Pylori DuoTect® *H. pylori* Rapid Test
for Whole Blood / Serum / Plasma

CE IVD

Intended Use

Pylori DuoTect® *H. pylori* Rapid Test is an in vitro immunochromatographic assay for the qualitative detection of antibodies specific to two *Helicobacter Pylori* (*H. pylori)* antigens in human whole blood, serum or plasma: FliD and CagA. The assay is intended to be used in first-line diagnostics to detect a *H. pylori* infection in adult subjects. Pylori DuoTect® is intended for near patient testing at Point-of-Care (POC) as well as for laboratory use. In the former case, personnel shall be well trained prior to performing the test. Pylori DuoTect® is not intended to be a tumor marker test.

Summary and Explanation

Helicobacter pylori, a gram-negative bacterium, colonizes the gastric mucosa in approximately 50% of the human population[1]. It is one of the most common infectious diseases worldwide and has been classified as a group-1 carcinogen by the IRA (*International Agency for Research on Cancer*) and the WHO (*World Health Organization)*[2]. Diagnosing and treating the infection early on lowers the risk of developing serious stomach diseases such as peptic ulcers, gastric MALT lymphoma and gastric cancer[3]. Around 20% to 30% of infected individuals may develop peptic ulcer disease.[4] *H. pylori* infection is the major risk factor associated with the development of gastric cancer.[5]

Pylori DuoTect® is an easy-to-perform, highly sensitive and specific immunochromatographic assay that uses two powerful markers to detect an infection with *H. pylori*.

Marker 1 – Indicator for a *H. pylori* infection:

FliD *(flagellar filament capping protein or flagellar hook-associated protein)* is an essential element in the assembly of *H. pylori´s* functional flagella. It is an efficient, highly sensitive and specific marker present in all *H. pylori* strains worldwide[6]. ImevaX holds the patent on the detection of FliD-specifc antibodies.

Marker 2 – Indicator for a *H. pylori* infection and additional risk factor for the development of peptic ulcer and gastric cancer:

CagA *(cytotoxin-associated antigen A)* is one of the best studied and most important virulence factors. Subjects infected with *H. pylori* strains expressing CagA have a significant higher risk to develop complications, including gastric cancer, compared to *H. pylori* infections alone.[7] However, Pylori DuoTect® cannot be considered as a tumor marker test.

Principle of the Test

Pylori DuoTect® contains colored particles conjugated with the specific *H. pylori* antigens FliD and CagA. When antibodies specific to FliD and/or CagA are present in the specimen, the corresponding antigen test lines will appear as colored lines (FliD: blue, CagA: red). If antibodies to these antigens are not present or are present below the detectable level, these lines will not develop.

Important: The red control line (C) must always appear regardless of the presence of antibodies to the *H. pylori* antigens. If this line does not appear, the test is invalid.

Materials

Materials supplied per kit:

- 25 test cassettes, each sealed in a pouch with transfer pipette and desiccant
- 2 bottles of assay buffer, each containing 3 ml, incl. 0.09% (w/v) sodium azide as a preservative
- 1 package insert (instructions for use)

Materials needed but not supplied:

- Lancet, alcohol swab and timer

Storage

- Test cassettes and assay buffer are stable until the expiration dates printed on their respective labels when stored at 4–27°C.
- Do not freeze or expose the kit to temperatures over 27°C.

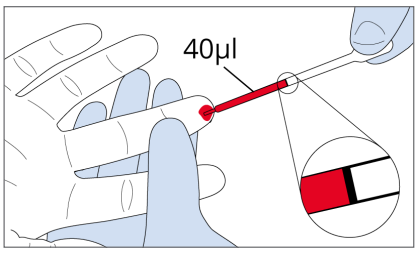
Precautions

1. This test is for professional qualitative in-vitro diagnostic use only.
2. The instructions must be followed strictly to obtain accurate results.
3. The test cassette must be used directly after opening the pouch.
4. Do not use the test cassette if the pouch is damaged.
5. Treat all specimens and used assay materials as potentially biohazardous materials.
6. Use a disposable transfer pipette and a test cassette for each specimen.
7. Inappropriate specimen volume (too low or too high) may lead to incorrect results.
8. Do not use the test cassette beyond the expiration date, which appears on the pouch label.

Specimen Collection /Storage

Whole blood:

- Fingerprick sampling is recommended for this assay.
- The middle or ring finger is the preferred puncture site.
- Clean patient's finger with a skin disinfectant. Wait until it is dry.
- Puncture the fingertip with the lancet.
- Gently rub the hand from palm to finger to help form a larger drop of blood at the punctured site.
- Use the provided transfer pipette, squeeze the bulb and release it to suck in the sample. Make sure it reaches the black marking in order to pipet the correct volume. Avoid air bubbles.



- Then, follow step by step procedures.
- Do not freeze whole blood specimens.

Serum & Plasma

- Venipuncture whole blood specimens: Collect anti-coagulated blood specimen (sodium or lithium heparin, potassium or sodium EDTA, sodium citrate) following standard laboratory procedures.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis.
- Use only clear, non-hemolyzed specimens.
- Do not leave the specimens at ambient room temperature for prolonged periods.
- Serum and plasma specimens can be stored at 2-8°C for up to 3 days. For long term storage, specimens must be kept at -20°C.
- Repeatedly freezing and thawing specimens must be avoided.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well before testing.

Performance Characteristics

Clinical Performance

Pylori DuoTect® *H. pylori* Rapid Test was evaluated with 246 serum samples from patients of which 53 were previously tested positive by gastric *H. pylori* histology and microbiology.

The 193 negative samples were obtained from patients, who were reported to be negative in the Urea Breath Test.

		Comfirmed Clinical Results	
		Positive	Negative
Pylori DuoTect® *H. pylori* Rapid Test	Positive	53	17
	Negative	0	176

The Pylori DuoTect® *H. pylori* Rapid Test has a diagnostic sensitivity of 100.0%, a diagnostic specificity of 91.2%, and a diagnostic trueness of 93.1%.

Cross-Reactivity

Pylori DuoTect® *H. pylori* Rapid Test has been tested with antibody positive sera to closely related antigens, namely *Campylobacter jejuni* and *Borrelia burgdorferi.* No cross reactivity was observed with these sera.

Interference

The following concentrations of interfering substances have been investigated:

Blood Component	Concentration(s) tested
Hemoglobin	100, 250, 500 and 1000 mg/dl
Bilirubin	50 and 100 mg/dl
Albumin	2, 4, and 7 g/dl
Hematocrit*	20, 30, 50 and 67%
Triglycerides	1000 mg/dl
Urea	2.4, 10, 25, and 50 mg/dl
Glucose	70, 150, 200, and 300 mg/dl

*In samples with very high, non-physiological packed cell volumes (≥ 67%) blocking of the sample flow from the sample port might be observed. In these cases, the use of serum or plasma samples is recommended instead. Other than that, no interferences were seen in testing of the listed substances and concentrations.

Repeatability and Reproducibility

For repeatability three replicates were tested with two batches each. Reproducibility used two batches with one replicate each by two users on three consecutive days. Agreement in repeatability and reproducibility were both 100%.

Matrix Equivalence

The sample matrix – whole blood obtained by venipuncture, capillary blood from the fingertip, serum and plasma – had no effect on the results as studied in matrix sets of five spiked samples with varying physiological packed cell volumes in whole blood.

Stability

Lot specific dates are printed on the box, the assay pouch and the assay buffer.

Period after Opening = 21 days for the assay buffer

References

[1] Marshall BJ, Warren JR; Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984 Jun 16;1(8390):1311-5. doi: 10.1016/s0140-6736(84)91816-6. PMID: 6145023.

[2] Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1-241. PMID: 7715068; PMCID: PMC7681621.

[3] Malfertheiner P. et al.; Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017 Jan;66(1):6-30. doi: 10.1136/gutjnl-2016-312288. Epub 2016 Oct 5. PMID: 27707777.

[4] Kazemi S. et al.; Diagnostic values of Helicobacter pylori diagnostic tests: stool antigen test, urea breath test, rapid urease test, serology and histology. J Res Med Sci. 2011 Sep;16(9):1097-104. PMID: 22973378; PMCID: PMC3430034.

[5] Díaz P. et al; Helicobacter pylori and Gastric Cancer: Adaptive Cellular Mechanisms Involved in Disease Progression. Front Microbiol. 2018 Jan 22;9:5. doi: 10.3389/fmicb.2018.00005. PMID: 29403459; PMCID: PMC5786524.

[6] Khalifeh Gholi M. et al; Helicobacter pylori FliD protein is a highly sensitive and specific marker for serologic diagnosis of H. pylori infection. Int J Med Microbiol. 2013 Dec;303(8):618-23. doi: 10.1016/j.ijmm.2013.08.005. Epub 2013 Sep 3. PMID: 24103649.

[7] Shiota S. et al; Biomarkers for Helicobacter pylori infection and gastroduodenal diseases. Biomark Med. 2014;8(9):1127-37. doi: 10.2217/bmm.14.72. PMID: 25402582; PMCID: PMC4254399.

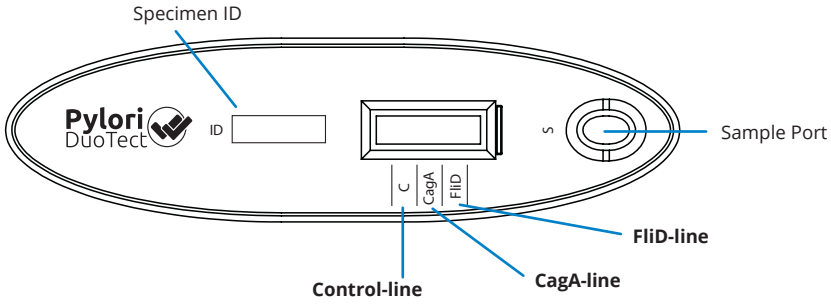
ImevaX

Structure of the Test & Quality Control

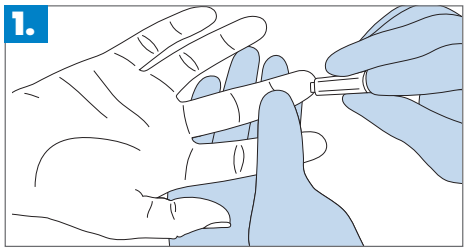
Built-in Control Features:
Pylori DuoTect® contains a built-in control feature, the C-line. The appearance of the red C-line indicates that the test performed properly. If the control line does not develop within 20 minutes, the test is invalid. In this case, repeat the test with a new specimen and new test cassette.

Step by Step Procedure

Preparation:
- Allow test cassette, specimen and assay buffer to reach room temperature (15–27°C) before testing.
- When ready to test, open the pouch at the notch and remove the test cassette. Place the cassette on a clean, flat surface and label it with the specimen ID number.
- Perform the test immediately after opening the pouch.

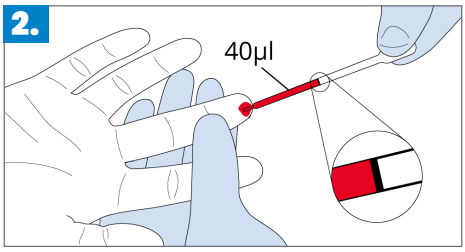


1.



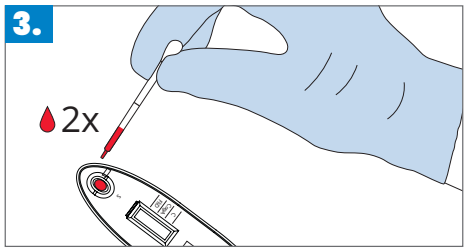
Clean the patient's fingertip with a skin disinfectant and puncture it with a sterile lancet.

2.



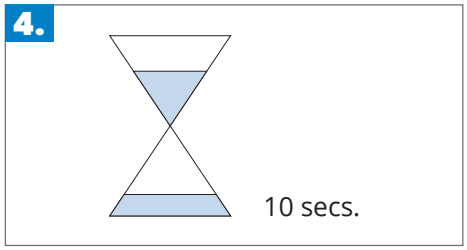
Gently rub the hand from palm to finger to help form a larger drop of blood at the punctured site. Then use the provided transfer pipette, squeeze the bulb and release it to suck in the sample. **Make sure it reaches the black marking in order to pipet the correct volume.** Avoid air bubbles.

3.



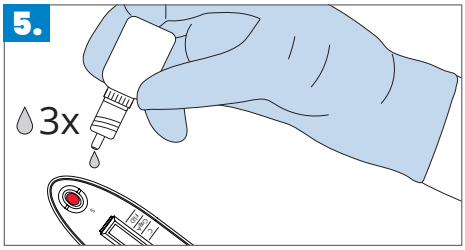
Immediately squeeze the bulb to dispense the full amount of whole blood **(≙ 2 drops / 40µl)** into the sample port (S) of the test cassette.
For serum or plasma specimens apply 1 drop to the sample port and discard the rest.

4.



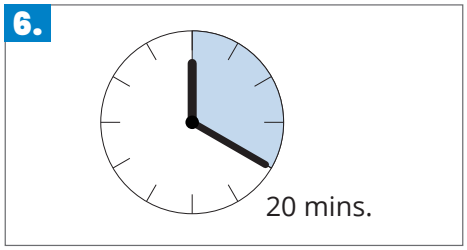
Wait 10 seconds until the sample is fully absorbed in the sample port.

5.



Add 3 drops of assay buffer to the sample port. Set the timer.

6.



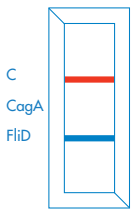
Read the results after 20 minutes.
Do not interpret the results after 25 minutes, as the accuracy cannot be guaranteed after this time.

Interpretation of Results

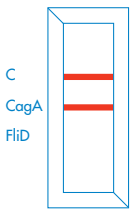
Positive
A positive result means that *H. pylori* specific antibodies were detected in the specimen and that either an active infection or a colonization with *H. pylori* is the present.

Negative
A negative result means that no *H. pylori* specific antibodies were detected in the specimen.

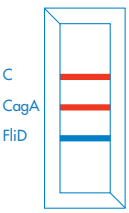
Invalid
An invalid result means that no Control Line (C) developed. In this case, repeat the test with a new specimen and new test cassette.



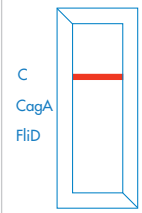
FliD and Control (C) Lines are visible.



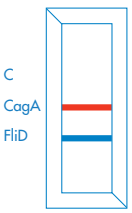
CagA and Control (C) Lines are visible.



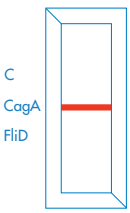
CagA, FliD and Control (C) Lines are visible.



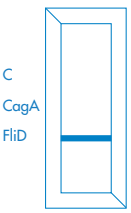
Only Control (C) Line is visible.



No Control (C) Line is present. CagA and FliD Lines are visible.



No Control (C) Line is present. CagA Line is visible.



No Control (C) Line is present. FliD Line is visible.

No Control (C), CagA and FliD Lines are present.

Note: Even a faint FliD and/or CagA line indicates a positive result.
Even a faint Control Line (C) indicates that the test performed properly.

Limitations

Pylori DuoTect® is for the qualitative detection of antibodies specific to two *H. pylori* antigens in human whole blood, serum or plasma: FliD and CagA. It does not indicate the antibody titer in the sample. A positive result does not distinguish between an active infection and colonization of *H. pylori.* The test should be used in first-line diagnostics to detect a *H. pylori* infection in adult subjects. The results must always be interpreted in context with the clinical picture and other diagnostic procedures. Any therapeutic decision must also be made on a case-by-case basis.

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