RAPID Hp StAR™

REF K671411-2 20 IVD CE

A rapid immunochromatographic assay for the detection of *H. pylori* antigen in stool specimens.

EN

1 INTENDED USE

RAPID Hp StAR™ is an *in vitro* qualitative immunochromatographic assay for the detection of *Helicobacter pylori* antigen in human stool samples. Test results are intended to aid the diagnosis of *Helicobacter pylori* infection and to monitor response post-therapy in adult patients.

2 SUMMARY

In 1984 Marshall and Warren described the presence of a Campylobacter-like organism in the antrum and corpus of patients with histological evidence of gastritis and peptic ulcers[1]. Today *H. pylori* is well recognized as a major cause of gastrointestinal diseases[2].

Infection by *H. pylori* leads to inflammation, which has a strong correlation with chronic gastritis, ulcers of the stomach and the duodenum, and gastric carcinoma[3,4]. Patients with successful eradication therapy show evidence for this cause and effect relationship - often gastritis and ulcers are cured.

These bacteria have adapted to live in the acidic environment of the stomach. The enzyme urease cleaves urea into ammonia and carbon dioxide, thus neutralizing the acid and enabling *H. pylori* to survive the bactericidal conditions of the stomach. The production of catalase and superoxide dismutase protects the bacteria from neutrophilic attack in the stomach mucosa[3].

Many *H. pylori* infected patients develop gastritis, and about 10% of them ulcers. 90% of patients suffering from ulcers of the duodenum or the stomach are *H. pylori* positive, regardless of age. The reasons for these phenomena as well as the mode of infection are subject to worldwide research[5].

There are two general methods of diagnosing *H. pylori* infection: the direct detection of the organism and the indirect determination by the detection of antibodies developed by the patient against *H. pylori*[1,6,7].

Direct, but invasive methods to detect *H. pylori* infection are the rapid urease test, histology or the culture of the organism from the biopsy material[8]. Culturing of *H. pylori* from biopsy material is difficult and time consuming. The technical difficulties can lead to false negative results and therefore to a reduced sensitivity. In addition, *H. pylori* tends to colonize the mucosa in patches and can therefore be missed during endoscopy[9].

Another direct way of diagnosing *H. pylori* is the use of a urea breath test, which detects carbon dioxide produced by the urease activity. Although highly sensitive and specific, it requires specialized instrumentation as well as the ingestion of isotope-labelled urea by the patient[8,10].

A commonly used method is the serological detection of antibodies specific for *H. pylori*. This is an indirect approach detecting *H. pylori* specific antibodies developed by the patient[10]. Sensitivity and specificity vary greatly among tests from different suppliers. Furthermore, eradication control with serological methods is insufficient, because a significant decrease of antibody level takes several months.

RAPID Hp StAR™ is a rapid immunochromatographic assay in a lateral flow (LAF) format for the direct, non-invasive detection of *H. pylori* antigen in human stool samples. Due to the direct detection of antigen this test can be used for the initial diagnosis of *H. pylori* infection as well as for monitoring eradication success four to six weeks after completion of eradication therapy and also for the diagnosis of reinfection.

3 PRINCIPLE OF THE TEST

RAPID Hp StAR™ is an immunochromatographic membrane based assay using amplification technology for the determination of *H. pylori* antigen in stool samples.

An amplified capture reagent incorporating streptavidin is bound to a nitrocellulose membrane. Two different monoclonal antibodies specific to *H. pylori* antigen are dried onto another membrane at the base of the strip. One of these monoclonal antibodies is conjugated to colloidal gold, and the other is conjugated to biotin. When the strip is dipped into the diluted stool sample, the liquid sample moves up the strip by capillary action, solubilising the two monoclonal antibody conjugates. Any *H. pylori* antigen present in the sample will bind to the two solubilised monoclonal antibody conjugates to form an antigen-antibody complex. Each complex will consist of antigen, bound to both monoclonal antibody conjugated to colloidal gold, and monoclonal antibody conjugated to biotin. This complex migrates further up the strip until it reaches the streptavidin capture reagent, which captures the biotin element of the antigen-antibody complex, immobilising the whole complex and forming a purple-pink line. Remaining unbound colloidal gold conjugate continues migrating up the strip and is captured by an anti-mouse polyclonal antibody that is immobilised on the control area of the nitrocellulose, forming a second purple-pink line, indicating that the test has performed correctly.

4 DEFINITIONS

The following symbols and definitions are used in the product information.

REF	Product code and catalogue number
	Consult the instructions for use
N	Contains sufficient for <N> tests
	Manufactured by
IVD	*In vitro* diagnostic medical device
	Use by
LOT	Batch Code
2°C-8°C	Storage temperature limitations
SPECIMEN NO	Specimen number/Patient ID
	Do not reuse

5 REAGENTS PROVIDED

20 - Each kit contains sufficient materials for 20 determinations. - The shelf life of the kit is as indicated on the outer box label.

5.1 *RAPID* Hp StAR™ TEST CONTENTS

One Instructions for Use booklet

25 x wooden sticks

20 x transparent test tubes

20 x plastic pasteur pipettes (graduated at 0.1mL)

TEST STRIPS x 20 20 x test strips provided ready to use in a resealable foil pouch

SAMPLE DILUENT 20 x 1.35mL Sample Diluent. PBS Buffer containing antimicrobial agent, animal serum and detergent

5.2 PREPARATION, STORAGE AND RE USE OF KIT COMPONENTS

In order to ensure optimal kit performance it is important that all unused kit components are stored according to the following instructions.

5.2.1 Test Strips TEST STRIPS x 20

Ready to use. For single use only. Store unused Test Strips in resealable foil pouch with silica gel at 2-8°C. Discard used test strips immediately after use.

5.2.2 Sample Diluent SAMPLE DILUENT

Ready to use. For single use only. Store unused Sample Diluent at 2-8°C.

6 EQUIPMENT

The following equipment is optional:

Vortex mixer

7 PRECAUTIONS

IVD For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

7.1 SAFETY PRECAUTIONS

7.1.1 Reagents of this kit contain antimicrobial agents. Avoid contact with skin and eyes. Rinse immediately with plenty of water if any contact occurs.

7.1.2 Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.

7.1.3 Do not pipette materials by mouth.

7.1.4 Wear disposable gloves while handling clinical specimens and reagents. Always wash hands after working with infectious materials.

7.1.5 Dispose of all clinical specimens in accordance with local legislation.

7.1.6 Safety data sheet available for professional user on request.

7.2 TECHNICAL PRECAUTIONS

7.2.1 Components must not be used after the expiry date printed on the labels. Do not mix or interchange different batches/lots of reagents.

7.2.2 DO NOT freeze any kit components.

7.2.3 Kit components should be examined visually for signs of contamination, deterioration or leakage.

7.2.4 If the Control line does not appear after 15 minutes this may indicate blockage of the membranes with particulate matter. In this case, it is recommended that a fresh dilution of sample is prepared and tested using a fresh test strip.

7.2.5 Samples collected into transport medium or other preservative media should not be tested.

7.2.6 Avoid contamination of reagents.

7.2.7 Test Strips cannot be re used.

8 COLLECTION AND PREPARATION OF STOOL SPECIMENS

8.1 SPECIMEN COLLECTION

The test can be performed on either fresh or frozen stool samples. Samples may be stored at 2-8°C for up to five days without interference with the assay performance. For longer periods store at -20°C or below. Repeated freezing and thawing of samples should be avoided.

Samples collected into transport medium or other preservative media should not be tested.

8.2 PREPARATION OF STOOL SPECIMENS

Label the required number of Sample Diluent vials with sample ID and remove the cap. Using a wooden stick applicator add a pea-sized stool sample (approx. 0.1g) to each vial of Sample Diluent. For liquid or semi-solid stool samples add 100µL of sample to the vial using a disposable pipette provided. 100µL is the volume to the first notch on the pipette. Replace cap. Homogenize for 15 seconds on a vortex mixer or manually mix by shaking vigorously.

Use a new wooden stick applicator or disposable pipette for each sample. NB: Addition of too much stool sample may cause restricted flow along the membrane and cause invalid results. In such a case a further sample preparation should be carried out in a fresh vial. Addition of too little stool sample may cause an incorrect test result.

Prepared stool suspension may be stored for up to 5 days at 2-8°C but not frozen.

9 TEST PROCEDURE

PLEASE REFER TO SECTION 7.2, TECHNICAL PRECAUTIONS, BEFORE PERFORMING THE TEST PROCEDURE.

Reagents and specimens should be brought to room temperature (18-30°C) before use.

9.1 ASSAY PROCEDURE

9.1.1 Specimen Addition

Place required number of transparent test tubes in rack provided and label with sample ID.

Using the disposable plastic pipette provided add 350µL of the stool suspension to the test tube, taking care to keep the sides of the tube clean. 350µL is the volume up to the base of the bulb of the disposable pipette.

9.1.2 Incubation

Open the foil pouch via the resealable strip, remove the required number of test strips and reseal pouch. Place test strip in tube with the uncovered end down, so that the base of the strip is immersed in the sample.

Leave the test strip to stand vertically at room temperature (18-30°C) for 15 minutes.

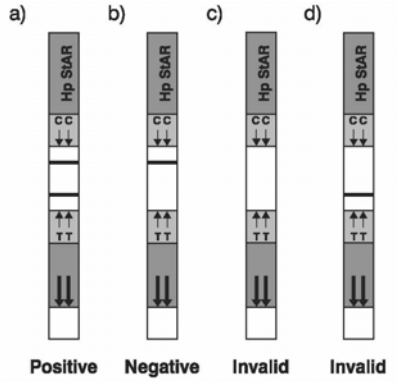
9.1.3 Reading the Test Results

Test results should be read visually within 5 minutes of the end of the 15 minute incubation period.

10 INTERPRETATION OF THE TEST RESULTS

Visually inspect the test strip through the transparent tube. If the test has functioned correctly at least one purple-pink line will appear.

Results should be interpreted as shown in the diagram below.



a) Two lines (T and C): Positive

b) One line (C only): Negative

c) No lines: Invalid Result – repeat test

d) One line (T only): Invalid Result – repeat test

11 PERFORMANCE LIMITATIONS

11.1 *RAPID* Hp StAR™ is for *in vitro* diagnostic use. Test results are intended to aid the diagnosis of *H. pylori* infection and to monitor response post-therapy in adult patients. Test results should be interpreted by the clinician in conjunction with clinical findings and/or other diagnostic procedures.

11.2 *RAPID* Hp StAR™ is a qualitative test and no quantitative interpretation should be made.

11.3 Antibiotics, proton pump inhibitors and bismuth preparations are known to suppress growth of *H. pylori*. Stool sampling must be performed not earlier than 2 weeks after termination of ingestion of proton pump inhibitors and bismuth preparations and 4 weeks after termination of ingestion of antibiotics, respectively.

11.4 A negative result does not exclude the possibility of *H. pylori* infection in the patient. Failure to detect *H. pylori* may be a result of factors such as collection of specimen at an improper time in the disease when too little antigen is present, improper sampling or handling of the specimen.

11.5 A positive test result alone does not justify an indication for eradication therapy. Other methods may be necessary to confirm *H. pylori* infection. Differential diagnosis with invasive endoscopic methods might be indicated in order to examine the presence of any other complicating conditions, eg ulcer, autoimmune gastritis and malignancies.

12 EXPECTED VALUES

Expected values depend on geographic location and type of population studied. The rate of positive test results may vary due to the type of test employed and the method of specimen collection and handling.

Epidemiological studies have shown that infection by *H. pylori* is prevalent throughout the world. In Europe and North America 25-50% of the population carries *H. pylori*. Even higher prevalence rates of 70-90% have been reported for Asia, Africa and South America[1,11].

The frequency of *H. pylori* infection has been shown to correlate with age, ethnic background, socioeconomic class and the general health environment eg the prevalence of infection in the United States increases with age at approximately 1% per year[12].

13 SPECIFIC PERFORMANCE CHARACTERISTICS

13.1 PERFORMANCE EVALUATIONS

Study 1: Primary diagnosis in adult patients

RAPID Hp StAR™ was evaluated externally on 198 patients (92 male, 106 female, age range 19-81 years) from a centre in Spain undergoing investigation of dyspeptic symptoms. Stool testing was performed in an independent laboratory in a blinded fashion. *H. pylori* infection status was confirmed by a panel of reference tests comprised of histology, rapid urease test, UBT and Amplified IDEIA™ Hp StAR stool antigen EIA. *H. pylori* infection was confirmed when at least two reference tests were positive. Results are shown in Table 1.

Table 1 Comparison of *RAPID* Hp StAR™ v Confirmed Status

		Confirmed Status	
		+	-
RAPID Hp StAR™	+	103	15
	-	10	70

Relative Sensitivity: 103/113	91.2%
95% Confidence Interval:	84.3%- 95.7%
Relative Specificity: 70/85	82.4%
95% Confidence Interval:	72.6% - 89.8%
PPV: 103/118	87.2%
95% Confidence Interval:	81.3% - 93.3%
NPV: 70/80	87.5%
95% Confidence Interval:	78.2% - 93.8%
Correlation: 173/198	87.4%
95% Confidence Interval:	82.7% - 92.0%

Study 2: Post eradication therapy in adult patients

RAPID Hp StAR™ test was evaluated externally on 88 *H. pylori* infected adult patients (26 female, 62 male, age range 21-86 years) from a centre in Spain who had undergone eradication therapy. *H. pylori* infection was confirmed by urea breath test (UBT) or gastric histology where required. Stool samples were collected 8 weeks after completion of eradication therapy. Stool testing was performed in an independent laboratory in a blinded fashion. Results are shown in Table 2.

Table 2 Control of eradication therapy in adults using *RAPID* Hp StAR™ and UBT

		Confirmed Status	
		+	-
RAPID Hp StAR™	+	10	6
	-	0	72

Relative Sensitivity: 10/10	100%
95% Confidence Interval:	69.2%-100%
Relative Specificity: 72/78	92.3%
95% Confidence Interval:	84.0%-97.1%
PPV: 10/16	62.5%
95% Confidence Interval:	35.4%-84.8%
NPV: 72/72	100%
95% Confidence Interval:	95.0%-100%
Correlation: 82/88	93.2%
95% Confidence Interval:	85.7%-97.5%

Study 3: Primary diagnosis of *H. pylori* infection

RAPID Hp StAR™ test was externally evaluated on 204 patients (107 male, 97 female, age range 11-80 years) from a centre in the UK undergoing investigation of dyspeptic symptoms. Stool testing was performed in an independent laboratory in a blinded fashion. Results are shown in Table 3.

Table 3 Primary diagnosis using *RAPID* Hp StAR™ and Amplified IDEIA™ Hp StAR™

		Amplified IDEIA™ Hp StAR™	
		+	-
RAPID Hp StAR™	+	56	5
	-	1	142

Relative Sensitivity: 56/57	98.25%
95% Confidence Interval:	90.6%- 100%
Relative Specificity: 142/147	96.60%
95% Confidence Interval:	92.2% - 98.9%
Correlation: 198/204	97.06%
95% Confidence Interval:	93.7% - 98.9%

13.2 CROSS REACTIVITY

RAPID Hp StAR™ is highly specific for antigens from *H. pylori*. No cross reactivity was observed when testing the micro-organisms listed below. Testing was performed on laboratory cultures of known organisms.

Acinetobacter sp.
Aeromonas sp.
Bacillus sp.
Bacteroides sp.
Beta haemolytic Streptococcus (group A)
Campylobacter sp.
Candida albicans
Citrobacter sp.
Clostridium difficile
Enterobacter sp.
Enterococcus faecalis
Escherichia coli
Haemophilus influenza
Klebsiella sp.
Lactobacillus sp.
Listeria sp.
Proteus sp.
Pseudomonas aeruginosa
Salmonella typhimurium
Serratia sp.
Shigella sonnei
Staphylococcus epidermidis
Staphylococcus aureus

13.3 TESTS FOR INTERFERING SUBSTANCES

The following substances were found to have no detrimental effect on stool sample results when tested at the following concentrations.

Stearic Acid	16mg/g
Palmitic Acid	16mg/g
Barium Sulphate	20mg/g
Human Haemoglobin	30mg/g
Ranitidine Hydrochloride	2mg/g
Omeprazole	2mg/g
Cimetidine	2mg/g
Calcium Carbonate	20mg/g
Bismuth (III) Subsalicylate	0.7mg/g
Mucin	14mg/g
Human Blood	200µL/g

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Oxoid (Ely) Ltd., Denmark House, Angel Drove, Ely, Cambs. CB7 4ET UK