IVD TECHNOLOGIES

Helicobacter pylori Antigen One Step Stool Test Kit

FOR DETECTION OF H. pylori ANTIGEN IN STOOL SAMPLES

REF HP03-3000 Research Use Only

H. pylori Stool Ag

INTENDED USE

The Helicobacter pylori Antigen One Step Stool Test Kit is used for the determination of *Helicobacter pylori* (H. pylori) antigen in stool samples.

ANALYTICAL PRINCIPLE

Diluted stool samples with potential H. pylori antigen and H. pylori-HRP conjugate is added to microwell coated with H. pylori antibody. H. pylori antigen if present will bind to the immobilized antibody on the surface of the microwell and H. pylori antibody-HRP will bind to any H. pylori antigen-H. pylori antibody complex on the surface of the microwell. Unbound HRP conjugate and antigens are removed by a wash step and TMB is allowed to react with any bound HRP conjugate. Color produced indicates the presence of H. Pylori antigen.

SPECIMEN REQUIREMENTS

Stool samples are acceptable. Avoid repetitive freezing and thawing of samples. See Sample Collection section.

REAGENTS

Precautions & Safety Notes

- WEAR LATEX GLOVES, FACE SHIELDS AND A LAB COAT WHEN HANDLING SPECIMENS AND OTHER HAZARDOUS REAGENTS
- FOR *IN VITRO* USE, POTENTIAL BIOHAZARDOUS MATERIAL. HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- Sample Diluent, Positive Control, Negative Control, and Wash Buffer reagents contain 0.05% ProClin 300. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	H. Pylori Antigen One Step Stool Test Kit	**HP03-3000**	96 Test
A	H. Pylori Microwell Plate [Contains: H. Pylori Antibody]	**HP03 - 3001**	96 Well Plate
B	HRP Conjugate [Contains: H. Pylori Antibody-HRP]	**HP03 - 3002**	5 mL
C1 - C6	Set of 6 Calibrators (0.0, 5.0, 10.0, 25, 50, 100 ng/mL)	**HP63-3003 (A-F)**	0.6 mL x 6
D	Control 1 Ready to Use	**HP63 - 3004A**	0.6 mL
E	Control 2 Ready to Use	**HP63 - 3004B**	0.6 mL
SD5	Sample Diluent	**STLD-1004**	100 mL
WBC	20X Wash Buffer Concentrate [Contains: Tris Buffer, 1% Tween-20, NaCl, 0.05% ProClin 150]	**WBFEA-1002**	25 mL
TS	TMB Solution [Contains TMB] Keep away from light	**TMBS-1001**	10 mL
SS	Stop Solution [Contains 1N Sulfuric Acid]	**SSFEA-1001**	10 mL

MATERIALS

The following materials are required but not supplied.

- Variable Pipettors and Tips
- Microplate reader at 450 nm
- Refrigerator (for kit storage)

PROCEDURE PRECAUTIONS

- Bring reagents to room temperature before use.
- Use clean instruments & equipments.
- Microwells can be snapped to select exact number of wells.
- Unused microwells should be sealed in pouch.
- Handle microwells with care.
- Minimize air bubbles in microwells.
- Microwells should not contain liquid after the washing steps. To ensure that liquids are removed from microwells after washing steps, microwells can be tapped against clean paper towels.
- It is recommended to perform calibrator, controls, and unknowns in duplicates.

REAGENT PREPARATION

1x Wash Buffer Preparation

1. Add 1 unit of volume of 20X Wash Buffer Concentrate (**WBC**) to 19 units of volume of DI water. For example, add **25 mL** 20X Wash Buffer Concentrate (**WBC**) to **475 mL** of DI water.
2. Mix the Wash Buffer Preparation well.

PROCEDURE

Sample Collection

- Collect sample in clean container.
- Always use fresh sample
- Keep sample stored at 2 - 8°C. Store at -20°C for longer term.

Sample Preparation

- Label test tube for each sample.
- Add 1mL of Sample Diluent (**SD5**) to each tube.
- Add **100 - 150 mg** (pea size) of stool to each tube. If stool is liquid, transfer **100 µL**.
- Vortex each tube for at least 3 seconds.
- Centrifuge at 3,000 RPM for 5 ± 2 minutes or let sample sediment by letting it sit for 30 minutes.

Assay Protocol

1. Dispense **100 µL** of diluted samples, standards (**C1 - C6**), Control 1 (**D**), and Control 2 (**E**) into designated wells of the Microwell Plate (**A**).
2. Dispense **50 µL** of HRP Conjugate (**B**) to each well.
3. Incubate for **30 minutes** at **room temperature on a shaker at 420 RPM.**
4. Wash each well **3 times** with **300 µL** of the **1X Wash Buffer Preparation**. Tap microwells against clean paper towels to ensure no liquid remains.
5. Dispense **100 µL** of TMB Solution (**TS**) to each well.
6. Incubate the wells for **15 minutes** at **room temperature** and **protect from light**.
7. Dispense **100 µL** of Stop Solution (**SS**) to each well.
8. Read wells on a microplate reader at **450 nm**.

CALCULATIONS

A standard curve is used to calculate the concentration of unknowns.

1.Use the mean OD values of the calibrators to plot a standard curve on a linear graph. Plot absorbance on y axis and concentration on x axis.

2. Plot the OD of the controls and unknowns using the standard curve to determine the concentration. Multiply the concentration found by the sample dilution factor, if a dilution was performed.

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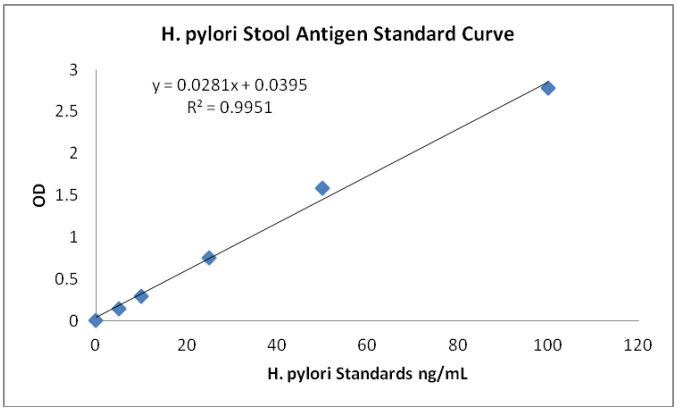
TYPICAL RESULTS

Standard Curve and Control Results			
ng/mL	**OD 1**	**OD 2**	**Mean OD**
0	0.008	0.009	0.0085
5	0.15	0.158	0.15
10	0.302	0.297	0.3
25	0.794	0.698	0.75
50	1.597	1.58	1.59
100	2.766	2.802	2.78

Control	**OD**	**Result**
C1	0.465	15.14
C2	1.362	47.06



RESULTS INTERPRETATION

Fifty-two (52) negative stool samples were tested for H. pylori. The OD values and H. pylori concentration ranged from 0.048 to 0.14. The concentration ranged from 1.2 to 4.4 ng/mL.

Negative	<5 ng/mL
Positive	> 10 ng/mL
Borderline	5 - 10 ng/mL

POSTPROCEDURE NOTES

- Results that are borderline should be repeated using fresh sample.

ASSAY PERFORMANCE

Reproducibility

Intra assay

Sample	A	B
N	20	20
Mean	4.28	26.59
SD	0.27	0.99
CV	6.4	3.67

Inter assay

Sample	A	B
N	20	20
Mean	4.42	26.47
SD	0.32	0.97
CV	7.32	3.68

Linearity

Sample 1			Sample 2		
Dilution	ng/mL	Recovery	Dilution	ng/mL	Recovery
Neat	85		Neat	57	
1:02	41.02	96.52	1:02	29	101.75
1:04	22.3	104.94	1:04	14.6	102.46
1:08	11.05	104.00	1:08	7.1	99.65
1:16	5.38	101.27	1:16	3.3	92.63
1:32	3.1	116.71	1:32	1.8	101.05
Mean		104.69	Mean		99.51

Sensitivity

Limit of detection: 0.5 ng/mL

Specificity

The H. pylori antibody used for coating and the HRP conjugate are specific to *Helicobacter pylori*.

Cross Reactivity

No cross reactivity with *Campylobacter spp, Clostridium difficile, Escherichia coli, Lisreria monocytogenes, Salmonella spp, Shigella spp, and Yersinia enterocolitica*.

Clinical Sensitivity and Specificity

Stool samples were collected from patients. 28 samples were confirmed positive and 45 samples were confirmed negative by clinical symptoms, diagnostic procedures, and other tests.

Clinical samples		**True Positive**	**True Negative**	**Total**
IVD Technologies' H. pylori Results	**Positive**	28	0	28
	Negative	0	45	45
	Total	28	45	73

Sensitivity	100%	28/28
Specificity	100%	45/45
Accuracy	100%	73/73

Manufacturer	
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