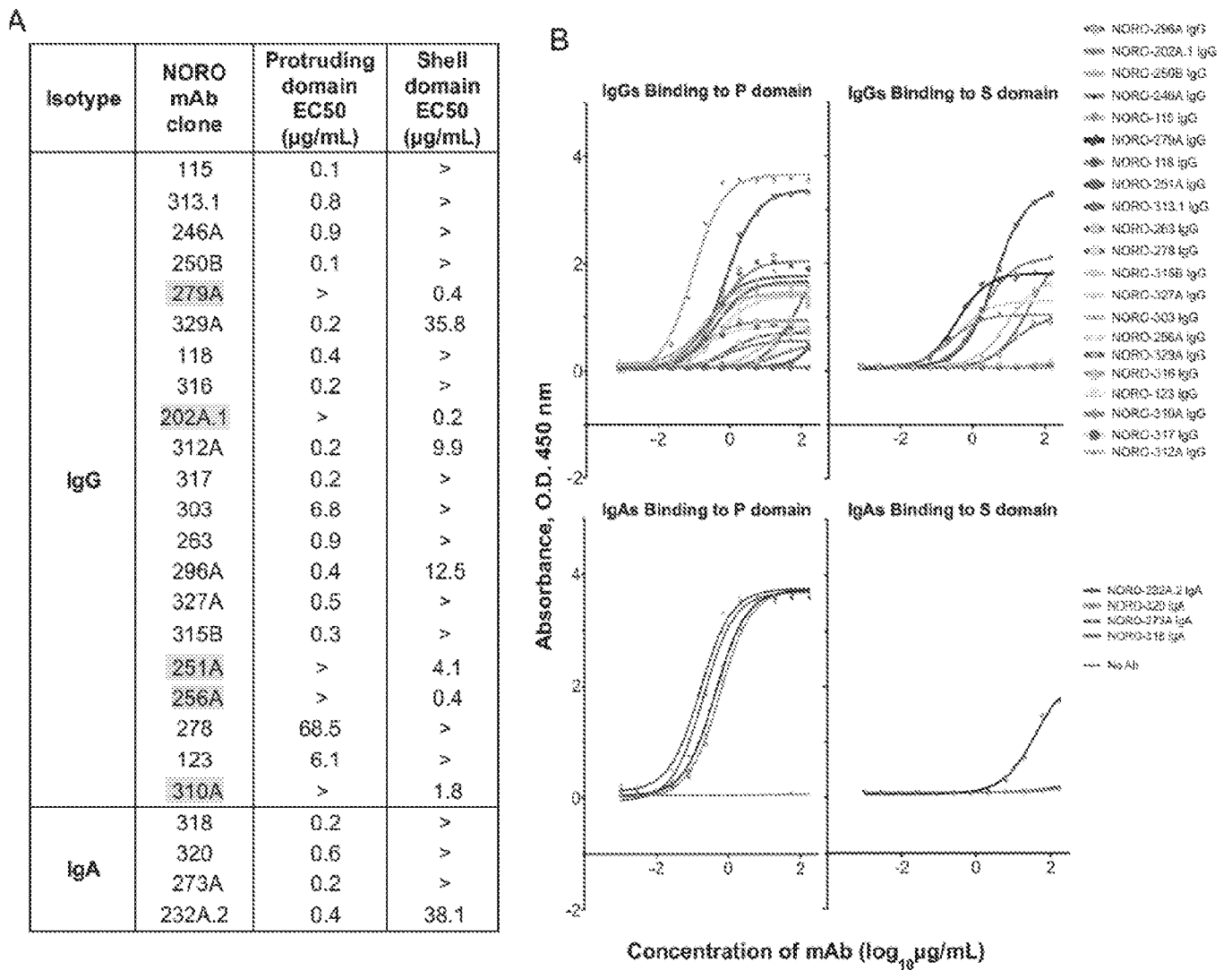


FIG. 4



*Shell domain reactive

FIG. 5A-B

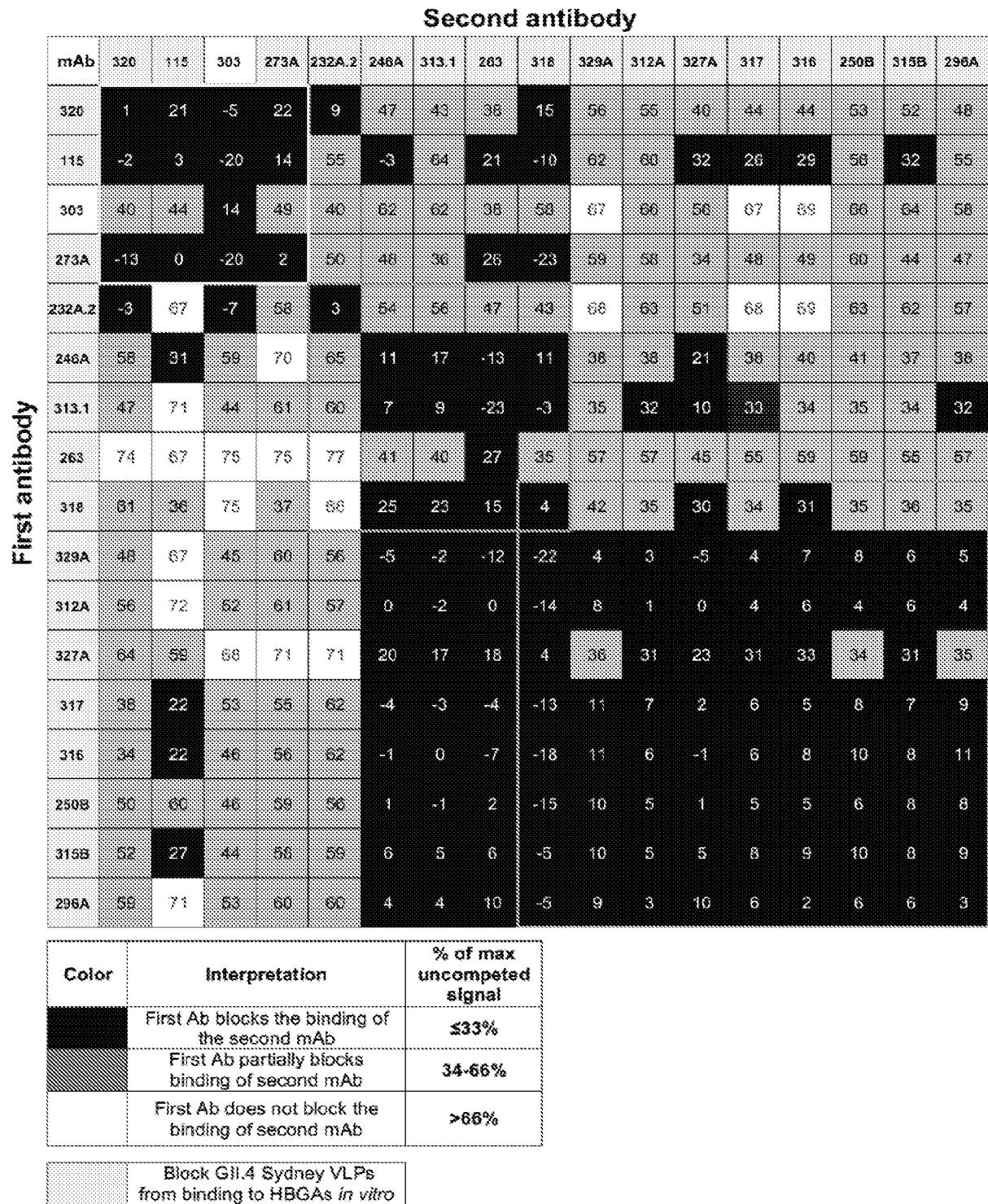
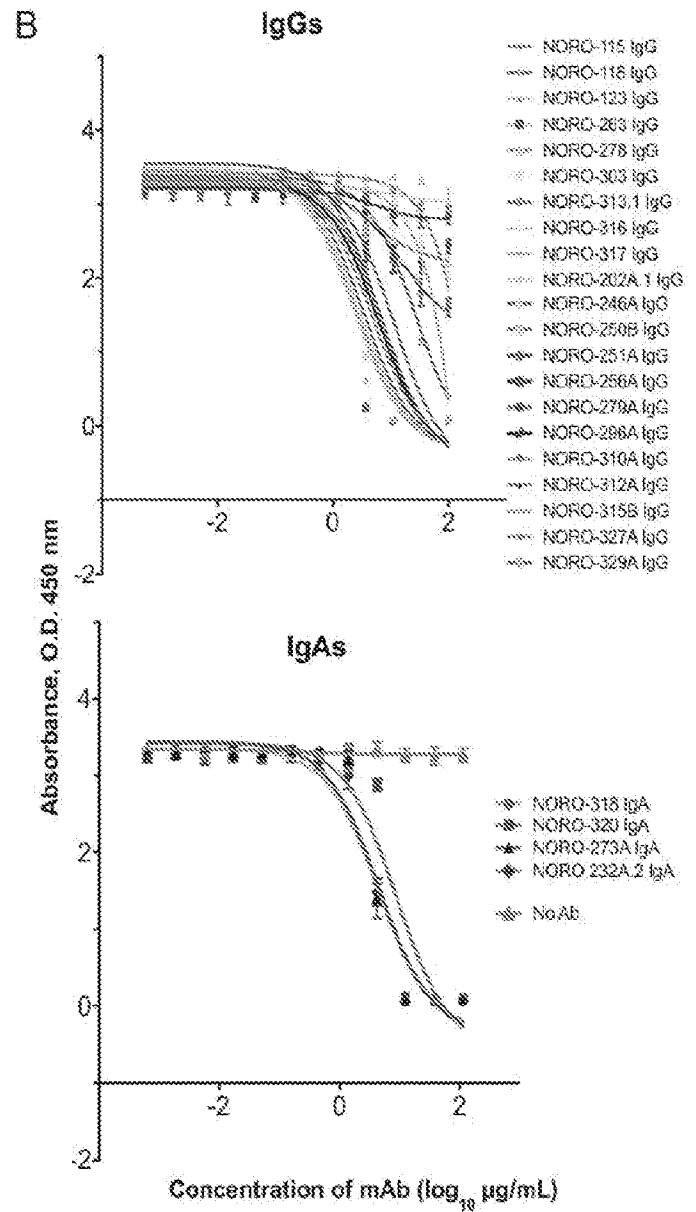


FIG. 6



A

Isotype	NORO mAb clone	Binding EC ₅₀ (μg/mL)	Blockade EC ₅₀ (μg/mL)
IgG	115	1.1	2.3
	313.1	0.1	2.1
	246A	0.2	2.1
	250B	0.1	17.9
	279A	0.3	>
	329A	5.5	3.0
	118	0.4	9.5
	316	0.6	2.1
	202A.1	0.1	>
	312A	0.6	3.0
	317	0.7	2.0
	303	0.5	>
	263	0.5	2.1
	296A	0.1	3.2
	327A	0.3	2.2
	315B	0.5	2.2
	251A	0.3	>
	256A	0.4	>
	278	0.5	>
	123	0.6	2.5
	310A	0.6	>
IgA	318	4.5	3.5
	320	0.3	3.3
	273A	0.2	5.2
	232A.2	0.2	5.4

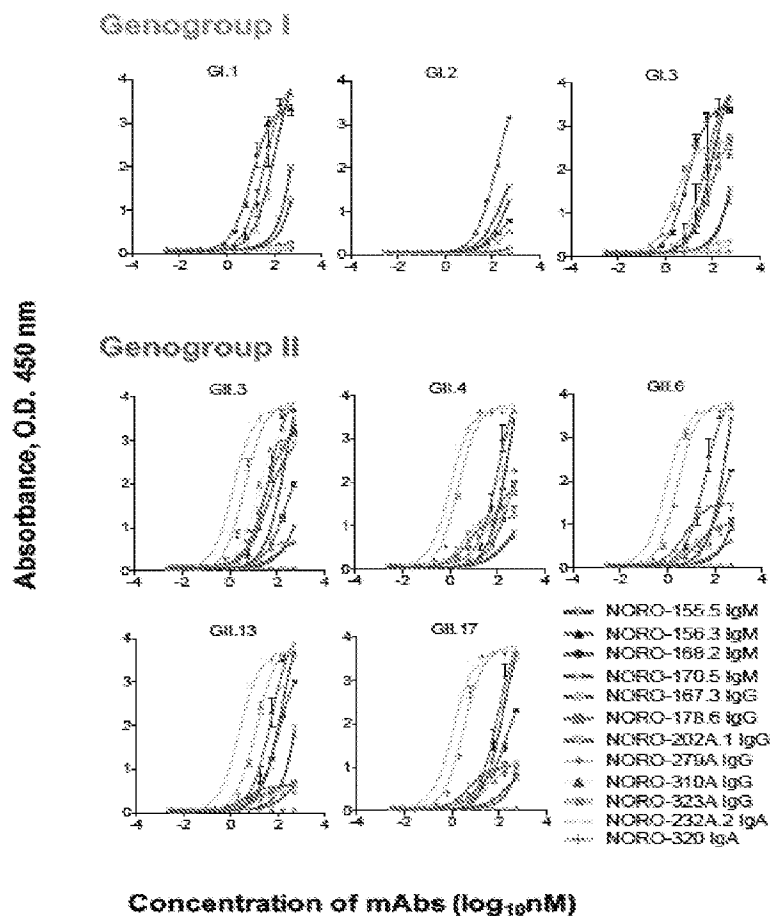


FIGS. 8A-B

A.

			EC ₅₀ (nM) for binding to indicated VLP							
Isotype	NORO-	K / A	Genogroup I			Genogroup II				
			GI.1	GI.2	GI.3	GI.3	GI.4	GI.6	GI.13	GI.17
IgM	155.5	K	>	415	>	>	332	>	>	>
	156.3	K	42	175	47	40	106	39	54	80
	166.2	K	11	285	8	224	285	199	107	153
	170.5	K	104	289	90	233	368	474	154	156
IgG	167.3	λ	>	>	>	115	152	175	263	226
	178.6	λ	>	>	83	29	43	29	18	16
	202A.1	λ	>	>	>	41	6	8	4	6
	279A	λ	>	>	70	14	5	14	10	18
	310A	K	>	>	24	15	72	44	53	40
	323A	λ	>	>	3	>	145	>	>	>
IgA	232A.2	K	>	>	>	1	1	1	2	1
	320	K	>	>	>	4	2	3	12	3

B.

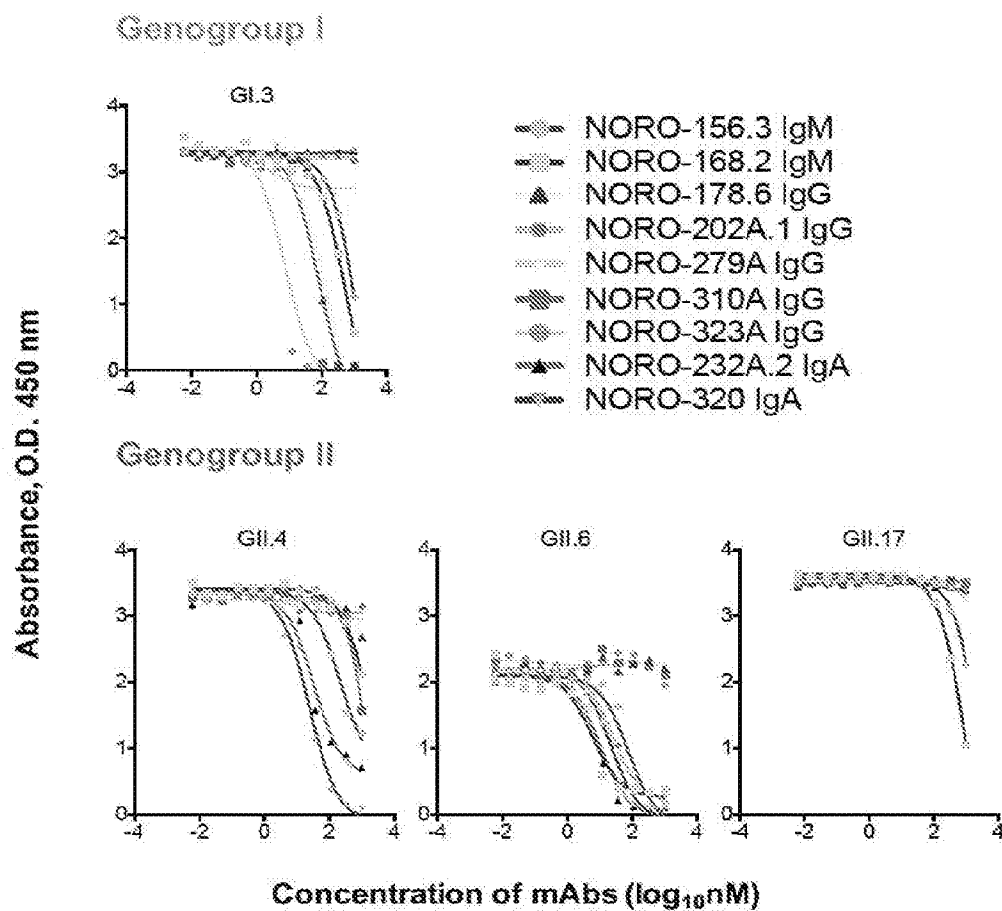


FIGS. 9A-B

A.

Isotype	NORO-	EC ₅₀ (nM) for blocking of binding to PGM			
		Genogroup I		Genogroup II	
		GI.3	GII.4	GII.6	GII.17
IgM	156.3	678	>	355	>
	168.2	>	>	7	>
IgG	178.6	470	>	>	>
	202A.2	>	>	47	>
	279A	>	>	32	>
	310A	192	>	>	>
	323A	15	>	>	>
IgA	232A.2	>	115	20	>
	320	>	73	36	>

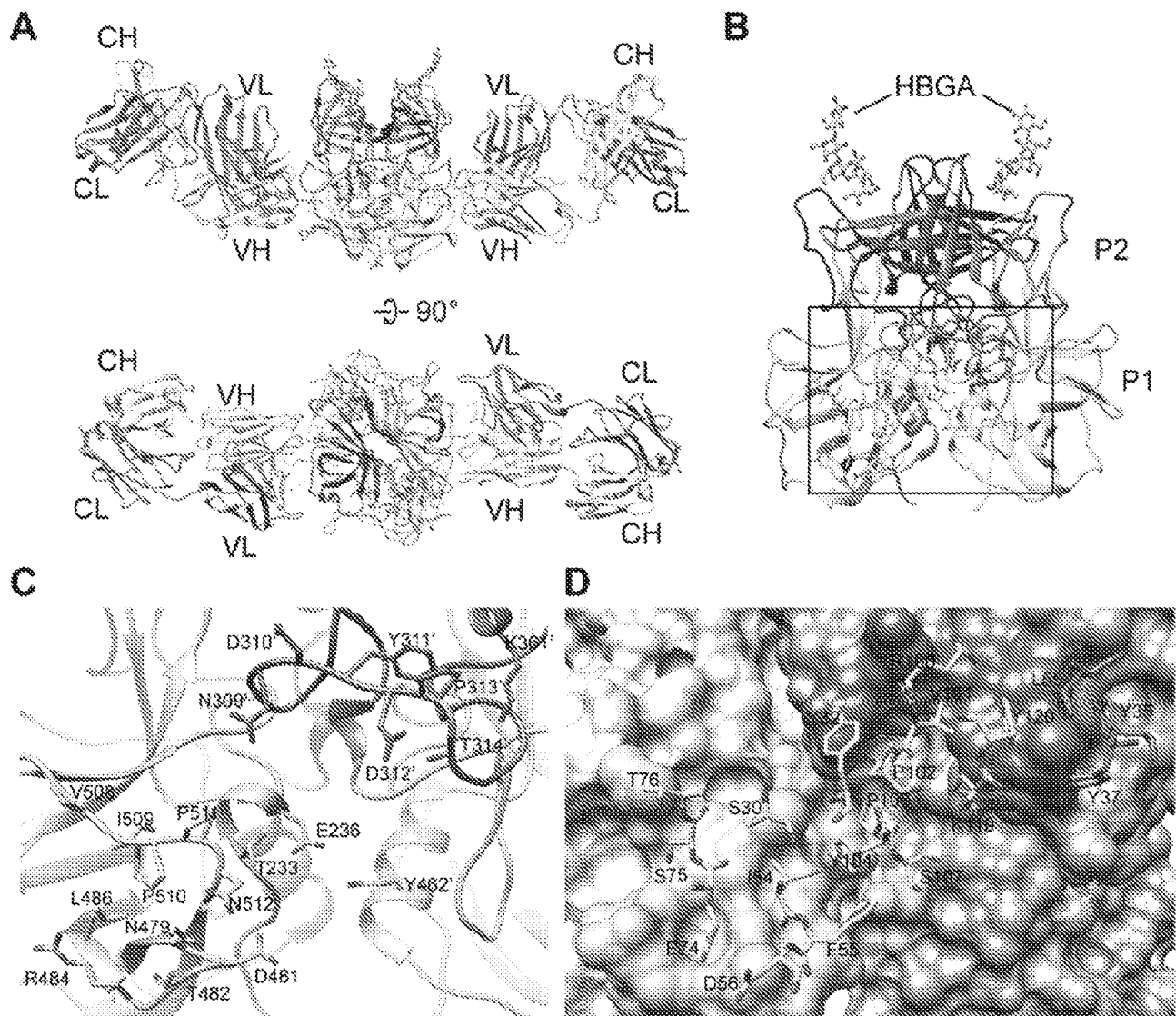
B.

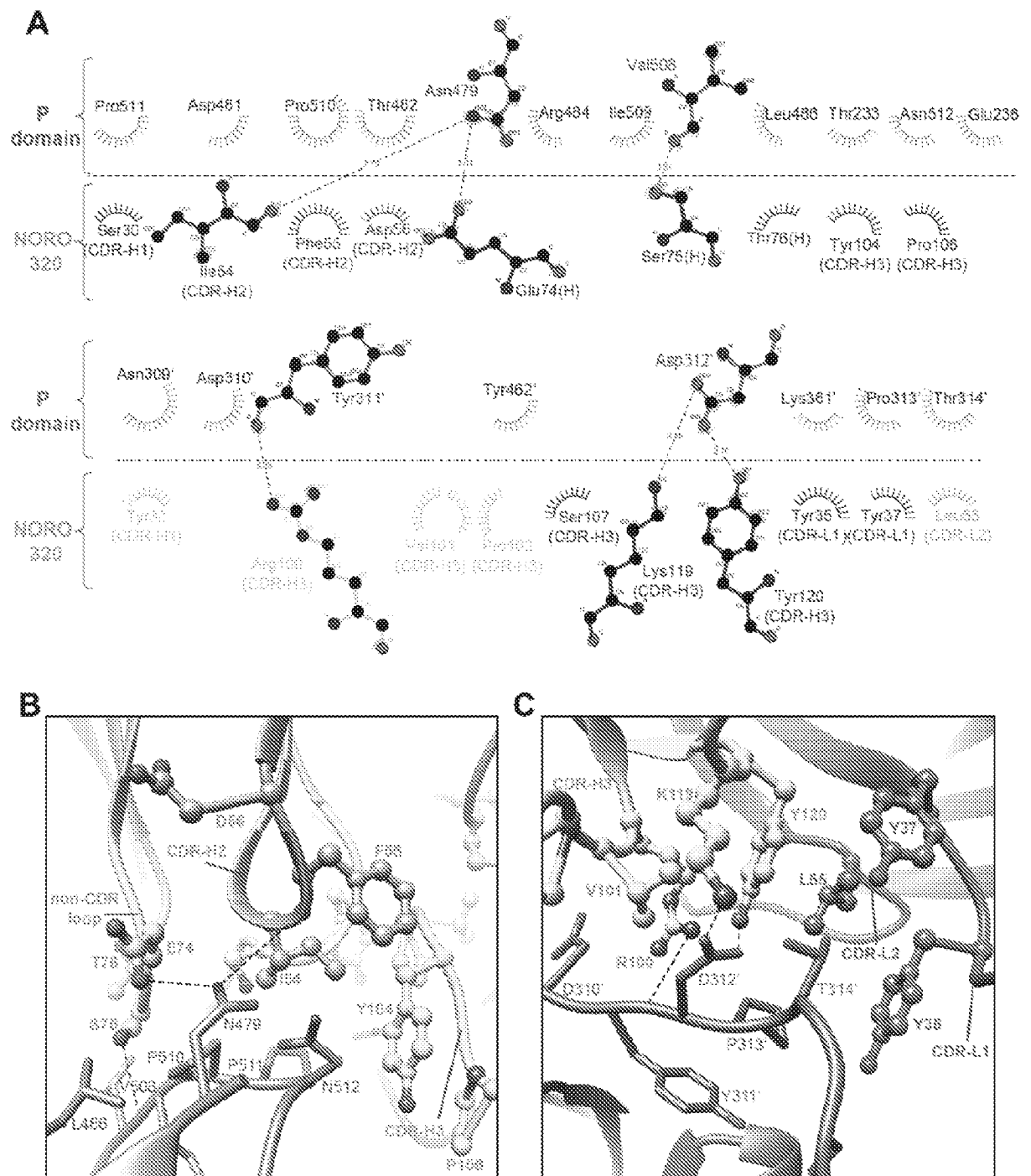


FIGS. 10A-B

			EC ₅₀ (nM) for binding to protruding or shell domain protein							
			Genogroup I		Genogroup II					
			GI.3		GI.4		GI.6		GI.17	
Isotype	NORO-	Domain specificity	Protruding	Shell	Protruding	Shell	Protruding	Shell	Protruding	Shell
IgM	155.5	ND	>	>	>	>	>	>	>	>
	156.3	ND	>	>	>	>	>	>	>	>
	168.2	P/S	143	37	238	78	135	162	390	183
	170.5	S	>	385	>	>	>	304	>	255
IgG	167.3	S	>	>	>	90	>	>	>	>
	178.6	P/S	336	>	>	8	>	>	>	>
	202A.2	S	>	>	>	3	>	>	>	>
	279A	S	>	>	>	3	>	>	>	>
	310A	P/S	60	>	219	19	>	>	>	>
	323A	P	7	>	172	>	>	>	>	>
IgA	232A.2	P	>	>	3	>	2	>	2	>
	320	P	>	>	2	>	3	>	3	>

FIG. 11





FIGS. 13A-C

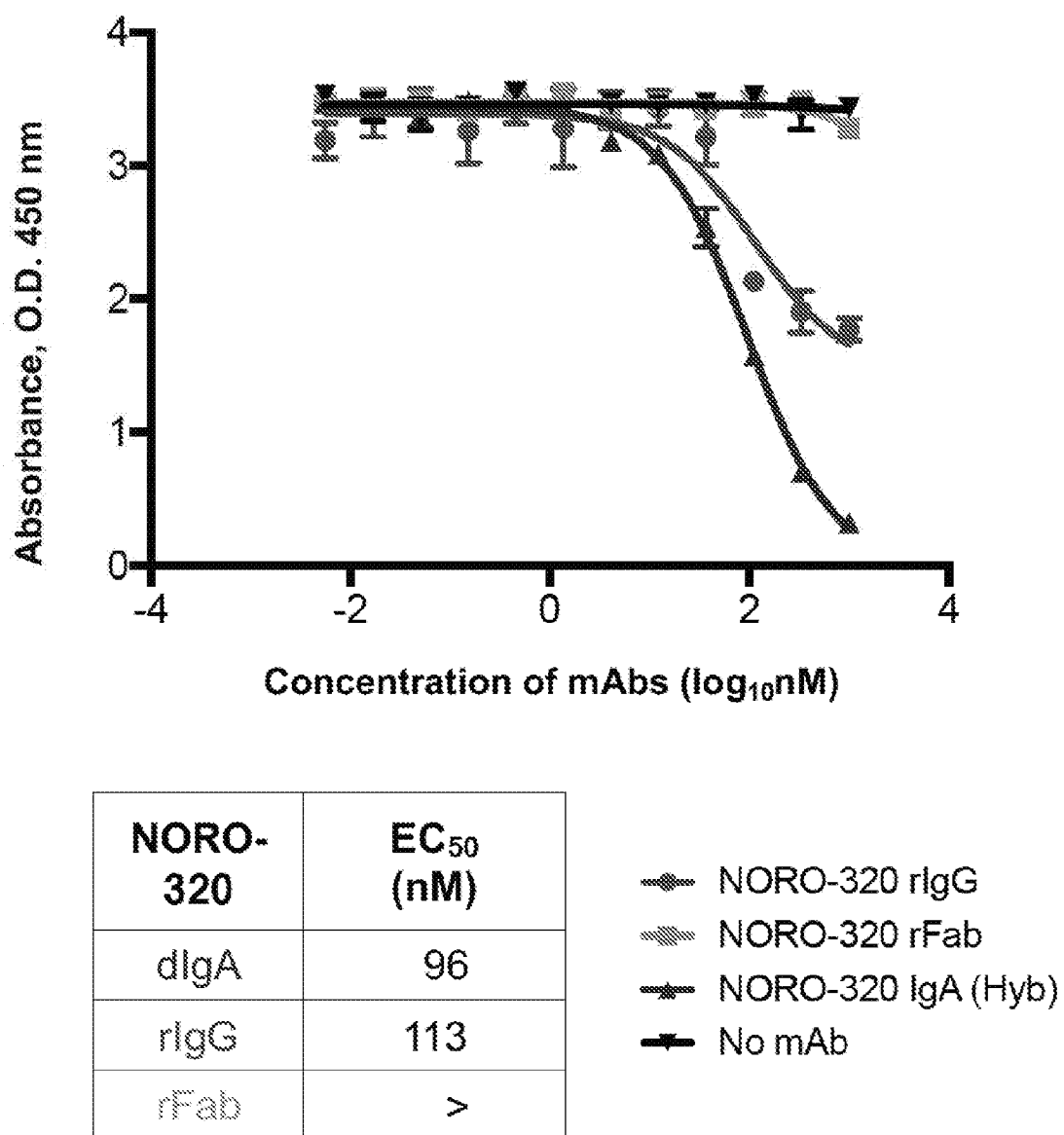


FIG. 15

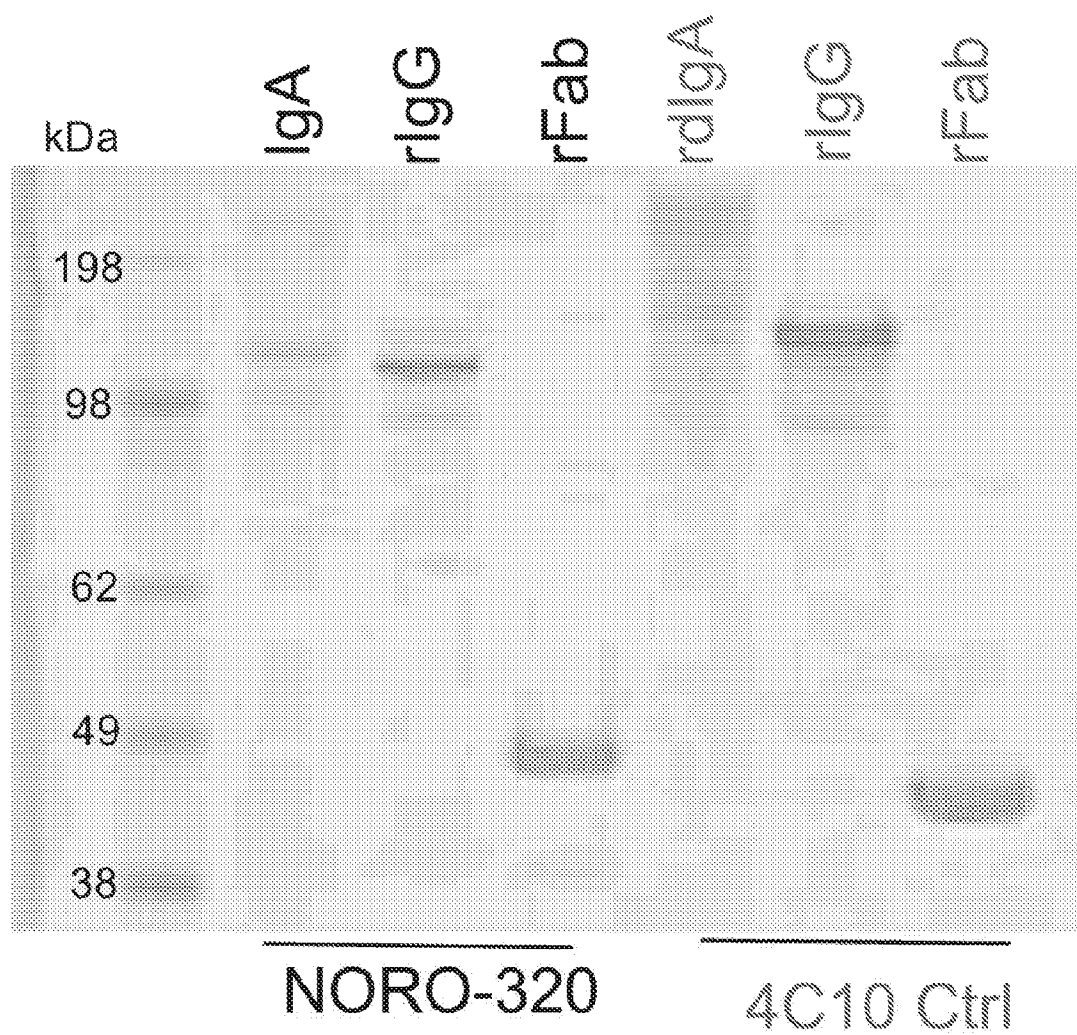
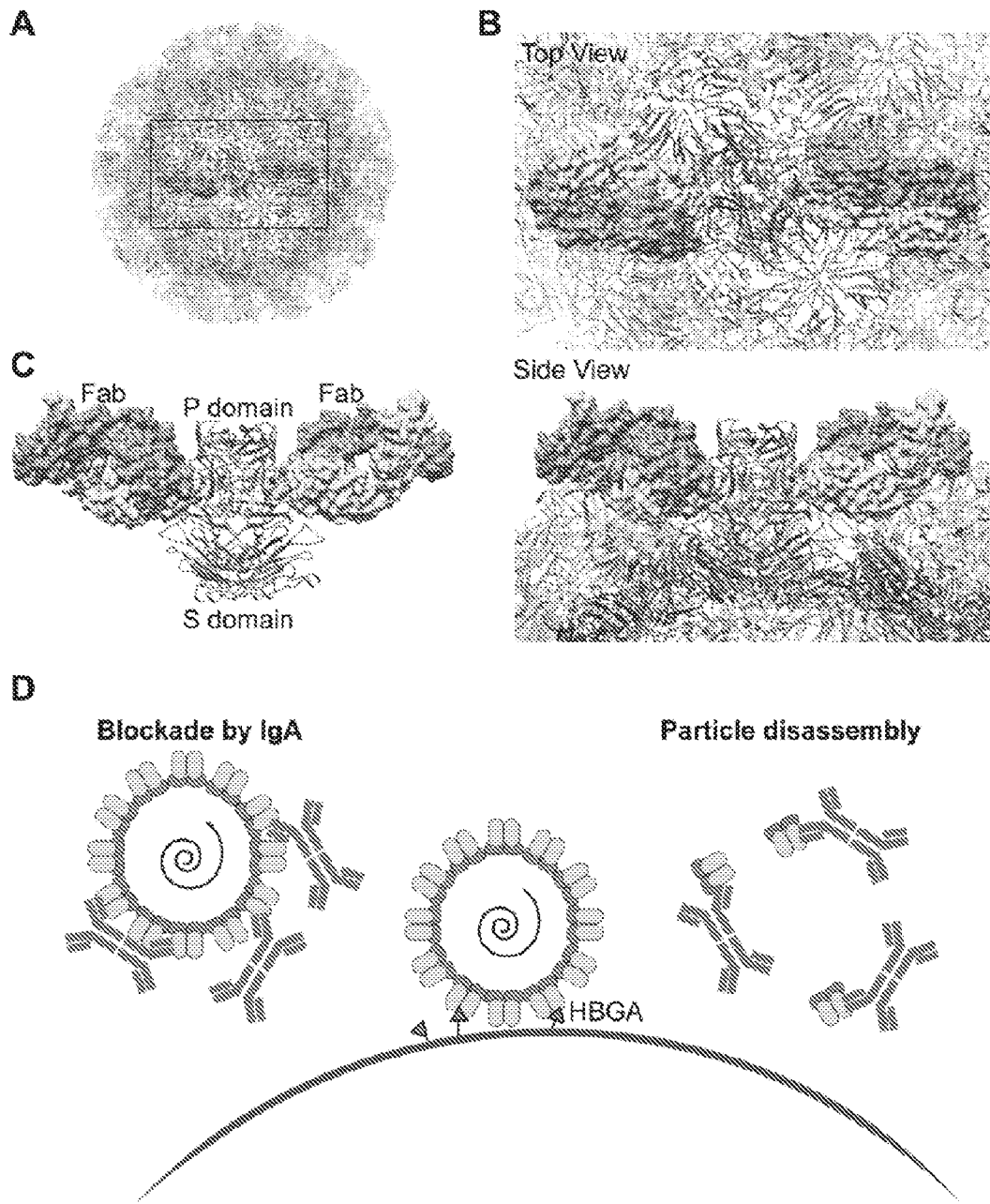


FIG. 16



FIGS. 17A-D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/048231

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/125; A61K 39/42; C07K 14/06; C07K 16/00; C07K 16/08 (2019.01)

CPC - A61K 39/00; A61K 39/125; A61K 2039/5258; C07K 14/005; C12N 2710/14043 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/159.1; 530/350; 530/388.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/011394 A1 (BAYLOR COLLEGE OF MEDICINE et al) 19 January 2017 (19.01.2017) entire document	97, 98, 100 ----- 99, 101
Y	PARRA et al. "Mapping and modeling of a strain-specific epitope in the Norwalk virus capsid inner shell," Virology, 21 March 2016 (21.03.2016), Vol. 492, Pgs. 232-241. entire document	99
Y	US 2005/0152911 A1 (HARDY) 14 July 2005 (14.07.2005) entire document	101
A	US 2017/0247434 A1 (DEUTSCHES KREBSFORSCHUNGSZENTRUM et al) 31 August 2017 (31.08.2017) entire document	1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, 97-101
A	US 6,610,305 B1 (ELBERS et al) 26 August 2003 (26.08.2003) entire document	1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, 97-101
A	US 9,244,072 B2 (MIKI) 26 January 2016 (26.01.2016) entire document	1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, 97-101
P, A	WO 2019/057755 A1 (UNIVERSITÄT HEIDELBERG et al) 28 March 2019 (28.03.2019) entire document	1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, 97-101

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 December 2019

Date of mailing of the international search report

07 JAN 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/048231

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 35-54 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/048231

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-12, 20-25, 34, 35, 44-46, 55-60, 68-73, 80-96
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, and 97-101 to the extent that they read on an anti-norovirus antibody of SEQ ID NOs: 1, 2, 35, 36, 70, 71, 72, 120, 121, and 122.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, and 97-101 are drawn to anti-norovirus antibodies, and methods, hybridomas, engineered cells, and vaccine formulations comprising the same.

The first invention of Group I+ is restricted to an anti-norovirus antibody, and methods, hybridomas, engineered cells, and vaccine formulations comprising the same, where the anti-norovirus antibody is selected to be NORO-115, wherein NORO-115 comprises a heavy chain variable region, wherein in the heavy chain variable region is SEQ ID NO:35, encoded by SEQ ID NO:1, the heavy chain further comprising heavy chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:69, CDR2 is SEQ ID NO:70, and CDR3 is SEQ ID NO:71; and a light chain variable region, wherein the light chain variable region is SEQ ID NO:36, encoded by SEQ ID NO:2, the light chain further comprising light chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:120, CDR2 is SEQ ID NO:121, and CDR3 is SEQ ID NO:122. It is believed that claims 1-3, 13-19, 26-33, 36-43, 47-54, 61-67, 74-79, and 97-101 read on this first named invention and thus these claims will be searched without fee to the extent that they read on an anti-norovirus antibody of SEQ ID NOs: 1, 2, 35, 36, 70, 71, 72, 120, 121, and 122.

Applicant is invited to elect additional anti-norovirus antibodies, each with specified SEQ ID NO for each heavy chain variable region and light chain variable region, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be an anti-norovirus antibody, and methods, hybridomas, engineered cells, and vaccine formulations comprising the same, where the anti-norovirus antibody is selected to be NORO-123, wherein NORO-123 comprises a heavy chain variable region, wherein in the heavy chain variable region is SEQ ID NO:37, encoded by SEQ ID NO:3, the heavy chain further comprising heavy chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:72, CDR2 is SEQ ID NO:73, and CDR3 is SEQ ID NO:74; and a light chain variable region, wherein the light chain variable region is SEQ ID NO:38, encoded by SEQ ID NO:4, the light chain further comprising light chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:123, CDR2 is SEQ ID NO:124, and CDR3 is SEQ ID NO:125. Additional anti-norovirus antibodies will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for binding a norovirus antigen, requiring the selection of alternatives for the anti-norovirus antibody variable heavy and light chain CDR sequences, where "said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1" and "said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2" and "the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively".

Additionally, even if Groups I+ were considered to share the technical features of a method of detecting a norovirus infection in a subject comprising: (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; and (b) detecting norovirus in said sample by binding of said antibody or antibody fragment to a norovirus antigen in said sample; a method of treating a subject infected with norovirus, or reducing the likelihood of infection of a subject at risk of contracting norovirus, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; a monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences; a hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences; a vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences; a method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with norovirus comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; a method of determining the antigenic integrity, correct conformation and/or correct sequence of a norovirus antigen comprising: (a) contacting a sample comprising said antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences, respectively; and (b) determining antigenic integrity, correct conformation and/or correct sequence of said antigen by detectable binding of said first antibody or antibody fragment to said antigen; a human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same, wherein said antibody or antibody fragment binds to norovirus capsid protein P domain and/or S domain; these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2017/011394 A1 to Baylor College of Medicine et al. discloses a method of detecting a norovirus infection in a subject (Compositions or antibodies described herein may be utilized in the treatment, prevention, and/or detection of Norovirus infection for a mammal, including at least a human, Para. [0070];) comprising: (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences (the method of evaluating an individual suspected of or having Norovirus comprises the step of detecting binding of an antibody that specifically binds to a particular Norovirus epitope in a biological sample from the individual, Para. [0074]; The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were identified, Para. [0029]); and (b) detecting norovirus in said sample by binding of said antibody or antibody fragment to a norovirus antigen in said sample (detecting binding of an antibody that specifically binds to a particular Norovirus epitope in a biological sample from the individual, wherein the detection in the biological sample is indicative of the presence of Norovirus, Para. [0074]); a method of treating a subject infected with norovirus, or

reducing the likelihood of infection of a subject at risk of contracting norovirus (there is a method for treating a Norovirus infection comprising the step of administering an effective amount of antibody that specifically binds an epitope, Para. [0079]), comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences (administering an effective amount of antibody that specifically binds an epitope comprising a conformational epitope formed by surface-exposed loop clusters in the P domain in the capsid protein, Para. [0079]; The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]); a monoclonal antibody (the monoclonal antibody is a human monoclonal antibody, Para. [0007]), wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences (The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]); a hybridoma or engineered cell encoding an antibody or antibody fragment (Certain embodiments are directed to a hybridoma cell and to a monoclonal antibody produced by a hybridoma, Para. [0057]) wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences (The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]); a vaccine formulation (vaccines and antiviral compounds against human noroviruses. The structural details relating how human antibody interacts with norovirus to block HBGA binding are useful for development of various therapeutic compounds, Para. [0007]; a vaccine composition, Para. [0114]) comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences (The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]); protecting a subject infected with or at risk of infection with norovirus (vaccines and antiviral compounds against human noroviruses. The structural details relating how human antibody interacts with norovirus to block HBGA binding are useful for development of various therapeutic compounds, Para. [0007]; a vaccine composition, Para. [0114]) comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences (The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]); a human monoclonal antibody or antibody fragment, or hybridoma or engineered cell producing the same (the monoclonal antibody is a human monoclonal antibody, Para. [0007]), wherein said antibody or antibody fragment binds to norovirus capsid protein P domain and/or S domain (the epitope is a conformational epitope formed by two surface-exposed loop clusters in the P domain of VP1 of human Norovirus... the disclosure comprises residues in the P domain in the capsid protein, Para. [0049]; an epitope recognized by inhibitory antibodies, Para. [0007]); a method of determining the antigenic integrity, correct conformation and/or correct sequence of a norovirus antigen (the inventors superimposed the Fab 512-P domain complex structure with an HBGA bound NV P domain structure (FIG. 16A, 16B). This comparison shows that Fab 512 binds in close proximity to the primary HBGA binding site (FIG. 16B) but does not alter the structural integrity of the HBGA binding site, including the sidechain orientations of the residues that participate in HBGA binding, Para. [0211]) comprising: (a) contacting a sample comprising said antigen with a first antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; and (b) determining antigenic integrity, correct conformation and/or correct sequence of said antigen by detectable binding of said first antibody or antibody fragment to said antigen (the method of evaluating an individual suspected of or having Norovirus comprises the step of detecting binding of an antibody that specifically binds to a particular Norovirus epitope in a biological sample from the individual, Para. [0074]; The VH domain was cloned, Para. [0220]; The VL domain was cloned, Para. [0220]; All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were Identified, Para. [0029]; Structural integrity and purity of the VLP preparations were confirmed, Para. [0234]).

US 6,610,305 B1 to Elbers et al. discloses a method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with a virus (a live vaccine for use in the prevention and/or treatment of BVDV infections in pregnant cows. This allows for the first time ever to prevent and protect the fetus itself against BVDV infections with a live vaccine, Col. 2, Lns. 62-66).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.