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(54) Titre : PROCEDE DE DETECTION D'UNE REPONSE IMMUNITAIRE CONTRE FLID ET SON UTILISATION EN TANT QUE BIOMARQUEUR D'UNE INFECTION PAR H. PYLORI

(54) Title: METHOD FOR THE DETECTION OF AN IMMUNE RESPONSE AGAINST FLID AND ITS USE AS A BIOMARKER FOR H. PYLORI INFECTION

(57) Abrégé/Abstract:
The present invention is related to a method for detecting H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject an immune response against FliD, wherein the immune response comprises an anti-FliD antibody.

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(54) Title: METHOD FOR THE DETECTION OF H.PYLORI INFECTION

(57) Abstract: The present invention is related to a method for detecting H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject an immune response against FliD, wherein the immune response comprises an anti-FliD antibody.

METHOD FOR THE DETECTION OF AN IMMUNE RESPONSE AGAINST FLID AND ITS USE AS A BIOMARKER FOR H. PYLORI INFECTION

The present invention is related to a method for detecting Helicobacter infection and more particularly H. pylori infection, use of an immune response as a biomarker, use of a Helicobacter protein as a biomarker, use of a nucleic acid coding for a Helicobacter protein as a biomarker, and a kit for use in the method for detecting Helicobacter infection and more particularly H. pylori infection.

Helicobacter pylori (H. pylori), a microaerophilic, Gram-negative and spiral bacterium is colonizing approximately half of the world population and considered to be a human-specific gastric pathogen (Michetti, et al., 1999). Most infected individuals develop asymptomatic chronic gastritis. However, in some subjects the infection causes chronic gastritis, peptic ulceration and atrophy, and plays an important role in the development of mucosa-associated lymphoid tissue (MALT) lymphoma, gastric adenocarcinoma and primary gastric non-Hodgkin's lymphoma (Suganuma, et al., 2001).

The World Health Organization has categorized H. pylori as a class I carcinogen (Goto, et al., 1999), and direct evidence of carcinogenesis has been demonstrated in animal models (Honda, et al., 1998; T. Watanabe, et al., 1998). Eradication of H. pylori can prevent gastric cancer in humans (Uemura, et al., 2001). Test & treat strategies have been considered in populations with high gastric cancer risk (Yamaoka, et al., 1998). However, such approach is hampered by the lack of efficient and affordable screening systems especially for countries of lower socioeconomic status. In these countries only serologic tests are applicable, most of which suffer from poor performance or are not well validated. For H. pylori serology there are several specific single markers known and described. These factors have been applied in many diagnostic approaches, but almost all of them have significant limitations which make them unsuitable for H. pylori diagnosis. For instance, the cytotoxin-associated protein (CagA) is a very well characterized H. pylori protein. It is encoded on the cag-PAI (cytotoxin associated gene Pathogenicity Island) and is described as an oncogenic protein (Franco, et al., 2005; Murata-Kamiya, et al., 2007). This protein is also a highly immunogenic antigen,

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making it a frequently employed marker for serologic tests. CagA positivity can be used as an indicator of H. pylori virulence because individuals infected with CagA positive strains are at a higher risk for developing gastroduodenal diseases. However, it is not suitable as a single marker, since only a subgroup of H. pylori strains are CagA positive. Moreover, CagA positivity is not a hallmark of active infection as H. pylori eradicated patients maintain antibodies against CagA for many years (Fusconi, et al., 1999). Therefore it should always be combined with other suitable antigens in serologic tests to confirm positivity. Another well-characterized H. pylori protein is the vacuolating cytotoxin (VacA). It was reported to induce vacuolation in cells exposed to H. pylori supernatants or purified protein (Cover & Blaser, 1992). The vacA gene codes for a 140 kDa pro-toxin, where the amino-terminal signal sequence and the carboxy-terminal fragment are proteolytically cleaved during secretion, leading to an active protein with a molecular mass of 88 kDa that aggregates to hexamers and forms a pore (Montecucco & de Bernard, 2003). This protein consists of two different regions. A signal sequence (s1a, s1b, s2) and a mid-region (m1, m2), both with high allelic variations which appear to regulate cytotoxic activity (Atherton, et al., 1995). The high diversity of VacA makes this protein unsuitable for serologic testing.

Another well characterized H. pylori protein, GroEL, belongs to the family of molecular chaperones, which are required for the proper folding of many proteins under stress conditions (Dunn, et al., 1992). In different studies it was shown that this protein is highly conserved among different H. pylori strains and that its seropositivity was even higher than for the CagA in infected patients (Macchia, et al., 1993; Suerbaum, et al., 1994). Also, in studies performed by the instant inventors it was observed that a positive serostatus for GroEL was more often found in German gastric cancer patients compared to matched controls (unpublished data). Also, it is suggested that antibodies against GroEL might persist longer after disease-related loss of H. pylori infection. Thus, GroEL may be a suitable marker of either current or past infection, and may be helpful to overcome the underestimation of H. pylori–related gastric cancer risk due to clearance of infection (Gao et al., 2009).

Therefore, the problem underlying the present invention was the provision of a method for detecting H. pylori infection with high sensitivity and/or high specificity. Another problem underlying the present invention was the provision of an assay which, compared to the assays

of the prior art, leads to less false positive and less false negative results, particularly in population-based approaches. A further problem underlying the present invention was to provide means for carrying out such methods and such assays, respectively. A still further problem underlying the present invention was the provision of a biomarker for H. pylori infected patients, whereby the biomarker preferably does not show any cross-reactivity with other bacteria and any proteins, peptides or nucleic acid molecules coding for such proteins and peptides in particular.

These and other problems underlying the present invention are solved by the subject matter of the attached independent claims. Preferred embodiments may be taken from the attached dependent claims.

These and other problems underlying the present invention are also solved by the following embodiments.

Embodiment 1: A method for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject an immune response against FliD.

Embodiment 2: The method of Embodiment 1, wherein if an immune response against FliD is detected in the sample from the subject, the subject is suffering from a Helicobacter infection, preferably an H. pylori infection, or the subject has undergone a Helicobacter infection, preferably an H. pylori infection, in the past.

Embodiment 3: The method of any one of Embodiments 1 to 2, wherein if no immune response against FliD is detected in the sample from the subject, the subject is not suffering from a Helicobacter infection, preferably an H. pylori infection.

Embodiment 4: The method of any one of Embodiments 1 to 2, wherein if no immune response against FliD is detected in the sample from the subject, the subject has undergone a Helicobacter infection, preferably an H. pylori infection, in the past.

Embodiment 5: The method of any one of Embodiments 1 to 4, wherein the immune response against FliD is an antibody response against FliD, preferably an anti-FliD antibody response.

Embodiment 6: The method of Embodiment 5, wherein the immune response against FliD is an antibody response against FliD and wherein the antibody response against FliD comprises at least one anti-FliD antibody selected from the group comprising an IgG antibody and an IgA antibody.

Embodiment 7: The method of Embodiment 5, wherein the immune response against FliD is an anti-FliD antibody response and wherein the anti-FliD antibody response comprises at least one anti-FliD antibody selected from the group comprising an IgG antibody and an IgA antibody.

Embodiment 8: The method of any one of Embodiments 1 to 7, wherein the subject is infected with Helicobacter, preferably H. pylori, expressing FliD.

Embodiment 9: The method of any one of Embodiments 1 to 8, wherein the subject is different from a subject which is immunosuppressed, preferably the subject is different from a subject which is under immunosuppressive therapy.

Embodiment 10: The method of any one of Embodiments 1 to 9, wherein the method further comprises detecting one or more antigens of Helicobacter, preferably of H. pylori.

Embodiment 11: The method of Embodiment 10, wherein the one or more antigens of Helicobacter, preferably H. pylori, is selected from the group comprising CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA.

Embodiment 12: The method of any one of Embodiments 1 to 11, wherein the method comprises reacting the sample with FliD or a fragment thereof.

Embodiment 13: The method of Embodiment 13, wherein the method comprises reacting the sample with a full-length FliD.

Embodiment 14: The method of any one of Embodiments 12 to 13, wherein the immune response against FliD comprises at least one of a humoral compound capable of interacting with FliD and a cellular compound capable of interacting with FliD, wherein the at least one humoral compound and/or cellular compound interacts with FliD, preferably the at least one humoral compound and/or cellular compound interacting with FliD forms an interaction product with FliD.

Embodiment 15: The method of Embodiment 14, wherein the immune response against FliD is an antibody response against FliD and wherein the antibody response against FliD forms an interaction product with FliD.

Embodiment 16: The method of Embodiment 14, wherein the immune response against FliD is an anti-FliD antibody response and wherein the anti-FliD response forms an interaction product with FliD.

Embodiment 17: The method of Embodiment 14, wherein the immune response against FliD comprises at least one anti-FliD antibody and wherein the anti-FliD antibody forms an interaction product with FliD.

Embodiment 18: The method of any one of Embodiments 14 to 17, wherein the interaction product is detected.

Embodiment 19: The method of Embodiment 18, wherein the interaction product is directly detected.

Embodiment 20: The method of Embodiment 18, wherein the interaction product is indirectly detected.

Embodiment 21: The method of any one of Embodiments 1 to 20, wherein the detection occurs by means of an ELISA or a line immunoassay.

Embodiment 22: The method of any one of Embodiments 1 to 20, wherein the detection occurs by means of a lateral flow assay.

Embodiment 23: The method of any one of Embodiments 1 to 22, wherein the sample is selected from the group comprising serum, plasma and whole blood.

Embodiment 24: The method of any one of Embodiments 1 to 23, wherein the subject is a human being and Helicobacter infection is H. pylori infection.

Embodiment 25: The method of Embodiment 24, wherein the FliD reacted with the sample is FliD from H. pylori.

Embodiment 26: The method of Embodiment 25, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 1.

Embodiment 27: The method of any one of Embodiments 1 to 23, wherein the subject is pig and Helicobacter infection is Helicobacter suis infection.

Embodiment 28: The method of Embodiment 27, wherein the FliD reacted with the sample is FliD from H. suis.

Embodiment 29: The method of Embodiment 28, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 3.

Embodiment 30: The method of any one of Embodiments 1 to 23, wherein the subject is cat and Helicobacter infection is Helicobacter felis infection. Preferably, the cat is selected from the group comprising domestic cat, wild cat, small cat and big cat.

Embodiment 31: The method of Embodiment 30, wherein the FliD reacted with the sample is FliD from H. felis.

Embodiment 32: The method of Embodiment 31, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 5.

Embodiment 33: The method of any one of Embodiments 1 to 32, wherein sensitivity of the method for detecting a Helicobacter infection, preferably a H. pylori infection in man, is more than 90 % and/or 97% or less.

Embodiment 34: The method of any one of Embodiments 1 to 33, wherein specificity of the method for detecting a Helicobacter infection, preferably a H. pylori infection in man, is more than 90 % and/or 99% or less.

Embodiment 35: Use of an immune response against FliD in a subject as a biomarker.

Embodiment 36: The use of Embodiment 35, wherein the biomarker is a biomarker for infection of the subject with Helicobacter.

Embodiment 37: The use of any one of Embodiment 35 to 36, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is man and Helicobacter is H. pylori.

Embodiment 38: The use of any one of Embodiments 35 to 36, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is pig and Helicobacter is H. suis.

Embodiment 39: The use of any one of Embodiments 35 to 36, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is cat and Helicobacter is H.felis.

Embodiment 40: The use according to any one of Embodiments 35 to 39, wherein the biomarker is a predictive biomarker.

Embodiment 41: The use according to any one of Embodiments 35 to 40, wherein the immune response is an antibody response against FliD.

Embodiment 42: The use according to any one of Embodiments 35 to 41, wherein the immune response is an anit-FliD antibody response against FliD.

Embodiment 43: A kit comprising FliD or a fragment thereof and at least one further constituent.

Embodiment 44: The kit of Embodiment 43, wherein the at least one further constituent is selected from the group comprising a buffer, a solid phase and an instruction leaflet.

Embodiment 45: The kit of any one of Embodiment 43 to 44, wherein FliD is full-length FliD.

Embodiment 46: The kit according to any one of Embodiments 43 to 44, wherein FliD comprises an amino acid sequence and wherein the amino acid sequence is selected from the group comprising an amino acid sequence according to SEQ ID NO: 1, an amino acid sequence according to SEQ ID NO: 3 and an amino acid sequence according to SEQ ID NO: 5.

Embodiment 47: The kit according to any one of Embodiments 43 to 46, wherein the kit is suitable for use or is for use in a method for detecting Helicobacter infection in a subject.

Embodiment 48: The kit according to Embodiment 47, wherein the kit is suitable for use or is for use in a method of any one of Embodiments 1 to 34.

Embodiment 49: A method for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting FliD in a sample from the subject.

Embodiment 50: The method of Embodiment 49, wherein if FliD is detected in the sample from the subject, the subject is suffering from a Helicobacter infection, preferably an H. pylori infection, or the subject has undergone a Helicobacter infection, preferably an H. pylori infection, in the past.

Embodiment 51: The method of any one of Embodiments 49 to 50, wherein if no FliD is detected in the sample from the subject, the subject is not suffering from a Helicobacter infection, preferably an H. pylori infection.

Embodiment 52: The method of any one of Embodiments 49 to 51, wherein if no FliD is detected in the sample from the subject, the subject has undergone a Helicobacter infection, preferably an H. pylori infection in the past.

Embodiment 53: The method of any one of Embodiments 49 to 52, wherein the subject is infected with Helicobacter, preferably H. pylori, expressing FliD.

Embodiment 54: The method of any one of Embodiments 49 to 53, wherein the method further comprises detecting one or more antigens of Helicobacter, preferably of H. pylori.

Embodiment 55: The method of Embodiment 54, wherein the one or more antigens of Helicobacter, preferably H. pylori, is selected from the group comprising CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA.

Embodiment 56: The method of any one of Embodiments 49 to 55, wherein FliD is full-length FliD or a fragment thereof.

Embodiment 57: The method of any one of Embodiments 49 to 56, wherein the method comprises reacting the sample with an interacting agent, wherein the interacting agent is interacting with FliD or a fragment thereof, preferably the interacting agent is specifically interacting with FliD or a fragment thereof.

Embodiment 58: The method of Embodiment 57, wherein the interacting agent is interacting with full-length FliD or a fragment of FliD.

Embodiment 59: The method of any one of Embodiments 57 to 58, wherein the interacting agent is selected from the group comprising an antibody, an aptamer and a spiegelmer.

Embodiment 60: The method of Embodiment 59, wherein the interacting agent is an antibody, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

Embodiment 61: The method of any one of Embodiments 56 to 60, wherein the interacting agent and the FliD present in the sample form an interaction product.

Embodiment 62: The method of Embodiment 61, wherein the interaction product is detected.

Embodiment 63: The method of Embodiment 62, wherein the interaction product is directly detected.

Embodiment 64: The method of Embodiment 62, wherein the interaction product is indirectly detected.

Embodiment 65: The method of any one of Embodiments 49 to 64, wherein the detection occurs by means of an ELISA or a line immunoassay.

Embodiment 66: The method of any one of Embodiments 49 to 63, wherein the detection occurs by means of a lateral flow assay.

Embodiment 67: The method according to any one of Embodiments 49 to 56, wherein FliD is detected by means of mass spectroscopy.

Embodiment 68: The method according to Embodiment 67, wherein mass spectroscopy is selected from the group comprising LC-ESI-MS/MS, MALDI-MS, tandem MS, TOF/TOF, TOF-MS, TOF-MS/MS, triple quadrupole MS, and triple quadrupole MS/MS.

Embodiment 69: The method of any one of Embodiments 49 to 68, wherein the subject is a human being and Helicobacter infection is H. pylori infection.

Embodiment 70: The method of Embodiment 69, wherein the FliD is from H. pylori.

Embodiment 71: The method of Embodiment 70 wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 1.

Embodiment 72: The method of any one of Embodiments 49 to 68, wherein the subject is pig and Helicobacter infection is Helicobacter suis infection.

Embodiment 73: The method of Embodiment 72, wherein the FliD is from H. suis.

Embodiment 74: The method of Embodiment 73, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 3.

Embodiment 75: The method of any one of Embodiments 49 to 68, wherein the subject is cat and Helicobacter infection is Helicobacter felis infection.

Embodiment 76: The method of Embodiment 75, wherein the FliD is from H. felis.

Embodiment 77: The method of Embodiment 76, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 5.

Embodiment 78: The method of any one of Embodiment 49 to 77, wherein the sample is selected from the group comprising stool, serum, plasma and whole blood, preferably the sample is stool.

Embodiment 79: Use of FliD as a biomarker.

Embodiment 80: The use of Embodiment 79, wherein the biomarker is a biomarker for infection of a subject with Helicobacter.

Embodiment 81: The use of any one of Embodiments 79 to 80, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is man and Helicobacter is H. pylori.

Embodiment 82: The use of Embodiment 81, wherein FliD comprises an amino acid sequence according to SEQ ID NO: 1.

Embodiment 83: The use of any one of Embodiments 80 to 81, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is pig and Helicobacter is H. suis.

Embodiment 84: The use of Embodiment 83, wherein FliD comprises an amino acid sequence according to SEQ ID NO: 3.

Embodiment 85: The use of any one of Embodiments 80 to 81, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is cat and Helicobacter is H.felis.

Embodiment 86: The use of Embodiment 85, wherein FliD comprises an amino acid sequence according to SEQ ID NO: 5.

Embodiment 87: The use according to any one of Embodiment 79 to 86, wherein the biomarker is a predictive biomarker.

Embodiment 88: A kit comprising an interacting agent capable of interacting with FliD or a fragment thereof and at least one further constituent.

Embodiment 89: The kit of Embodiment 88, wherein the at least one further constituent is selected from the group comprising a buffer, a solid phase and an instruction leaflet.

Embodiment 90: The kit of Embodiment 89, wherein the interacting agent is capable of specifically interacting with FliD or a fragment thereof.

Embodiment 91: The kit of any one of Embodiments 88 to 90, wherein the interacting agent is selected from the group comprising an antibody, an aptamer and a spiegelmer.

Embodiment 92: The kit according to any one of Embodiments 88 to 91, wherein the kit is suitable for use or is for use in a method for detecting Helicobacter infection in a subject.

Embodiment 93: The kit according to Embodiment 92, wherein the kit is suitable for use or is for use in a method of any one of Embodiments 49 to 78.

Embodiment 94: A method for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject a nucleic acid coding for FliD.

Embodiment 95: The method of Embodiment 94, wherein the nucleic acid is a genomic nucleic acid coding for FliD, preferably DNA

Embodiment 96: The method of Embodiment 94, wherein the nucleic acid is an mRNA coding for FliD.

Embodiment 97: The method of any one of Embodiments 94 to 96, wherein if a nucleic acid coding for FliD is detected in the sample from the subject, the subject is suffering from a Helicobacter infection, preferably an H. pylori infection, or the subject has undergone a Helicobacter infection, preferably an H. pylori infection, in the past.

Embodiment 98: The method of any one of Embodiment 94 to 97, wherein if no nucleic acid coding for FliD is detected in the sample from the subject, the subject is not suffering from a Helicobacter infection, preferably an H. pylori infection.

Embodiment 99: The method of any one of Embodiments 94 to 98, wherein if no nucleic acid coding for FliD is detected in the sample from the subject, the subject has undergone a Helicobacter infection, preferably an H. pylori infection in the past.

Embodiment 100: The method of any one of Embodiments 94 to 99, wherein the subject is infected with Helicobacter, preferably H. pylori, expressing FliD.

Embodiment 101: The method of any one of Embodiment 94 to 100, wherein the method further comprises detecting one or more antigens of Helicobacter, preferably of H. pylori, and/or a nucleic acid coding for one or more antigens of Helicobacter, preferably of H. pylori.

Embodiment 102: The method of Embodiment 101, wherein the one or more antigens of Helicobacter, preferably H. pylori, is selected from the group comprising CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA.

Embodiment 103: The method of any one of Embodiments 94 to 102, wherein FliD is full-length FliD or a fragment thereof.

Embodiment 104: The method of any one of Embodiments 94 to 103, wherein the method comprises reacting the sample with an interacting agent, wherein the interacting agent is interacting with a nucleic acid coding for FliD, preferably the interacting agent is specifically interacting with a nucleic acid coding for FliD.

Embodiment 105: The method of Embodiment 104, wherein the interacting agent is interacting with a nucleic acid coding for full-length FliD or a fragment of FilD.

Embodiment 106: The method of any one of Embodiments 104 to 105, wherein the interacting agent is selected from the group comprising a primer and a probe.

Embodiment 107: The method of any one of Embodiments 104 to 106, wherein the interacting agent and the nucleic acid coding for FliD present in the sample form an interaction product.

Embodiment 108: The method of Embodiment 107, wherein the interaction product is detected.

Embodiment 109: The method of Embodiment 108, wherein the interaction product is directly detected.

Embodiment 110: The method of Embodiment 108, wherein the interaction product is indirectly detected.

Embodiment 111: The method according to any one of Embodiments 94 to 103, wherein a nucleic acid molecule coding for FliD is detected by means of mass spectroscopy, PCR or a hybridization assay.

Embodiment 112: The method according to Embodiment 111, wherein mass spectroscopy is selected from the group comprising LC-ESI-MS/MS, MALDI-MS, tandem MS, TOF/TOF, TOF-MS, TOF-MS/MS, triple quadrupole MS, and triple quadrupole MS/MS.

Embodiment 113: The method of any one of Embodiments 94 to 112, wherein the subject is a human being and Helicobacter infection is H. pylori infection.

Embodiment 114: The method of Embodiment 113, wherein the nucleic acid coding for FliD is from H. pylori.

Embodiment 115: The method of Embodiment 114, wherein the nucleic acid coding for FliD comprises a nucleotide sequence according to SEQ ID NO: 2.

Embodiment 116: The method of any one of Embodiments 94 to 112, wherein the subject is pig and Helicobacter infection is Helicobacter suis infection.

Embodiment 117: The method of Embodiment 116, wherein the nucleic acid coding for FliD is from H. suis.

Embodiment 118: The method of Embodiment 117, wherein the nucleic acid coding for FliD comprises a nucleotide sequence according to SEQ ID NO: 4.

Embodiment 119: The method of any one of Embodiments 94 to 112, wherein the subject is cat and Helicobacter infection is Helicobacter felis infection.

Embodiment 120: The method of Embodiment 119, wherein the nucleic acid coding for FliD is from H. felis.

Embodiment 121: The method of Embodiment 120, wherein the FliD comprises an amino acid sequence according to SEQ ID NO: 6.

Embodiment 122: The method of any one of Embodiments 94 to 121, wherein the sample is selected from the group comprising stool, serum, plasma and whole blood, preferably the sample is stool.

Embodiment 123: Use of a nucleic acid coding for FliD as a biomarker.

Embodiment 124: The use of Embodiment 123, wherein the biomarker is a biomarker for infection of a subject with Helicobacter.

Embodiment 125: The use of any one of Embodiments 123 to 124, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is man and Helicobacter is H. pylori.

Embodiment 126: The use of Embodiment 125, wherein the nucleic acid coding for FliD comprises a nucleotide sequence according to SEQ ID NO: 2.

Embodiment 127: The use of any one of Embodiments 124 to 125, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is pig and Helicobacter is H. suis.

Embodiment 128: The use of Embodiment 127, wherein the nucleic acid coding for FliD comprises a nucleotide sequence according to SEQ ID NO: 4.

Embodiment 129: The use of any one of Embodiments 124 to 125, wherein the biomarker is a biomarker for infection of the subject with Helicobacter, wherein the subject is cat and Helicobacter is H. felis.

Embodiment 130: The use of Embodiment 129, wherein the nucleic acid coding for FliD comprises a nucleotide sequence according to SEQ ID NO: 6.

Embodiment 131: The use according to any one of Embodiments 123 to 130, wherein the biomarker is a predictive biomarker.

Embodiment 132: A kit comprising an interacting agent capable of interacting with a nucleic acid coding for FliD or a fragment thereof and at least one further constituent.

Embodiment 133: The kit of Embodiment 132, wherein the at least one further constituent is selected from the group comprising a buffer, a solid phase and an instruction leaflet.

Embodiment 134: The kit of Embodiment 133, wherein the interacting agent is capable of specifically interacting with FliD or a fragment thereof.

Embodiment 135: The kit of any one of Embodiments 132 to 134, wherein the interacting agent is selected from the group comprising a primer and a probe.

Embodiment 136: The kit according to any one of Embodiments 132 to 135, wherein the kit is suitable for use or is for use in a method for detecting Helicobacter infection in a subject.

Embodiment 137: The kit according to Embodiment 136, wherein the kit is suitable for use or is for use in a method of any one of Embodiments 94 to 122.

The present inventors have surprisingly found that FliD which is a protein also referred to as "hook-associated protein 2 homologue", is a marker for infection with Helicobacter and H. pylori in particular. The present inventors have also surprisingly found that FliD and/or an immune response against FliD can be advantageously used as a marker in serological analysis and, accordingly, in any method and assay, respectively, which is based on or makes use of a sample of a subject to be tested for Helicobacter and H. pylori infection in particular, whereby the sample is preferably selected from the group comprising a serum sample, a plasma sample, a blood sample and a stool sample. Finally, the present inventors have surprisingly found that infection of a subject with Helicobacter and H. pylori in particular can be detected based on FliD and/or a nucleic acid coding for FliD, whereby FliD and/or the nucleic acid coding for FliD are used as the sole marker. In other words, according to the present

invention, an infection of a subject with Helicobacter and H. pylori in particular can be diagnosed solely based and, respectively, relying on FliD and/or a nucleic acid coding therefor. The same is also true for an immune response against FliD developed by a subject infected with Helicobacter and H. pylori in particular: According to the present invention, an infection of a subject with Helicobacter and H. pylori in particular can be diagnosed solely based and, respectively, relying on an immune response against FliD, whereby the immune response against FliD was generated by the subject. A further advantage of the present invention is that the immune response against FliD and FliD as such can be determined in a sample which is typically obtained by non-invasive methods which is in contrast to many detection methods of the prior art where the sample has to be taken by an invasive method such as a biopsy.

It will be acknowledged by a person skilled in the art that the present invention can in principle be applied to the detection of any infection of a subject with Helicobacter as long as such Helicobacter codes for and/or expresses FliD. It will also be acknowledged by a person skilled in the art that, typically, a distinct species of a subject such as, e.g., man, will be infected by a distinct species of Helicobacter. In case the subject is man, the species of Helicobacter is H. pylori. In case the subject is pig, the species of Helicobacter is H. suis. In case the subject is cat, including big cats, the species of Helicobacter is H. felis. The instant specification particularly refers to the detection of H. pylori in man. Such reference to H. pylori and man, however, is made solely for reasons of clarity and given the above, any embodiment referring to H. pylori and man, equally applies to any other Helicobacter expressing FliD, or a homologue thereof, and any other species of the subject. Preferably, the other species of the subject is any mammal which suffers or may suffer from an infection with Helicobacter and a species homolog to H. pylori, whereby such Helicobacter and species homolog to H. pylori expresses FliD or a homologue thereof.

It will also be acknowledged by a person skilled in the art that for each species of Helicobacter typically various strains exist. The amino acid sequence and the nucleic acid sequence of FliD of such strains of the Helicobacter species typically show a very high identity in terms of amino acid sequence. More specifically, bioinformatic analysis revealed that FliD amino acid sequence is present and highly conserved in all (>200) H. pylori strains.

FliD has a homology of 97% in around 200 H. pylori strains which were analyzed by the present inventors. Interestingly, except for some other non-pylori Helicobacter species with partial homology, there is no other known organism with a significant genomic or proteomic homology to FliD of H. pylori. Comparison of the H. pylori FliD protein shows the high conservation of FliD in Helicobacter species, while it is distinct from most other bacteria as well as eukaryotic organisms. This analysis together with high antigenicity prediction of this protein provides the rational for factually no cross-reactivity.

Furthermore, FliD is expressed by factually all strains which infect or which are capable of infecting a subject. This explains why according to the present invention FliD is a marker for factually each strain of H. pylori and, respectively, each strain of the Helicobacter species infecting the respective subject species. In other words, nearly all H. pylori positive patients show an immune response against FliD.

The H. pylori FliD protein is an essential element in the assembly of the functional flagella and a FliD mutant strain is completely non-motile. Flagellin plays a central role in bacterial motility and is necessary for colonization and persistence of H. pylori infection (Eaton, et al., 1996). Motility of H. pylori is a virulent factor in the pathogenesis of gastric mucosal injury (S. Watanabe, et al., 1997). The H. pylori FliD gene encodes a 76-kDa protein (Kim, et al., 1999). The FliD operon of H. pylori consists of FlaG, FliD, and FliS genes, in the order stated, under the control of a Sigma (28)-dependent promoter. An entry for FliD from H. pylori can be found in databanks UniProtKB/Swiss-Prot as P96786.4 providing, among others, the amino acid sequence thereof and mutations of FliD as found in various strains of H. pylori.

The method of the invention for detecting Helicobacter infection in a subject, preferably an H. pylori infection in a subject, can also be characterized such that it comprises the step of determining whether a sample from the subject contains an immune response against FliD, FliD or a nucleic acid coding for FliD. If the sample from the subject matter contains an antibody response against FliD, FliD or a nucleic acid coding for FliD, the subject is suffering from Helicobacter infection, preferably an H. pylori infection, or has undergone a Helicobacter infection in the past, preferably an H. pylori infection in the past.

The methods of the invention for detecting Helicobacter infection in a subject, preferably an H. pylori infection in a subject, can also be applied to a subject of which it is unknown whether it is suffering from a Helicobacter infection, preferably H. pylori infection, or whether such subject has undergone a Helicobacter infection, preferably H. pylori infection. Insofar, the present invention is related in a further aspect to methods for determining whether a subject is suffering from a Helicobacter infection, preferably H. pylori infection, or has undergone a Helicobacter infection, preferably H. pylori infection in the past.

As preferably used herein, the expression "in the past" refers to a point in time which is prior to the point in time when a sample has been or is taken from a subject, whereby such sample is a sample used in connection with the various aspects and/or the various embodiments of the present invention and in particular in detecting H. pylori and/or H. pylori infection in a subject and in the diagnosis of H. pylori and/or H. pylori infection in a subject.

In connection with the various aspects of the present invention and the various methods of the invention in particular, it will be acknowledged by a person skilled in the art that the immune response and the anti-FliD antibody response generated by the subject infected by Helicobacter and H.pylori in particular persists over some years. The prevalence of such anti-FliD antibody response is typically about 50 % after 1 to 5 years after eradication of H. pylori, about 50 % after 6 to 10 years after eradication of H. pylori, about 25 % after 11 to 15 years after eradication of H. pylori and about 25 % after 16 to 20 years after eradication of H. pylori. In light thereof, a subject which is diagnosed as H. pylori positive may be a subject which is actually suffering from H. pylori infection at the time the sample was taken, or a subject which had undergone an H. pylori infection in the past with the anti-FliD immune response still prevailing.

To the extent that immune response against FliD is an antibody response against FliD and more specifically an anti-FliD antibody response, the anti-FliD antibodies are typically IgG or IgA. This class specificity can be used in detection the anti-FliD antibodies by using, as the detecting antibodies or capture antibodies, anti-IgG and/or anti-IgA antibodies. In the

embodiment where the subject is man, the detecting antibodies and capture antibodies are preferably anti-human IgG and/or anti-human IgA.

In connection with the various aspects of the present invention and the various methods of the invention in particular, the methods may, in an embodiment, additionally comprise the detection of one or more Helicobacter antigens or a nucleic acid coding for such Helicobacter antigens. In an embodiment, such Helicobacter antigens are H. pylori antigens. In a further embodiment, the antigens are selected from the group comprising CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA which are all known in the art, and described, for example, in Yakoob J et al. (Yakoob J et al., Gut and Liver, Vol. 4, No. 3, September2010, pp. 345-350), Sabarth N et al. (Sabarth N et al., Infection and Immunity, Nov. 2002, p. 6499-6503), Gao L. et al. (Gao L. et al., Cancer Res 2009; 69: (15). August 1, 2009, p. 6164 – 6170), Yamaoka Y (Yamaoka Y, J Med Microbiol. 2008 May; 57 (Pt5): 545-553), Miehlke S et al. (Miehlke S et al., Int. J. Cancer: 87, 322-327 (2000)), and Atherton JC et al. (Atherton JC et al., Current Microbiology, Vol. 39(1999), pp 211-218). An amino acid sequence of CagA is disclosed herein as SEQ ID NO: 7, a nucleotide sequence of CagA is disclosed herein as SEQ ID NO:8, an amino acid sequence of VacA is disclosed herein as SEQ ID NO: 9, a nucleotide sequence of VacA is disclosed herein as SEQ ID NO:10, an amino acid sequence of GroEL is disclosed herein as SEQ ID NO: 11, a nucleotide sequence of GroEL is disclosed herein as SEQ ID NO:12, an amino acid sequence of Hp0231 is disclosed herein as SEQ ID NO:13, a nucleotide sequence of Hp0231 is disclosed herein as SEQ ID NO:14, an amino acid sequence of JHp0940 is disclosed herein as SEQ ID NO:15, a nucleotide sequence of JHp0940 is disclosed herein as SEQ ID NO:16, an amino acid sequence of HtrA is disclosed herein as SEQ ID NO:17, and a nucleotide sequence of HtrA is disclosed herein as SEQ ID NO:18.

In an embodiment of the method of the invention where Helicobacter infection and more preferably an H. pylori infection in a subject is detected by detecting in a sample from the subject an immune response against FliD and in particular an anti-FliD antibody in the sample, the sample and FliD are reacted. In one embodiment, the sample is added to FliD. Preferably, FliD is attached to a solid phase in such method. It is also within the present invention that FliD is added to the sample. Preferably, FliD is added as a solution, more

preferably as an aqueous solution such as a buffered solution. In a preferred embodiment, FliD is reacted with the sample with FliD being attached to a solid phase. It will be acknowledged by a person skilled in the art that FliD and the sample are reacted under conditions such that, if the sample contains an immune response against FliD and anti-FliD antibodies in particular, an interaction product is formed. Preferably, such interaction product is a complex of FliD and an anti-FliD antibody contained in the sample.

The interaction product thus formed can be either directly or indirectly detected. In the embodiment where the interaction product is detected directly, the FliD reacted with the sample comprises a label which allows the detection of FliD, particularly when interacting with an anti-FliD antibody. Labels of this type are known to the ones skilled in the art and encompass radiolabels, fluorescence labels, dyes, nanoparticles as Gold and enzymes such as horseradish peroxidase. Further labels are those disclosed herein in connection with the labeling of antibodies. In the embodiment where the interaction product is detected indirectly, the interaction product is subsequently reacted with a detection agent, whereby the detection agent specifically binds to the interaction product. Such detection agent may be an antibody, preferably an anti-IgG or an anti-IgA antibody. The detection agent itself is typically comprising a label which allows the detection of the detection agent, preferably when the detection agent is specifically bound to the interaction product.

In preferred embodiments of the methods of the invention the interaction product is detected by means of an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay which are known to a person skilled in the art (Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998). The ELISA may be an indirect ELISA, a sandwich ELISA, a competitive ELISA or a non-competitive ELISA.

In an alternative preferred embodiments of the methods of the invention the interaction product is detected by means of a lateral flow test which is also known as lateral flow immunochromatographic assays which are, for example, described in US 6,485,982. Such lateral flow test is, in an embodiment, used in any method of the invention where either an anti-FliD antibody is and, respectively, anti-FliD antibodies are detected in a sample from a subject. The lateral flow test will be described for illustrative purposes for the embodiment of

the method of the invention where anti-FliD antibodies in a sample from a subject are detected, wherein the subject is man.

The technology is based on a series of capillary beds, such as pieces of porous paper or polymer. Each of these components has the capability to transport fluid, e.g. serum, plasma or blood, precipitately. The sample pad acts as a sponge and holds an excess of sample fluid. When the sample pad is saturated, the fluid moves to the conjugate pad in which nanoparticles, preferably gold nanoparticles, conjugated with anti-human antibody is located. When the sample fluid migrate to this element, it dissolves the particles and in one combined reaction, the sample and conjugate mix flow through the porous structure. In this way, antibody immobilized on the surface of nanoparicles, binds to human IgG existing in the sample while migrating further through the next capillary matrix. On this element which is typically a hydrophobic membrane like nitrocellulose antigens as well as a control (e.g. human IgG) are immobilized as test or control lines. Once human IgG which is now bound to the conjugate particles reaches these lines, antigen immobilized on the membrane will capture antibody complex specifically. After a while, more and more particles accumulate at an antigen site and a simply detectable colored band appears. In one embodiment there is only one antigen, namely FliD. In another embodiment there are, in addition to FliD, one or more antigens. Preferably, the one or more antigens is/are selected from the group comprising CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA.

In a further alternative preferred embodiments of the methods of the invention the interaction product is detected by means of a line assay. Such line assay typically comprises a plurality of strips. On said strips, highly purified recombinant either FliD or an interactin agent which is capable of interacting with FliD is fixed on the strips. Such strips are preferably made of nitrocellulose membrane. The strips are incubated with the sample, preferably with a diluted serum or plasma sample, and the anti-FliD antibodies bind to FliD, in case FliD is immobilized for detecting anti-FliD antibodies in the sample, or FliD binds to the anti-FliD antibodies, in case anti-FliD antibodies are immobilized for detecting FliD in the sample, on the test strips. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG and IgA), which are coupled to horse radish peroxidase. Specifically bound antibodies are detected with a staining reaction catalyzed by the peroxidase. If an antigen-

antibody reaction has taken place forming an interaction product, a dark band will appear on the strip at the corresponding point. In an embodiment control bands at the upper end of the test strips are:

a) The reaction control band under the strip number, which must show a reaction for every sample.
b) The conjugate control bands (IgG, IgA) are used to check the detected antibody class. If, for example, the test strip for the detection of IgG antibodies is used, the IgG conjugate will show a clear band.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of the individual antigen bands.

An assay having this kind of design, with antigens different from FliD, is basically available from Mikrogen GmbH, Neuried, Germany, as "*recom*Line Helicobacter IgG" or "*recom*Line Helicobacter IgA".

In an embodiment of the method of the invention for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject FliD, FliD is detected by means of mass spectrometry which is, e.g., described in Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998.

In those embodiments of the methods of the invention where FliD is detected in a sample from the subject, the interacting agent forming together with FliD the interaction product is preferably one selected from the group comprising an antibody, an aptamer and a spiegelmer. The generation of such interacting agent is within the skills of a person of the art.

The generation of an antibody binding and more particularly specifically binding to FliD, is known to the one skilled in the art and, for example, described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,(1988). Preferably, monoclonal antibodies may be used in connection with the present

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invention which may be manufactured according to the protocol of Cesar and Milstein and further developments based thereon. Antibodies as used herein, include, but are not limited to, complete antibodies, antibody fragments or derivatives such as Fab fragments, Fc fragments and single-stranded antibodies, as long as they are suitable and capable of binding to FliD. Apart from monoclonal antibodies also polyclonal antibodies may be used and/or generated. The generation of polyclonal antibodies is also known to the one skilled in the art and, for example, described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1988).

The antibodies which may be used according to the present invention may have one or several markers or labels. Such markers or labels may be useful for detecting the antibody. Preferably the markers and labels are selected from the group comprising avidine, streptavidine, biotin, gold, enzymes as HRP and fluorescein and used, e. g., in ELISA methods. These and further markers as well as methods are, e. g. described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,(1988).

Aptamers are D-nucleic acids which are either single stranded or double stranded and which specifically interact with a target molecule such as, in the instant invention, FliD. The manufacture or selection of aptamers is, e. g., described in European patent EP 0 533 838. Basically the following steps are realized. First, a mixture of nucleic acids, i. e. potential aptamers, is provided whereby each nucleic acid typically comprises a segment of several, preferably at least eight subsequent randomised nucleotides. This mixture is subsequently contacted with the target molecule whereby the nucleic acid(s) bind to the target molecule, such as based on an increased affinity towards the target or with a bigger force thereto, compared to the candidate mixture. The binding nucleic acid(s) are/is subsequently separated from the remainder of the mixture. Optionally, the thus obtained nucleic acid(s) is amplified using, e. g., polymerase chain reaction. These steps may be repeated several times giving at the end a mixture having an increased ratio of nucleic acids specifically binding to the target from which the final binding nucleic acid is then optionally selected. These specifically binding nucleic acid(s) are referred to as aptamers. It is obvious that at any stage of the method for the generation or identification of the aptamers samples of the mixture of individual nucleic acids may be taken to determine the sequence thereof using standard

techniques. It is within the present invention that the aptamers may be stabilized such as, e. g., by introducing defined chemical groups which are known to the one skilled in the art of generating aptamers. Such modification may for example reside in the introduction of an amino group at the 2'-position of the sugar moiety of the nucleotides.

The generation or manufacture of spiegelmers binding to and more particularly specifically binding to FliD as a target molecule is based on a similar principle. The manufacture of spiegelmers is described in international patent application WO 98/08856. Spiegelmers are L-nucleic acids, which means that they are composed of L-nucleotides rather than D-nucleotides as aptamers are. Spiegelmers are characterized by the fact that they have a very high stability in biological system and, comparable to aptamers, specifically interact with the target molecule against which they are directed. In the purpose of generating spiegelmers, a heterogonous population of D-nucleic acids is created and this population is contacted with the optical antipode of the target molecule, in the present case for example with the D-enantiomer of the naturally occurring L-enantiomer of FliD. Subsequently, those D-nucleic acids are separated which do not interact with the optical antipode of the target molecule. However, those D-nucleic acids interacting with the optical antipode of the target molecule are separated, optionally determined and/or sequenced and subsequently the corresponding L-nucleic acids are synthesized based on the nucleic acid sequence information obtained from the D-nucleic acids. These L-nucleic acids which are identical in terms of sequence with the aforementioned D-nucleic acids interacting with the optical antipode of the target molecule, will specifically interact with the naturally occurring target molecule rather than with the optical antipode thereof. Similar to the method for the generation of aptamers it is also possible to repeat the various steps several times and thus to enrich those nucleic acids specifically interacting with the optical antipode of the target molecule.

In the embodiments of the method of the invention for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject a nucleic acid coding for FliD, the interacting agent is selected from the group comprising a primer and a probe. Given the nucleotide and amino acid sequences of FliD disclosed herein, it is within the skills of a person of the art to design and prepare such primer and probe (see, for example, Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum

Akademischer Verlag Heidelberg, 1998). Such interacting agent can be labeled. The various labels and ways how to label the interacting agent are known to a person skilled in the art. In an embodiment the labels are the same as outlined above in connection with the antibodies.

The interaction product comprising a nucleic acid molecule coding for FliD or a fragment thereof and an interaction agent can be detected by means known to a person skilled in the art and, for example, described in Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998.

In an embodiment of the method of the invention for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject a nucleic acid coding for FliD, the nucleic acid coding for FliD is detected by means of mass spectrometry which is, e.g. described in Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998.

In an embodiment of the method of the invention for detecting Helicobacter infection and more preferably an H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject a nucleic acid coding for FliD, the nucleic acid coding for FliD is detected by means of polymerase chain reaction (PCR) in its diverse forms which are, e.g., described in Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998. Alternatively, the nucleic acid coding for FliD is detected by a hybridization assay as, e.g., described in Lottspeich F. and Zorbas H (eds.), Bioanalytik, Spektrum Akademischer Verlag Heidelberg, 1998.

In those aspects of the invention which are related to biomarker, it will be acknowledged that the immune response against FliD as defined herein, FliD and a nucleic acid coding for FliD each act as a predictive biomarker as the presence of said immune response against FliD as defined herein, FliD and/or nucleic acid coding for FliD is correlated with histology and inflammation in untreated patients.

It will be acknowledged by a person skilled in the art that given the disclosure provided herein the particular design of the kit of the invention is within the common skills of a person skilled in the art. In an embodiment, the kit is a ready-for-use kit.

In a further aspect of the present invention, the present invention is related to the use of the interacting agents as disclosed herein for the detection of FliD as disclosed herein.

As preferably used herein a sample is a sample as immediately obtained from a or the subject, or a sample which has been processed prior to being used in connection with the invention and in particular with the methods of the invention.

In an embodiment of the various aspects and embodiments of the invention the subject is a subject which is assumed to suffer from or suspected of suffering from a H. pylori infection.

In an embodiment Helicobacter infection is infection with Helicobacter or an assumed or suspected infection with Helicobacter.

In an embodiment of any aspect of the present invention where a first compound specifically interacts with or specifically binds to a second compound, the interaction or binding between said first compound and said second compound is characterized by a K_D of 1 μM or less, preferably a K_D of 0.25 μM or less and more preferably a K_D of 0.1 or less.

It will be understood by a person skilled in the art that in those embodiment where FliD is detected, FliD may be present either as a full-length FliD or a fragment of FliD or a fragment of full-length FliD. As preferably used herein a full-length FliD is a FliD as produced by Helicobacter which is active as a virulence factor. In an embodiment a full-length FliD is preferably a FliD as produced by Helicobacter. A fragment of full-length FliD is a fragment the amino acid sequence of which is shorter than the amino acid sequence of full-length FliD, whereby the fragment of FliD is still active as a virulence factor. A fragment of FliD is preferably a fragment of FliD, preferably of full-length FliD, whereby the fragment has an amino acid sequence which is long enough so as to allow a person skilled in the art to identify the fragment to be a fragment of FliD and full-length FliD in particular and to exclude that the

fragment is a fragment of a protein or polypeptide different from FliD and full-length FliD in particular. In a preferred embodiment full-length FliD comprises an amino acid sequence according to SEQ ID NO:1.

The same considerations and definitions equally apply to a nucleic acid coding for FliD. In accordance therewith, it will be understood by a person skilled in the art that in those embodiment where a nucleic acid coding for FliD is detected, a nucleic acid coding for FliD may be present either as a nucleic acid coding for a full-length FliD or a nucleic acid coding for fragment of FliD or a nucleic acid coding for fragment of full-length FliD. As preferably used herein a full-length FliD is a FliD as produced by Helicobacter which is active as a virulence factor. In an embodiment a full-length FliD is preferably a FliD as produced by Helicobacter. A fragment of full-length FliD is a fragment the amino acid sequence of which is shorter than the amino acid sequence of full-length FliD, whereby the fragment of FliD is still active as a virulence factor. A fragment of FliD is preferably a fragment of FliD, preferably of full-length FliD, whereby the fragment has an amino acid sequence which is long enough so as to allow a person skilled in the art to identify the fragment to be a fragment of FliD and full-length FliD in particular and to exclude that the fragment is a fragment of a protein or polypeptide different from FliD and full-length FliD in particular. In a preferred embodiment the nucleic acid coding for a full-length FliD comprises a nucleotide sequence according to SEQ ID NO:2.

A fragment of a nucleic acid coding for FliD is preferably a fragment of a nucleic acid coding for FliD, preferably for full-length FliD, whereby the fragment of the nucleic acid has a nucleotide sequence which is long enough so as to allow a person skilled in the art to identify the fragment to be a fragment of a nucleic acid coding for FliD and full-length FliD in particular and to exclude that the fragment of the nucleic acid is a fragment of a nucleic acid coding for a protein or polypeptide different from FliD and full-length FliD in particular.

It will also be understood by a person skilled in the art that in those embodiments of the methods of the invention where an immune response against FliD as defined herein is detected, FliD which is reacted with the immune response against FliD as defined herein, can be FliD as produced by the Helicobacter species infecting the subject or presumably infecting

the subject, can be a full-length FliD as defined herein or can be a fragment of FliD as defined herein. Furthermore, a fragment of FliD is, in an embodiment, a fragment of FliD having a shorter amino acid sequence than FliD, wherein the fragment can be used in said embodiments of the methods of the invention, while allowing specific interaction with or specific detection of the immune response against FliD as defined herein.

In connection with the instant invention a primer targeting a nucleic acid coding for FliD as used in connection with the various aspects of the invention and/or in connection with the various embodiments of the present invention is one selected from the group comprising a primer comprising a nucleotide sequence according to SEQ ID NO: 21, a primer comprising a nucleotide sequence according to SEQ ID NO: 22, a primer comprising a nucleotide sequence according to SEQ ID NO: 23, a primer comprising a nucleotide sequence according to SEQ ID NO: 24, a primer comprising a nucleotide sequence according to SEQ ID NO: 25, a primer comprising a nucleotide sequence according to SEQ ID NO: 26, a primer comprising a nucleotide sequence according to SEQ ID NO: 27 and a primer comprising a nucleotide sequence according to SEQ ID NO: 28. Preferably, the primer is a combination at least two primers, whereby

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 21 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 22;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 21 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 24;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 21 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 26;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 21 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 28;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 23 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 22;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 23 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 24;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 23 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 26;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 23 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 28;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 25 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 22;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 25 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 24;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 25 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 26;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 25 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 28;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 27 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 22;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 27 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 24;

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 27 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 26; or

a first primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 27 and a second primer of the at least two primers is a primer comprising a nucleotide sequence according to SEQ ID NO: 28.

The various SEQ ID NOs: to which it is referred herein, the compound represented by said SEQ ID NOs:, the organisms from which said sequences were taken and, in some cases, an indication of the corresponding entry of the sequence in publicly available databanks is summarized in the following Table 1 :

Table 1:

SEQ ID NO:1 is the amino acid sequence of FliD expressed by H. pylori which corresponds to GenBank entry ACI27464.1.
SEQ ID NO:2 is the nucleotide sequence (cDNA) of FliD expressed by H. pylori which corresponds to Genbank entry CP001173.1.

SEQ ID NO:3 is the amino acid sequence of FliD expressed by H. suis which corresponds to NCBI Reference Sequence WP_006563874.1.

SEQ ID NO:4 is the nucleotide sequence (cDNA) of FliD expressed by H. suis which corresponds to GenBank entry ADGY01000008.1.

SEQ ID NO:5 is the amino acid sequence of FliD expressed by H. felis which corresponds to NCBI Reference Sequence YP_004073770.1.

SEQ ID NO:6 is the nucleotide sequence (cDNA) of FliD expressed by H. felis which corresponds to GenBank entry FQ670179.2.

SEQ ID NO:7 is the amino acid sequence of CagA of H. pylori G27 which corresponds to NCBI reference sequence YP_002266135.1.

SEQ ID NO:8 is the nucleotide sequence (cDNA) of CagA of H. pylori G27 which corresponds to GenBank entry JQ318032.1.

SEQ ID NO:9 is the amino acid sequence of VacA of H. pylori G27 which corresponds to NCBI reference sequence YP_002266461.1.

SEQ ID NO:10 is the nucleic acid sequence (cDNA) of VacA of H. pylori G27 which corresponds to NCBI reference sequence NC_011333.1.

SEQ ID NO:11 is the amino acid sequence of GroEL of H. pylori G27 which corresponds to NCBI reference sequence YP_002265651.1.

SEQ ID NO:12 is the nucleotide sequence (cDNA) of GroEL of H. pylori G27 which corresponds to NCBI reference sequence NC_011333.1.

SEQ ID NO:13 is the amino acid sequence of Hp0231 of H. pylori 26695 which corresponds to NCBI reference sequence NP_207029.1.

SEQ ID NO:14 is the nucleotide sequence (cDNA) of Hp0231 of H. pylori 26695 which corresponds to NCBI reference sequence NC_000915.1.

SEQ ID NO:15 is the amino acid sequence of JHp0940 of H. pylori J99 which corresponds to NCBI reference sequence NP_223657.1.

SEQ ID NO:16 is the nucleotide sequence (cDNA) of JHp0940 of H. pylori J99 which corresponds to NCBI reference sequence NC_000921.1.

SEQ ID NO:17 is the amino acid sequence of HtrA of H. pylori G27 which corresponds to NCBI reference sequence YP_002266040.1.

SEQ ID NO:18 is the nucleotide sequence (cDNA) of HtrA of H. pylori G27 which corresponds to NCBI reference sequence NC_011333.1.

SEQ ID NO: 19 is a primer used in the cloning of the FliD gene from H. pylori.
SEQ ID NO: 20 is a primer used in the cloning of the FliD gene from H. pylori.
SEQ ID NO: 21 is a forward primer used in PCR1 of Example 9.
SEQ ID NO: 22 is a reverse primer used in PCR1 of Example 9.
SEQ ID NO: 23 is a forward primer used in PCR2 of Example 9.
SEQ ID NO: 24 is a reverse primer used in PCR2 of Example 9.
SEQ ID NO: 25 is a forward primer used in PCR3 of Example 9.
SEQ ID NO: 26 is a reverse primer used in PCR3 of Example 9.
SEQ ID NO: 27 is a forward primer used in PCR4 of Example 9.
SEQ ID NO: 28 is a reverse primer used in PCR4 of Example 9.

It will be understood by a person skilled in the art that in case the nucleotide sequence is a DNA sequence and a cDNA sequence in particular, also disclosed herein is a RNA sequence differing from such DNA sequence and cDNA sequence only insofar that the sugar moiety is a ribonucleotide rather than a deoxyribonucleotide.

The present invention is now further illustrated by the following figures and examples which are not intended to limit the scope of protection. From said figures and examples further features, embodiments and advantages may be taken, wherein

Fig. 1 shows an embodiment of a line assay used in the methods of the invention for detecting anti-FliD antibodies in serum sample from 20 human patients histologically diagnosed as H. pylori positive;

Fig. 2 shows an embodiment of a lateral flow assay which can be used in the methods of the present invention for detecting anti-FliD antibodies in a sample such as a whole blood sample from a human subject, whereby Fig. 2A illustrates the schematic design of the assay, and Fig. 2B depicts a result of the assay;

Fig. 3 is a diagram indicating prevalence of an anti-FliD response in samples from man as a function of years after H. pylori eradication;

Fig. 4 shows ROC curves for FliD compared to two well-known antigens;

Fig. 5 shows the result of a Western blot analysis detecting FliD at various concentrations using mouse anti-FliD serum, but not Tig or gGT;

Fig. 6 shows a series of Southern blots using polymerase chain reaction 1 (PCR1), polymerase chain reaction 2 (PCR2), polymerase chain reaction 3 (PCR3) or polymerase chain reaction 4 (PCR4) for the detection of genomic DNA present in representative samples from patients having been diagnosed as H. pylori-positive;

Fig. 7 shows the result of a representative Western blot analysis performed using whole protein lysates of the cultured H. pylori; and

Fig. 8 shows the result of two Western blot analyses for determining whether FliD was expressed by the microorganisms indicated underneath each of the Western blots.

Example 1: Cloning of the H. pylori FliD Gene

All DNA manipulations were performed under standard conditions as described by Sambrook et al. (Sambrook, et al., 1989). Briefly, the FliD gene was amplified by PCR using genomic DNA from H. pylori strain J99 as the template. Following oligonucleotides were used as primers: 5′- CAT ATG GCA ATA GGT TCA TTA A-3′ (SEQ ID NO: 19) and 5′- CTC GAG ATT CTT TTT AGC CGC TGC-3′ (SEQ ID NO: 20). Using this approach a NdeI site was introduced at the 5′-end of forward primers and a XhoI site at 5′-end of the reverse primers. After PCR amplification, the product (2058 bp) was ligated into the pTZ57R / T cloning vector (InsTAclone™ PCR Cloning Kit, MBI Fermentas, Lithuania). Subsequently, the insert was confirmed via PCR and sequencing, and was cloned into a PET-28a(+) expression vector (Qiagen, USA) using NdeI and XhoI restriction enzymes.

Example 2: Expression, purification and recognition of recombinant FliD

E. coli BL21 (Qiagen, USA) competent cells were transformed with pET-28a(+)-fliD and inoculated in LB broth with antibiotic (kanamycin, 50μg /ml). Expression was induced by addition of 1 mmol/L Isopropyl β-D-1-thiogalactopyranoside (IPTG) at an optical density (OD600) of 0.6. After 4 hours cells were harvested and protein analysis of whole lysate was carried out by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The soluble histidine-tagged proteins were purified using affinity chromatography (HisTrap™ crude, GE Healthcare). As a second polishing step and for buffer exchange, size exclusion chromatography (Superdex™ 75, GE Helthcare) was performed. The relevant fractions were collected and concentrated with a centrifugal filter device (Millipore) with a cut off of 10 kDa and stored at - 80°C. Purified recombinant protein was evaluated by Western blot using an anti-His Tag-HRP antibody and also a mouse anti-H. pylori-HRP antibody (Pierce, Rockford, USA) and detected by ECL system (GE Healthcare, Uppsala, Sweden).

Amplification of the FliD gene from H. pylori strain J99 DNA revealed a single PCR product of 2.05 kb (data not shown) which was confirmed by sequencing and ligated into the expression vector pET-28a(+). After transformation into E. coli expression strain BL21 DE3 and induction with IPTG, a clear single band could be observed on Western blot using a commercial polyclonal anti-H. pylori antiserum. The protein was purified to >90% purity (data not shown) and again confirmed by Western blot (data not shown).

Example 3: Production and purification of rFliD specific antibody

A mature white New Zealand rabbit was immunized with purified protein according to the protocol of Hay et al. with light modifications (Hay, et al., 2002). Briefly, immunization was carried out by i.m. injection of 250 μg purified recombinant protein (0.5 ml) with the same volume (0.5 ml) of Freund's complete adjuvant. For the recall immunizations, the rabbit was boosted with 125 μg purified protein prepared in the same volume (0.5 ml) of Freund's

incomplete adjuvant 4, 6, 8 and 10 weeks later. As a negative control a serum sample was taken prior to immunization. Finally, two weeks after the last immunization, blood was collected and sera separated. Polyclonal IgG antibody was purified by sepharose™-4B affinity chromatography using rFliD conjugated columns prepared according to the manufacturer's protocol (Pharmacia, 1988). FliD expression of H. pylori (J99) was detected by Western blot using ultrasonic supernatant at the protein concentration of 50 µg/ml. The rabbit polyclonal IgG antibody raised against rFliD protein was used as the first antibody (1:5000 dilution), HRP-labeling sheep antibody against rabbit IgG (Avicenna Research Institute, Tehran, Iran) as the second antibody (1:3000 dilution) and ECL system were used for the detection (Chen, et al., 2001).

Furthermore, to test the antigenicity of the recombinant FliD and to compare it to the native protein, rabbit polyclonal antiserum was produced. Antibody titers were already determined after the third immunization and reached high levels after the fourth boost, confirming the good immunogenicity of FliD. The rabbit antiserum was able to recognize the purified rFliD and FliD in H. pylori lysate (data not shown).

Example 4: Development of an ELISA

ELISA plates were coated with 100 µl rFliD protein at a concentration of 1 µg/ml in PBS and incubated overnight at 4°C. The coated wells were blocked with phosphate buffered saline (PBS) containing 2.5% bovine serum albumin (BSA, Sigma) for two hours at 37°C. All H. pylori positive and negative serologic samples used in this study were screened for antibodies against FliD by using optimal dilution of patients' sera (1:100 dilution) as the first antibody, HRP-conjugated anti-human IgG (Promega, Mannheim, Germany) (1:100 dilution) as the secondary antibody and TMB (3,3',5,5'- tetra methyl benzidine) as a substrate. Moreover, wells were left uncoated as a control for each serum. The result of ELISA for a patient's serum sample was considered to be positive if its OD450 value was over the mean plus 3 SD of negative serum samples (Chen, et al., 2001).

Date Reçue/Date Received 2020-11-11

Example 5: Development of an FliD line assay

A line immunoassay based on recombinant H. pylori proteins immobilized on nitrocellulose was prepared. In contrast to ELISA, the test principle allows the identification of specific antibodies against various antigens of H. pylori through separate application of different single antigens.

rFliD was immobilized on nitrocellulose membrane strips together with other highly purified recombinant H. pylori antigens (CagA, VacA, GroEL, UreA (urease A), HcpC (Cysteine rich protein C) (Mittel et al., 2003) and gGT (gamma glutamyl transferase). The appropriate line conditions for rFliD were determined empirically with a selection of standard serum samples from a previously described study population comprising 20 defined H. pylori histologically positive samples and 20 defined H. pylori histologically negative samples. The optimal antigen concentration and ideal choice of additives like detergent, dithiothreitol, and NaCl was adjusted for each antigen by repeated cycles of lining and screening. The conditions with best presentation of antigen epitopes and optimal binding to the membrane, observable by perfect band appearance and best discrimination of negative and positive samples, were selected for ideal product specifications of first lots. Control bands were added on the upper end of the strip comprising rabbit anti-human IgG/IgM/IgA antibodies as incubation controls and human IgG, IgM or IgA antibodies as conjugate control as well as a cut off control that allows the assessment of the reactivity of the individual antigen bands.

After scanning and densitometric analysis of the band intensities, the control was used as internal reference to calculate ratios for each band. Usually, cut off control bands are scored between 20 and 30, while strong positive bands can score up to 600 points. Every band scoring above the individual control of the each stripe is considered positive (ratio >1).

The respective line assay is depicted in Fig. 1

Example 6: Prototype of a lateral flow assay for the diagnosis of H. pylori

Using the materials defined above a lateral flow assay was developed based on the principles disclosed herein related to design of a lateral flow assay.

The prototype of such lateral flow assay is depicted in Fig. 2, whereby Fig. 2A illustrates the schematic design of the assay, and Fig. 2B depicts the result of an analysis of a sample obtained from a human being using the assay, wherein anti-FliD antibodies were detected.

As may be taken from Fig. 2A, the assay used anti-hIgG coated gold nanoparticles. rFliD as well as recombinant CagA were present as antigens. hIgG was also immobilized serving as a control. The porous structure was formed by nitrocellulose. Control band indicated that the system work properly. FliD band indicated that the patient had an active or newly treated infection. CagA band, in case of active infection (+FliD band), indicates that this infection must be treated.

Example 7: Analysis of samples from man

A total of six hundred and eighteen (618) human patients (308 men, 310 women) were enrolled in the study. After receiving an explanation of the purpose of the study, informed consent was obtained from each patient and a blood sample was taken at the time of endoscopy, before any therapy was initiated. Sera were separated and stored at -20°C. Diagnosis of infection was based on the histopathology as gold standard. Patients were considered H. pylori positive when the results of histopathology were positive. All patients were screened by FliD Line assay, and a subset of 246 sera was tested by FliD ELISA as described above and by line assay as described above.

Table 2 shows the results of using said FliD ELISA. More specifically, Table 2 shows FliD serologic response in ELISA comparing *H. pylori* negative and positive human patients.

		Histology		Total
		Negative	Positive	
ELISA	Negative	73	8	81
	Positive	3	162	165
Total		76	170	246

Table 3 shows the results of using said line assay for a subgroup of the group of patients. More specifically, Table 3 shows FliD serologic response in the line assay comparing *H. pylori* negative and positive patients.

		Histology		Total
		Negative	Positive	
Line Assay	Negative	76	14	90
	Positive	0	156	156
Total		76	170	246

Using the FliD ELISA, among 170 positive reported samples, 165 positive samples were detected, whereas among 76 samples reported negative 73 were reconfirmed as negative by ELISA (Table 2). Taken together, application of FliD in ELISA based diagnosis of H. pylori infection has a specificity of 96% and a sensitivity of 97%. Interestingly, the five cases which were ELISA negative had also low but barely positive scores in the line blot which were just above the cut off (ratios ranging from 1.2 to 2.2). One of these was also regarded H. pylori negative by line blot, while the other four were line blot positive, reacting with several other antigens (data not shown). It is important to note that only one sample was negative by both tests.

The entire group of 618 human patients (part of which had been screened by ELISA) was analyzed using the line assay as to antibody response against FliD. a high sensitivity of 97,4% with 310 out of 318 patients evaluated positive in histopathology being positive by line assay,

whereas the line assay reaches a specificity of 99% (Table 2). The results from the patients in which discrepant results were obtained, was carefully examined. 8 sera were negative for FliD in the line assay but showed reactivity with other antigens, indicating that here, indeed, FliD was not recognized as antigen. Within these 8 samples, one had no reactivity against the FliD band at all. Seven had a weak reactivity which was barely below the cut off (ratios between 0,6 and 0,95), and four of these had weak reactivities against all other recognized antigens in general (not shown). All three samples in which FliD gave a "false positive" result showed reactivities with other bands as well. All these bands including FliD were relatively weak, but clearly above cut off.

From said samples the prevalence of an anti-FliD antibody response was determined as a function of years after eradication. The result is shown in Fig. 3. As may be taken from Fig. 3 there is still a prevalence of an anti-FliD antibody response of about 25 % after 16 to 20 years after eradication of H. pylori.

From said samples receiver operating characteristics (ROC) curves have been prepared for FliD, CagA and UreA. The result is shown in Fig. 4. From said Fig. 4 it is evident that FliD is advantageous over the two antigens of the prior art used in the detection of H. pylori infection.

Example 8: Bioinformatic analysis of FliD sequences

Using bioinformatics tools, FliD protein of H. pylori G27 strain was widely compared to other organisms, mainly prokaryotes. This analysis shows more than 97% homology between over 200 H. pylori strains.

The results are shown in Table 4.

Entry	Entry name	Protein names	Organism	Length	Identity	Score
B5Z7B5	B5Z7B5_HELPG	Putative flagellar hook-associated protein 2	Helicobacter pylori (strain G27)	685	100.0%	3412
J0KLR1	J0KLR1_HELPX	Putative flagellar hook-associated protein 2	Helicobacter pylori Hp H-27	685	99.0%	3383
I9RP80	I9RP80_HELPX	Flagellar capping protein	Helicobacter pylori Hp A-20	685	99.0%	3381
J0MV71	J0MV71_HELPX	Putative flagellar hook-associated protein 2	Helicobacter pylori Hp A-27	685	99.0%	3379
J0DL62	J0DL62_HELPX	Flagellar capping protein	Helicobacter pylori Hp H-11	685	99.0%	3375
J0A5P7	J0A5P7_HELPX	Flagellar capping protein	Helicobacter pylori Hp A-9	685	98.0%	3372
J0IU02	J0IU02_HELPX	Flagellar capping protein	Helicobacter pylori NQ4228	685	99.0%	3371
K2L7H4	K2L7H4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R036d	685	98.0%	3370
J0TQK4	J0TQK4_HELPX	Putative flagellar hook-associated protein 2	Helicobacter pylori Hp P-30	685	99.0%	3369
M7RTJ4	M7RTJ4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori UMB_G1	685	98.0%	3367
K2L537	K2L537_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R055a	685	99.0%	3367
J0SAM4	J0SAM4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-15b	685	99.0%	3367
J0M138	J0M138_HELPX	Putative flagellar hook-associated protein 2	Helicobacter pylori Hp H-45	685	99.0%	3367
I9WVW7	I9WVW7_HELPX	Flagellar capping protein	Helicobacter pylori Hp P-15	685	99.0%	3367
K2KUE2	K2KUE2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R030b	685	99.0%	3365
I0EHS2	I0EHS2_HELPX	Flagellar capping protein	Helicobacter pylori PeCan18	685	98.0%	3365
H8H4E1	H8H4E1_HELPX	Flagellar capping protein	Helicobacter pylori ELS37	685	98.0%	3362
K2LNG6	K2LNG6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R038b	685	98.0%	3361
D0IS88	D0IS88_HELP1	Flagellar hook-associated protein 2	Helicobacter pylori (strain 51)	685	98.0%	3360
N4T9B5	N4T9B5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-11	685	98.0%	3359
E1PZL2	E1PZL2_HELPM	Flagellar capping protein	Helicobacter pylori (strain SJM180)	685	98.0%	3358
J0IVU8	J0IVU8_HELPX	Flagellar capping protein	Helicobacter pylori NQ4099	685	98.0%	3358
J0FGE4	J0FGE4_HELPX	Putative flagellar hook-associated protein 2	Helicobacter pylori Hp P-16	685	98.0%	3358
Q1CTB8	Q1CTB8_HELPH	Putative flagellar hook-associated protein 2	Helicobacter pylori (strain HPAG1)	685	98.0%	3357
E8QPN8	E8QPN8_HELPR	Flagellar capping protein	Helicobacter pylori (strain Lithuania75)	685	98.0%	3355
K2KUX6	K2KUX6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R32b	685	98.0%	3355

K2KMU9	K2KMU9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R037c	685	98.0%	3355
J0HQJ3	J0HQJ3_HELPX	Flagellar capping protein	Helicobacter pylori CPY1124	685	98.0%	3355
I9XF52	I9XF52_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-74	685	98.0%	3355
I9U4H5	I9U4H5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-26	685	98.0%	3355
D7FEA9	D7FEA9_HELP3	Flagellar hook-associated protein 2	Helicobacter pylori (strain B8)	685	98.0%	3354
I9US75	I9US75_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-9	685	98.0%	3354
B9XZK1	B9XZK1_HELPX	Putative uncharacterized protein	Helicobacter pylori B128	685	98.0%	3354
J0J9Q0	J0J9Q0_HELPX	Flagellar capping protein	Helicobacter pylori NQ4076	685	98.0%	3353
I9QZB4	I9QZB4_HELPX	Flagellar capping protein	Helicobacter pylori NQ4110	685	98.0%	3353
G2M3P3	G2M3P3_HELPX	Flagellar capping protein	Helicobacter pylori Puno120	685	98.0%	3352
E1QBB7	E1QBB7_HELPC	Flagellar capping protein	Helicobacter pylori (strain Cuz20)	685	98.0%	3351
J0LRM5	J0LRM5_HELPX	Flagellar capping protein	Helicobacter pylori Hp H-43	685	98.0%	3351
E6NRT1	E6NRT1_HELPQ	Flagellar capping protein	Helicobacter pylori (strain F57)	685	98.0%	3350
M3MVK5	M3MVK5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM114Ai	685	98.0%	3350
K2KRX5	K2KRX5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R018c	685	98.0%	3350
K2KFQ1	K2KFQ1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R056a	685	98.0%	3350
J0IGN4	J0IGN4_HELPX	Flagellar capping protein	Helicobacter pylori NQ4216	685	98.0%	3349
E6S1Q8	E6S1Q8_HELPF	Flagellar hook-associated protein 2	Helicobacter pylori (strain 35A)	685	98.0%	3348
I9YJR2	I9YJR2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-13b	685	98.0%	3348
I9WT57	I9WT57_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-13	685	98.0%	3348
I9U161	I9U161_HELPX	Flagellar capping protein	Helicobacter pylori Hp A-14	685	98.0%	3348
B6JLY6	B6JLY6_HELP2	Flagellar hook-associated protein 2	Helicobacter pylori (strain P12)	685	98.0%	3347
K2K5F9	K2K5F9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori R046Wa	685	98.0%	3347
I9XUJ1	I9XUJ1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori CPY1313	685	98.0%	3347
I9PV13	I9PV13_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori CPY6311	685	98.0%	3347
I9PLR1	I9PLR1_HELPX	Flagellar capping protein	Helicobacter pylori CPY6261	685	98.0%	3347

L8VWS3	L8VWS3_HELPX	Flagellar capping protein	Helicobacter pylori A45	685	98.0%	3346
K7Y5K8	K7Y5K8_HELPX	Flagellar capping protein	Helicobacter pylori Aklavik117	685	98.0%	3346
J0T145	J0T145_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M2	685	98.0%	3346
I9T4Z9	I9T4Z9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-44	685	98.0%	3346
E8QFQ7	E8QFQ7_HELP7	Flagellar capping protein	Helicobacter pylori (strain India7)	685	98.0%	3345
C7BX84	C7BX84_HELPB	Flagellar hook-associated protein 2 FliD	Helicobacter pylori (strain B38)	685	98.0%	3345
I9W7Z2	I9W7Z2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-2	685	98.0%	3345
I0ZBA9	I0ZBA9_HELPX	Flagellar capping protein	Helicobacter pylori P79	685	98.0%	3345
F4D5I7	F4D5I7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori 83	685	98.0%	3345
B9XUM1	B9XUM1_HELPX	Putative uncharacterized protein	Helicobacter pylori 98-10	685	98.0%	3345
P96786	FLID_HELPY	Flagellar hook-associated protein 2 (HAP2) (Filament cap protein) (Flagellar cap protein)	Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter pylori)	685	98.0%	3345
M3SDI9	M3SDI9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAMchJs106B	685	98.0%	3344
I9XAU3	I9XAU3_HELPX	Flagellar capping protein	Helicobacter pylori Hp P-23	685	98.0%	3344
I9PTN1	I9PTN1_HELPX	Flagellar capping protein	Helicobacter pylori CPY6271	685	98.0%	3344
G2M8C7	G2M8C7_HELPX	Flagellar capping protein	Helicobacter pylori Puno135	685	98.0%	3344
E1Q6P5	E1Q6P5_HELPP	Flagellar capping protein	Helicobacter pylori (strain PeCan4)	685	98.0%	3343
M3KWT6	M3KWT6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM119Bi	685	98.0%	3343
I0ZGY9	I0ZGY9_HELPX	Flagellar capping protein	Helicobacter pylori NCTC 11637 = CCUG 17874	685	98.0%	3343
I2DFT2	I2DFT2_HELPX	Flagellar capping protein	Helicobacter pylori XZ274	685	98.0%	3342
E6NKD5	E6NKD5_HELPL	Flagellar capping protein	Helicobacter pylori (strain F32)	685	98.0%	3341
E6NIS5	E6NIS5_HELPK	Flagellar capping protein	Helicobacter pylori (strain F30)	685	98.0%	3341
I9ZP80	I9ZP80_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori NQ4161	685	98.0%	3341
I9RRM1	I9RRM1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-17	685	98.0%	3341
J0A0N9	J0A0N9_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp P-26	685	97.0%	3340
I9QGH5	I9QGH5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori NQ4053	685	98.0%	3340

D6XPZ1	D6XPZ1_HELPV	Flagellar hook-associated protein 2	Helicobacter pylori (strain v225d)	685	98.0%	3339
M5YZL4	M5YZL4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAMchJs124i	685	97.0%	3339
M5YMA1	M5YMA1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAMchJs114i	685	97.0%	3339
M4ZNA5	M4ZNA5_HELPX	Flagellar capping protein	Helicobacter pylori OK310	685	97.0%	3339
M3NNS0	M3NNS0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM246Ai	685	97.0%	3339
M3MBN7	M3MBN7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM105Ai	685	97.0%	3339
I9S784	I9S784_HELPX	Flagellar capping protein	Helicobacter pylori Hp H-28	685	98.0%	3339
I0E4K1	I0E4K1_HELPX	Flagellar capping protein	Helicobacter pylori Shi417	685	98.0%	3339
J0P747	J0P747_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-23	685	97.0%	3338
J0N2H0	J0N2H0_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-4	685	97.0%	3338
I0ED42	I0ED42_HELPX	Flagellar capping protein	Helicobacter pylori Shi112	685	98.0%	3338
M7SSG1	M7SSG1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori CPY1662	685	97.0%	3337
M5Y955	M5Y955_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAMchJs117Ai	685	97.0%	3337
M4ZKA3	M4ZKA3_HELPX	Flagellar capping protein	Helicobacter pylori OK113	685	98.0%	3337
M3LA33	M3LA33_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM231Ai	685	97.0%	3337
I9Z0G2	I9Z0G2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-28b	685	97.0%	3337
I9S3M7	I9S3M7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-24	685	98.0%	3337
I0EWG9	I0EWG9_HELPX	Flagellar capping protein	Helicobacter pylori HUP-B14	685	97.0%	3337
M3PSG4	M3PSG4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM96Ai	685	97.0%	3336
J0U8I3	J0U8I3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-3b	685	97.0%	3336
J0RUS2	J0RUS2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-5b	685	97.0%	3336
J0Q0D5	J0Q0D5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-4	685	97.0%	3336
J0PSB5	J0PSB5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-3	685	97.0%	3336
I9Y932	I9Y932_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-4c	685	97.0%	3336
I9XWQ4	I9XWQ4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-4d	685	97.0%	3336
E6NDJ6	E6NDJ6_HELPI	Flagellar capping protein	Helicobacter pylori (strain F16)	685	97.0%	3334

M3QDF1	M3QDF1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM80Ai	685	97.0%	3334
M3Q5B9	M3Q5B9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM42Ai	685	97.0%	3334
M3P646	M3P646_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM245Ai	685	97.0%	3334
M3LV71	M3LV71_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM112Ai	685	97.0%	3334
M3L655	M3L655_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM101Biv	685	97.0%	3334
E1S8R1	E1S8R1_HELP9	Flagellar hook-associated protein	Helicobacter pylori (strain 908)	685	97.0%	3333
E1PVI4	E1PVI4_HELPT	Flagellar capping protein	Helicobacter pylori (strain Sat464)	685	98.0%	3333
D0JZC3	D0JZC3_HELP5	Flagellar capping protein	Helicobacter pylori (strain 52)	685	97.0%	3333
M3RS44	M3RS44_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP116Bi	685	97.0%	3333
M3R005	M3R005_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM264Ai	685	97.0%	3333
M3MJ19	M3MJ19_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM103Bi	685	97.0%	3333
J0I156	J0I156_HELPX	Flagellar capping protein	Helicobacter pylori CPY3281	685	98.0%	3333
J0AJS5	J0AJS5_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-16	685	97.0%	3333
I0E947	I0E947_HELPX	Flagellar capping protein	Helicobacter pylori Shi169	685	98.0%	3333
F2JET0	F2JET0_HELP9	Flagellar hook-associated protein	Helicobacter pylori 2018	685	97.0%	3333
F2JAT7	F2JAT7_HELP9	Flagellar hook-associated protein	Helicobacter pylori 2017	685	97.0%	3333
Q9ZL91	FLID_HELPJ	Flagellar hook-associated protein 2 (HAP2) (Filament cap protein) (Flagellar cap protein)	Helicobacter pylori (strain J99) (Campylobacter pylori J99)	685	97.0%	3333
M3NID0	M3NID0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM270ASi	685	97.0%	3332
J0DCU5	J0DCU5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-6	685	97.0%	3332
I9V408	I9V408_HELPX	Flagellar capping protein	Helicobacter pylori Hp H-10	685	97.0%	3332
J0U3G8	J0U3G8_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-62	685	97.0%	3331
I9SL37	I9SL37_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-29	685	97.0%	3331
E8QM56	E8QM56_HELP4	Flagellar capping protein	Helicobacter pylori (strain Gambia94/24)	685	97.0%	3330
M5YNV6	M5YNV6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAMchJs136i	685	97.0%	3330
M3TQ89	M3TQ89_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP260Bi	685	97.0%	3330

M3QIV4	M3QIV4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM260Bi	685	97.0%	3330
M3Q2L5	M3Q2L5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM263BFi	685	97.0%	3330
M3M583	M3M583_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM115Ai	685	97.0%	3330
J0SFX5	J0SFX5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-25c	685	97.0%	3330
J0HGQ0	J0HGQ0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-25d	685	97.0%	3330
I9X9I1	I9X9I1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-25	685	97.0%	3330
I9VCT9	I9VCT9_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-19	685	97.0%	3330
M3S7G6	M3S7G6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM83T	685	97.0%	3329
M3PEV1	M3PEV1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM244Ai	685	97.0%	3329
M3P9F3	M3P9F3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM83Bi	685	97.0%	3329
M3NFC4	M3NFC4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM118Bi	685	97.0%	3329
K8GY42	K8GY42_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM100Ai	685	97.0%	3329
J0UFU9	J0UFU9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M9	685	97.0%	3329
J0T5P3	J0T5P3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M4	685	97.0%	3329
J0REL3	J0REL3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-24c	685	97.0%	3329
J0I743	J0I743_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M5	685	97.0%	3329
J0I1J2	J0I1J2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M3	685	97.0%	3329
J0HJK5	J0HJK5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M1	685	97.0%	3329
I9ZYP3	I9ZYP3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp M6	685	97.0%	3329
I9XJ16	I9XJ16_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-24b	685	97.0%	3329
M3UI84	M3UI84_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP260BFii	685	97.0%	3328
M3U8F0	M3U8F0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250BSi	685	97.0%	3328
M3T9M6	M3T9M6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250ASi	685	97.0%	3328
M3T443	M3T443_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250ASii	685	97.0%	3328
M3T0U7	M3T0U7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250AFiV	685	97.0%	3328
M3SWF6	M3SWF6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250BFiV	685	97.0%	3328

M3SP57	M3SP57_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250AFiii	685	97.0%	3328
M3S6F4	M3S6F4_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250BFiii	685	97.0%	3328
M3R7T2	M3R7T2_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250AFii	685	97.0%	3328
M3QV83	M3QV83_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM260BSi	685	97.0%	3328
M3QS41	M3QS41_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250BFii	685	97.0%	3328
M3QQ64	M3QQ64_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP250BFi	685	97.0%	3328
M3Q6I7	M3Q6I7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM250T	685	97.0%	3328
M3NV58	M3NV58_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM252Bi	685	97.0%	3328
M3NKC5	M3NKC5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM252T	685	97.0%	3328
M3LZX8	M3LZX8_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM250AFi	685	97.0%	3328
J0CLQ3	J0CLQ3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-16	685	97.0%	3328
I9XTZ6	I9XTZ6_HELPX	Flagellar capping protein	Helicobacter pylori CPY1962	685	98.0%	3328
B2UT80	B2UT80_HELPS	Flagellar capping protein	Helicobacter pylori (strain Shi470)	685	97.0%	3327
M7SW73	M7SW73_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-1	685	97.0%	3327
M3P129	M3P129_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM254Ai	685	97.0%	3327
I9P985	I9P985_HELPX	Flagellar capping protein	Helicobacter pylori CPY6081	685	97.0%	3326
K7YA88	K7YA88_HELPX	Flagellar capping protein	Helicobacter pylori Aklavik86	685	97.0%	3325
M3RIK8	M3RIK8_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM93Bi	685	97.0%	3324
J0M8U8	J0M8U8_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-6	685	97.0%	3324
M3NGP1	M3NGP1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM265BSii	685	97.0%	3323
M3KZM7	M3KZM7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM120Ai	685	97.0%	3323
M3PUQ6	M3PUQ6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM249T	685	97.0%	3322
M3PCL7	M3PCL7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM239Bi	685	97.0%	3322
M3NM23	M3NM23_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM121Aii	685	97.0%	3322
J0IWR3	J0IWR3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori NQ4200	685	97.0%	3322
J0PFP0	J0PFP0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-1	685	97.0%	3321

I9XPS7	I9XPS7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-1b	685	97.0%	3321
J0N254	J0N254_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-3	685	97.0%	3320
M3U8B7	M3U8B7_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP260AFii	685	97.0%	3319
M3U287	M3U287_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP260AFi	685	97.0%	3319
M3RLI9	M3RLI9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori HP260ASii	685	97.0%	3319
M3Q751	M3Q751_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM268Bii	685	97.0%	3319
M3P4U3	M3P4U3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM260ASi	685	97.0%	3319
M3LLE6	M3LLE6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM201Ai	685	97.0%	3318
J0JT98	J0JT98_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-5	680	98.0%	3318
I9SDQ6	I9SDQ6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-30	677	97.0%	3314
J0BNB3	J0BNB3_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-42	680	97.0%	3311
G2MEG6	G2MEG6_HELPX	Flagellar capping protein	Helicobacter pylori SNT49	685	97.0%	3311
J0UBP3	J0UBP3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-2b	677	97.0%	3308
I9QNB9	I9QNB9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori NQ4044	685	96.0%	3302
I9ZZM5	I9ZZM5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-4	677	97.0%	3300
M7SHH3	M7SHH3_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori CCHI33	677	97.0%	3297
I9TJA1	I9TJA1_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp A-8	677	97.0%	3297
M3NMW6	M3NMW6_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori GAM210Bi	685	96.0%	3296
I9YL06	I9YL06_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-11b	677	97.0%	3293
I9WS67	I9WS67_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-11	677	97.0%	3293
J0PC82	J0PC82_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-34	677	97.0%	3292
I9VJH4	I9VJH4_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-21	677	97.0%	3292
J0PV65	J0PV65_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-8	677	97.0%	3291
I9YHM9	I9YHM9_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-8b	677	97.0%	3291
J0TUW0	J0TUW0_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp P-41	677	97.0%	3289

J0NSR5	J0NSR5_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-18	677	97.0%	3289	
J0BAE3	J0BAE3_HELPX	Flagellar hook-associated protein	Helicobacter pylori Hp H-36	677	97.0%	3289	
J0LI78	J0LI78_HELPX	Flagellar hook-associated protein 2	Helicobacter pylori Hp H-41	677	96.0%	3278	
E8QRV3	E8QRV3_HELPW	Flagellar capping protein	Helicobacter pylori (strain SouthAfrica7)	685	95.0%	3264	
Q17Y06	Q17Y06_HELAH	Flagellar hook-associated protein	Helicobacter acinonychis (strain Sheeba)	685	94.0%	3249	
K4NRS2	K4NRS2_HELPY	Flagellar capping protein	Helicobacter pylori (strain ATCC 700392 / 26695) (Campylobacter pylori)	674	97.0%	3190	
K4NL36	K4NL36_HELPX	Flagellar capping protein	Helicobacter pylori Rif2	674	97.0%	3190	
K4NJJ9	K4NJJ9_HELPX	Flagellar capping protein	Helicobacter pylori Rif1	674	97.0%	3190	
M3QVV4	M3QVV4_HELPX	Flagellar hook-associated protein 2 (Fragment)	Helicobacter pylori GAM71Ai	647	97.0%	3146	
I0ETW0	I0ETW0_HELCM	Flagellar capping protein	Helicobacter cetorum (strain ATCC BAA-540 / MIT 99-5656)	685	88.0%	3065	
I0EMR1	I0EMR1_HELC0	Flagellar capping protein	Helicobacter cetorum (strain ATCC BAA-429 / MIT 00-7128)	685	81.0%	2861	
E7ADC3	E7ADC3_HELFC	Flagellar hook-associated protein	Helicobacter felis (strain ATCC 49179 / NCTC 12436 / CS1)	684	63.0%	2190	
E7FYJ6	E7FYJ6_9HELI	Flagellar capping protein	Helicobacter suis HS1	689	61.0%	2158	
F8KTH3	F8KTH3_HELBC	Flagellar hook-associated protein FliD	Helicobacter bizzozeronii (strain CIII-1)	694	59.0%	2091	
K4RHP3	K4RHP3_HELHE	Flagellar hook-associated protein FliD	Helicobacter heilmannii ASB1.4	691	58.0%	2073	
D3UGM5	D3UGM5_HELM1	Putative flagellar hook-associated protein	Helicobacter mustelae (strain ATCC 43772 / LMG 18044 / NCTC 12198 / 12198) (Campylobacter mustelae)	674	52.0%	1778	
Q7VI19	Q7VI19_HELHP	Flagellar filament capping protein FliD	Helicobacter hepaticus (strain ATCC 51449 / 3B1)	682	51.0%	1698	
I2FDC5	I2FDC5_HELCP	Flagellar capping protein	Helicobacter cinaedi (strain PAGU611)	682	51.0%	1690	
I7GZJ0	I7GZJ0_9HELI	Flagellar capping protein	Helicobacter cinaedi ATCC BAA-847	682	51.0%	1689	
E4VHL6	E4VHL6_9HELI	Flagellar hook-protein 2	Helicobacter cinaedi CCUG 18818	682	51.0%	1689	
N2BQN7	N2BQN7_9HELI	Uncharacterized protein	Helicobacter bilis WiWa	679	45.0%	1589	
C3XDT1	C3XDT1_9HELI	Flagellar capping protein	Helicobacter bilis ATCC 43879	679	45.0%	1583	

Q7MAM3	Q7MAM3_WOLSU	FLAGELLAR HOOK-ASSOCIATED PROTEIN 2	Wolinella succinogenes (strain ATCC 29543 / DSM 1740 / LMG 7466 / NCTC 11488 / FDC 602W) (Vibrio succinogenes)	682	45.0%		1479
C5EXF0	C5EXF0_9HELI	Flagellar hook-protein 2	Helicobacter pullorum MIT 98-5489	685	39.0%		1272
C5ZWT4	C5ZWT4_9HELI	Flagellar hook-associated protein (Flagellar hook-protein 2)	Helicobacter canadensis MIT 98-5491	689	39.0%		1262
H5VEC0	H5VEC0_HELBI	Flagellar hook-associated protein FliD	Helicobacter bizzozeronii CCUG 35545	458	53.0%		1185
C3XLS4	C3XLS4_9HELI	Flagellar hook-associated protein 2	Helicobacter winghamensis ATCC BAA-430	689	37.0%		1175
H5VEC1	H5VEC1_HELBI	Flagellar hook-associated protein FliD	Helicobacter bizzozeronii CCUG 35545	231	66.0%		807
H8CS11	H8CS11_CAMJU	Flagellar capping protein	Campylobacter jejuni subsp. jejuni LMG 9872	645	27.0%		474
B9KGA6	B9KGA6_CAMLR	Flagellar filament cap protein FliD	Campylobacter lari (strain RM2100 / D67 / ATCC BAA-1060)	766	26.0%		471
C6RGG2	C6RGG2_9PROT	SMR-type multidrug efflux transporter	Campylobacter showae RM3277	577	29.0%		465
D2MX77	D2MX77_CAMJU	Flagellar hook-associated protein	Campylobacter jejuni subsp. jejuni 414	642	28.0%		461
M3I083	M3I083_9PROT	Flagellar capping protein	Campylobacter showae CC57C	577	28.0%		457
H7SA14	H7SA14_CAMCO	Flagellar capping protein	Campylobacter coli 84-2	644	26.0%		452
D2MS44	D2MS44_CAMJU	Flagellar hook-associated protein FliD	Campylobacter jejuni subsp. jejuni 1336	647	26.0%		451
H7XBH0	H7XBH0_CAMJU	Flagellar capping protein	Campylobacter jejuni subsp. jejuni LMG 23216	648	26.0%		451
H7YRN9	H7YRN9_CAMJU	Flagellar capping protein	Campylobacter jejuni subsp. jejuni LMG 23357	648	27.0%		449
Q30U48	Q30U48_SULDN	Flagellar hook-associated protein 2-like protein	Sulfurimonas denitrificans (strain ATCC 33889 / DSM 1251) (Thiomicrospira denitrificans (strain ATCC 33889 / DSM 1251))	462	31.0%		441
H8BWB9	H8BWB9_CAMJU	Flagellar capping protein	Campylobacter jejuni subsp. jejuni 1213	642	27.0%		447
H8AWN7	H8AWN7_CAMJU	Flagellar capping protein	Campylobacter jejuni subsp. jejuni 1997-11	643	26.0%		447
A3ZDR2	A3ZDR2_CAMJU	Flagellar hook-associated protein FliD	Campylobacter jejuni subsp. jejuni HB93-13	643	26.0%		447
A7H4J4	A7H4J4_CAMJD	Flagellar hook-associated protein FliD	Campylobacter jejuni subsp. doylei (strain ATCC BAA-1458 / RM4099 /	646	26.0%		447

		269.97)					
A3YRI3	A3YRI3_CAMJU	Flagellar hook-associated protein FliD	Campylobacter jejuni subsp. jejuni 260.94	642	25.0%	442
H7WEH0	H7WEH0_CAMCO	Flagellar capping protein	Campylobacter coli H8	637	26.0%	441
E1PLQ8	E1PLQ8_CAMJM	Flagellar hook-associated protein 2	Campylobacter jejuni subsp. jejuni serotype HS21 (strain M1 / 99/308)	643	27.0%	441

Example 9: Presence and expression of the FliD in H. pylori

Samples

81 H. pylori isolates from human patients were enrolled in the study. The samples were diagnosed as positive by conventional bacterial culture on selective plates. In such testing, bacteria were grown on Wilkins–Chalgren blood agar plates under microaerobic conditions (10% CO2, 5% O2, 8.5% N2, and 37°C) for 36 hours, and positivity for oxidase, catalase and urease was confirmed by biochemical testing. A part of the cultured bacteria was used for DNA isolation and the remainder was applied for preparation of protein lysate for Western blot analysis.

Polyclonal mouse anti - FliD sera

Three C57BL6 mice were immunized 3 times (weekly) with 30µg of recombinant H. pylori FliD as antigen and 10µg CT (cholera toxin) as adjuvant re-suspended in PBS. One week after the last immunization boost, mice were bleed and sera were pooled. The antigenicity and specificity of the pooled sera was tested in a Western blot analysis.

Western blot analysis

To establish the optimal conditions of the assay, different concentration of the recombinant FliD protein as well as other recombinant control proteins (Tig (Trigger factor (Tomb et al., 1997)) and gGT) generated and purified under the same conditions, were applied on 8% SDS gels. After blotting of the proteins on nitrocellulose membrane (Whatman/GE Healthcare, Freiburg, Germany), membranes were blocked in 5% non-fat milk for 1 h at room temperature and incubated overnight with different dilutions of the anti-sera as primary antibodies. After incubation of the membranes with HRP-labeled anti-mouse IgG, bands were detectable by adding of ECL Western Blotting Detection reagents.

The results are shown in Fig. 5, whereby on the right side of the depicted SDS gel the antigen and its amount applied to the individual lanes is indicated. An optimal dilution (1:2000) of mouse anti serum was used.

PCR analysis of the presence of the FliD's ORF in H. pylori's genome

Four PCRs were designed based on the DNA sequence of the FliD as subject to SEQ ID NO: 2. Specificity of each primer pair as indicated in Table 5 was confirmed by blast analysis against all bacterial nucleotide sequences of the gene bank. PCRs were established using H. pylori DNA as positive control and genomic DNA of 10 other microorganisms as negative controls. PCRs were performed using GoTaq polymerase master mix (Promega), annealing temperature of 56°C and 30 seconds extension time.

Table 5: Primers used for PCR analysis.

	Forward primer	**Reverse primer**	**Length of the amplicon (bp)**
PCR1	AGC TCA TTA GGG CTT GGC AG (SEQ ID NO: 21)	GCT CGC GCT CAA CGC ATC (SEQ ID NO: 22)	246
PCR2	ATC ACG GAC GCT ACC AAT GG (SEQ ID NO: 23)	AGG GAC TTC ATG CAT GCT CC (SEQ ID NO: 24)	288
PCR3	CAC AGA CGC TAT CAT TCA AGC (SEQ ID NO: 25)	CCC GCT GAT CAC ATC ATT GAC (SEQ ID NO: 26)	300
PCR4	CGC TAA CCT CAT AGA TGG AGG (SEQ ID NO: 27)	TAA GCG GCA AAG CGC TCC G (SEQ ID NO: 28)	150

Results

ORF of the FliD is presented in all H. pylori patient isolates (cultured bacteria isolated from patient biopsies). Presence of the ORF of the FliD could be confirmed by all four PCRs used for this assay. PCR1, PCR2 and PCR3 performed by isolated DNA from 81 H. pylori samples

were overall positive. Whereas the PCR4 was positive for 79 samples (Fig. 6). Specificity of the assay was confirmed by applying DNA isolated from P. aeruginosa (ATCC 27813), Klebsiella oxytoca (ATCC 700324), Candida albicans (ATCC 90028), Entrococcus faecalis (ATCC 29292), Strep. Group A (ATCC 19615), S.thyphimurium (ATCC 13311), S.aureus (ATCC 25923), S.epidermidis (ATCC 18228), H.influensae (ATCC 49247) and E.coli (ATCC 25922).

As may be taken from Fig. 6 depicting the results of a representative PCR analysis performed using genomic DNA isolated from cultured H. pylori isolated from patient biopsies, FliD's ORF (open reading frame) is presented in almost all H. pylori isolates. Thus, PCR results confirm the presence of the FliD in genomic DNA. In Fig. 6, numbers above lanes indicate internal sample number.

As to the detection of FliD protein in samples from patients having been diagnosed as H. pylori-positive, FliD protein is detectable in 97.5% of the samples. Using Western blot analysis it could be demonstrated that the expression of the FliD protein is detectable in 79 out of 81 H.pylori protein lysates. The results are shown in Fig. 7. In Fig. 7 numbers above lanes indicate internal sample number.

The specificity of the assay was confirmed through negative results when protein lysates from other microorganisms were analyzed by Western blot analysis. The results thereof are indicated in Fig. 8. As may be taken from Fig. 8 apart from recombinant FliD with streptavidin tag (lanes 2 of both Western Blots) and without streptavidin tag (lanes 3 of both Western blots) protein lysates from P. aeruginosa (ATCC 27813) (left Western blot, lane 4), Klebsiella oxytoca (ATCC 700324) (left Western blot, lane 5), Candida albicans (ATCC 90024) (left Western blot, lane 6), Enterococcus faecalis (ATCC 29292) (left Western blot, lane 7), Streptococcus Group A (ATCC 19615) (left Western blot, lane 8), S. thyphimurium (ATCC 13311) (right Western blot, lane 4), S. aureus (ATCC 25923) (right Western blot, lane 5), S. epidermidis (ATCC 18228) (right Western blot, lane 6), H. influensae (ATCC 49247) (right Western blot, lane 7), and E. coli (ATCC 25922) (right Western blot, lane 8).

In the instant specification it is referred to various documents of the prior art the complete reference of which reads as follows.

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The features of the present invention disclosed in the specification, the sequence listing, the claims and/or the drawings may both separately and in any combination thereof be material for realizing the invention in various forms thereof.

Claims

1. A method for detecting H. pylori infection in a subject, wherein the method comprises detecting in a sample from the subject an immune response against FliD, wherein the immune response comprises an anti-FliD antibody, wherein the method comprises reacting the sample with a full-length FliD.

2. The method of claim 1, wherein the anti-FliD antibody is an IgG antibody or an IgA antibody.

3. The method of claim 1 or 2, wherein the detection occurs by means of an ELISA, a lateral flow assay or a line assay.

4. The method of any one of claims 1 to 3, wherein the method further comprises detecting one or more antigens of *Helicobacter*, wherein the one or more antigens are selected from the group consisting of CagA, VacA, GroEL, Hp 0231, JHp 0940 and HtrA.

5. The method of claim 4, wherein the *Helicobacter* is *H. pylori.*

6. The method of any one of claims 1 to 5, wherein the sample is blood serum, blood plasma, whole blood or stool.

7. Use of a kit comprising a full-length FliD and at least one further constituent, in a method of any one of claims 1 to 6, wherein the at least one further constituent is a buffer, a solid phase or an instruction leaflet.

8. A kit for use in a method of any one of claims 1 to 6, said kit comprising full-length FliD and at least one further constituent,
wherein the at least one further consistuent is a buffer, a solid phase or an instruction leaflet.

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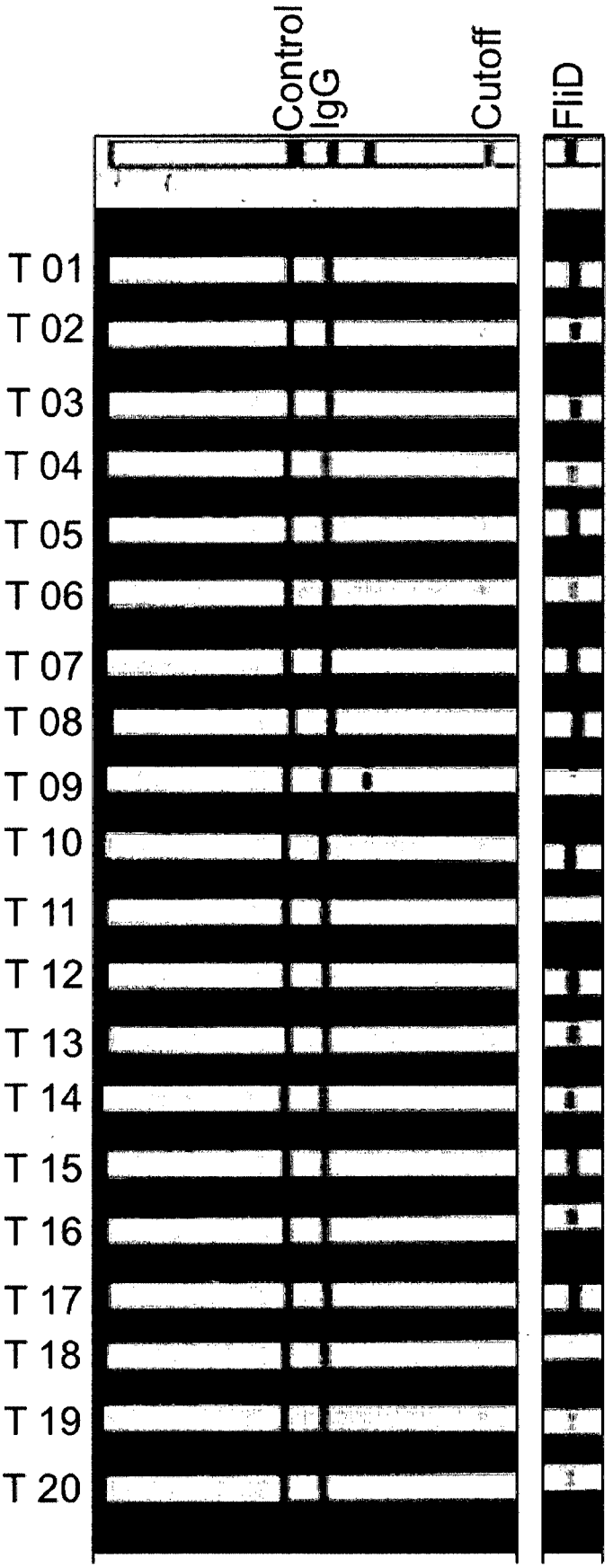
Control
IgG
Cutoff
FliD
T 01
T 02
T 03
T 04
T 05
T 06
T 07
T 08
T 09
T 10
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T 15
T 16
T 17
T 18
T 19
T 20

Fig. 1

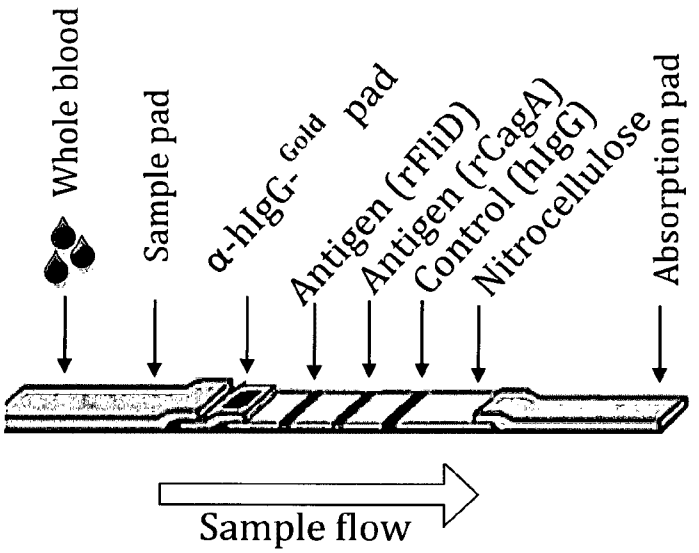

Whole blood
Sample pad
α-hIgG-Gold pad
Antigen (rFliD)
Antigen (rCagA)
Control (hIgG)
Nitrocellulose
Absorption pad
Sample flow

Fig. 2A

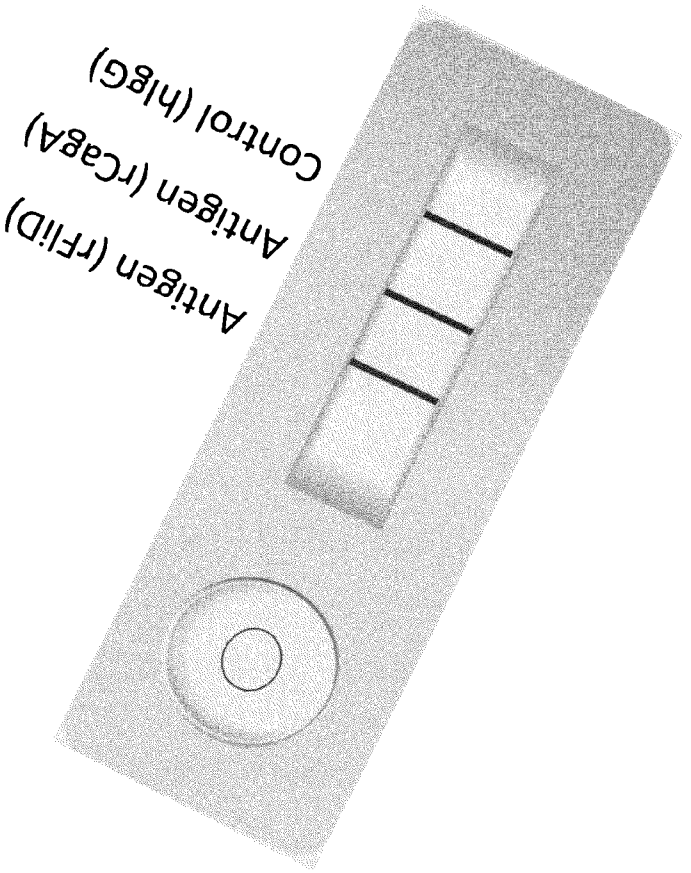


Fig. 2B

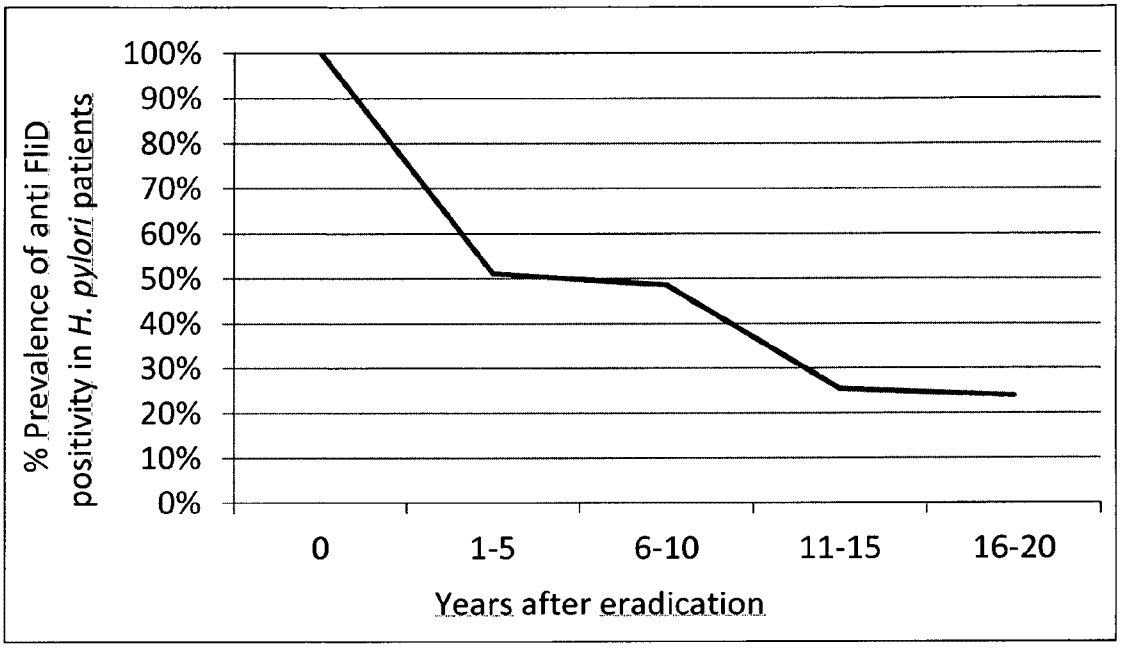

% Prevalence of anti FliD positivity in H. pylori patients
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1-5
6-10
11-15
16-20
Years after eradication

Fig. 3

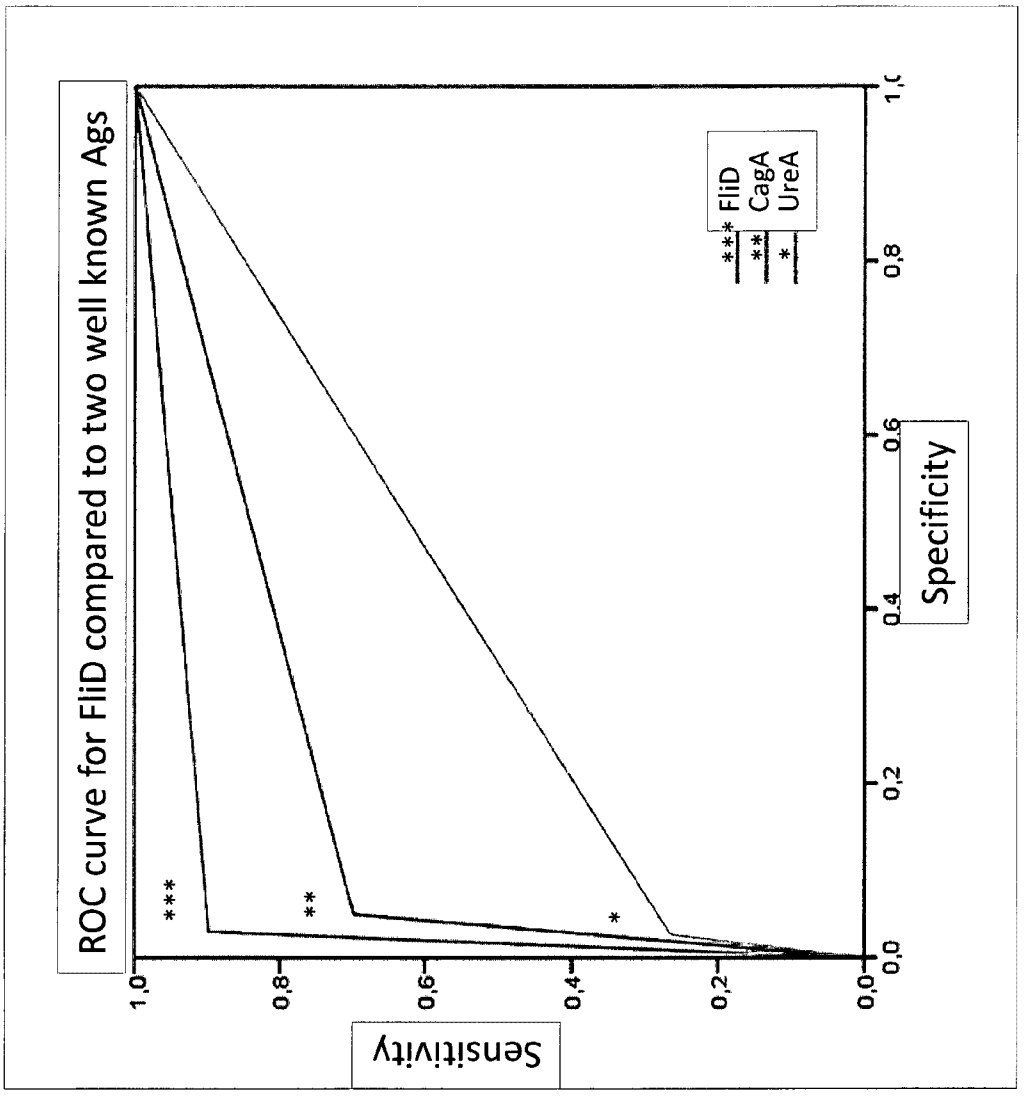
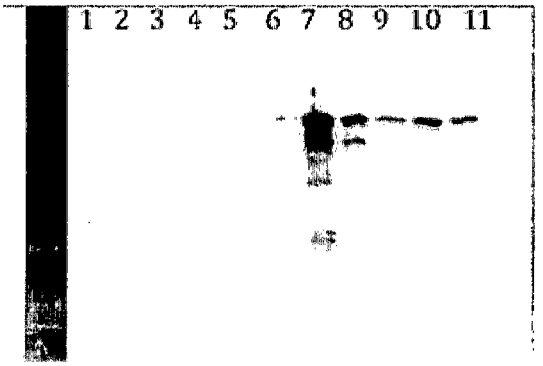

ROC curve for FliD compared to two well known Ags
Sensitivity
Specificity
1,0
0,8
0,6
0,4
0,2
0,0
0,0
0,2
0,4
0,6
0,8
1,0
***
**
*
*** FliD
** CagA
* UreA

Fig. 4



1. Tig 100 ng
2. Tig 10 ng
3. Tig 1ng
4. gGT 100 ng
5. gGT 10 ng
6. gGT 1ng
7. FliD 100 ng
8. FliD 10 ng
9. FliD 1ng
10. FliDstrep 100 ng
11. FliDstrep 10 ng

Fig. 5

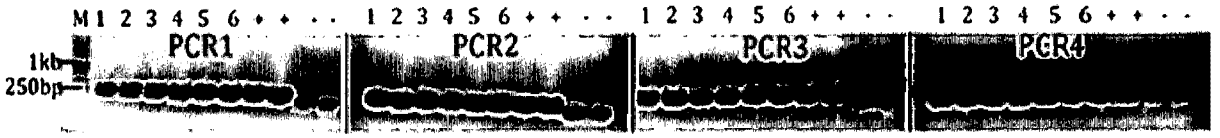


Fig. 6

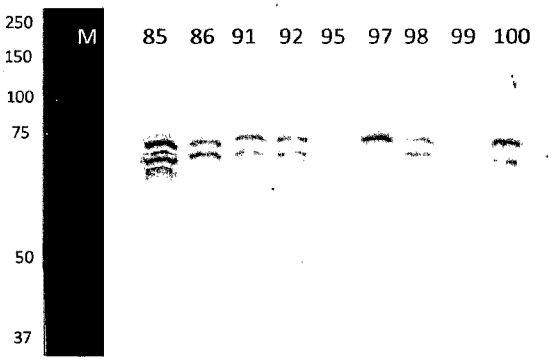
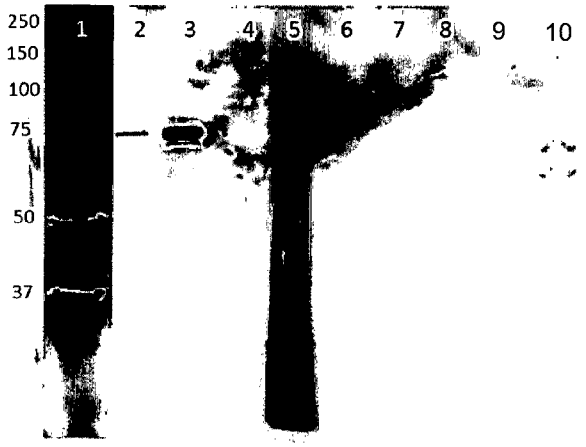
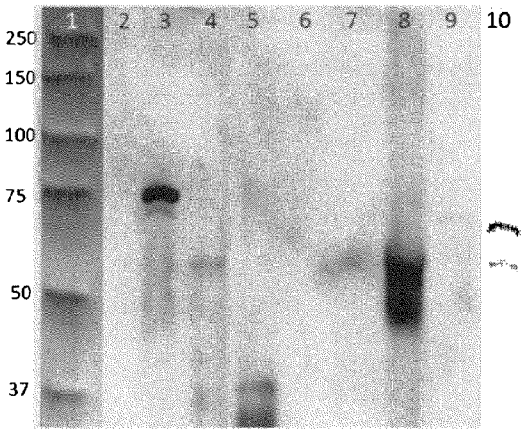


Fig. 7



1. *Protein marker (kD)*
2. *Rec. FliD (w. strep.tag)*
3. *Rec. FliD*
4. *P.aeruginosa(ATCC 27813)*
5. *Klebsiella oxytoca (ATCC 700324)*
6. *Candida albicans (ATCC 90028)*
7. *Entrococcus faecalis (ATCC 29292)*
8. *Strep. Group A (ATCC 19615)*
9. *H. Pylori pos. Ctrl.*
10. *H. Pylori pos. Ctrl.*



1. *Protein marker (kD)*
2. *Rec. FliD (w. strep.tag)*
3. *Rec. FliD*
4. *S.thyphimurium (ATCC 13311)*
5. *S.aureus (ATCC 25923)*
6. *S.epidermidis (ATCC 18228)*
7. *H.influensae (ATCC 49247)*
8. *E.coli (ATCC 25922)*
9. *H. Pylori pos. Ctrl.*
10. *H. Pylori pos. Ctrl.*

Fig. 8

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