K-*ASSAY*®

H. pylori Test Reagent

For the Qualitative Determination of *H. pylori* Antibodies in Serum and Plasma on Automated Chemistry Analyzers

Cat. No. KAI-240

INTENDED USE

The **K-*ASSAY*®** H. pylori Test Reagent is for the qualitative determination of anti-*Helicobacter pylori* antibodies in human serum and plasma by immunoturbidimetric assay on automated clinical chemistry analyzers. The **K-*ASSAY*®** H. pylori Test Reagent is used as an aid in the diagnosis and treatment of *H. pylori* infection. Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions. FOR RESEARCH USE ONLY IN THE U.S. NOT FOR USE IN DIAGNOSTIC PROCEDURES IN THE U.S.

INTRODUCTION AND SUMMARY

Helicobacter pylori, previously *Campylobacter pylori*, is a gram-negative bacterium usually found in the stomach. *H. pylori* infection may be present in more than half the people in the world.[1,2] In general, people infected with *H. pylori* are asymptomatic. Individuals infected with *H. pylori* have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring stomach cancer.[2] Anti-*H. pylori* antibody detection is useful in the diagnosis and treatment of *H. pylori* infection.

The **K-*ASSAY*®** H. pylori Test Reagent is a latex-enhanced immunoturbidimetric assay, developed to qualitatively determine levels of antibody to *H. pylori* in serum and plasma samples.

PRINCIPLE OF TEST

An antigen-antibody reaction occurs between anti-*H. pylori* antibodies in the sample and *H. pylori* antigens conjugated to latex particles which produces agglutination. This agglutination is detected as an absorbance change on an automated clinical chemistry analyzer. The magnitude of the change is proportional to the quantity of anti-*H. pylori* antibodies in the sample. The actual concentration is then determined by interpolation from a standard curve prepared from calibrators of known concentrations.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent
Good's buffer solution (50 mM)

R2: Latex Suspension
0.15 w/v% suspension of latex particles sensitized with *H. pylori* antigens.

WARNINGS AND PRECAUTIONS

For research use only in the U.S. Not for use in diagnostic procedures in the U.S.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain less than 0.1 w/v% sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution. **Do not shake the reagent bottles when putting on the analyzer.**

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for 12 months from the date of manufacture as indicated on the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for 8 weeks if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at approximately 570 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

Serum
Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot tube. It is recommended that the specimen collection be carried out in accordance with the NCCLS document M29-A2. After sampling, the specimen should be immediately stored at 2-8°C and assayed as soon as possible. If the assay cannot be performed immediately, then the sample should be tightly capped and frozen at -20°C or below. Avoid more than 2 freeze-thaw cycles.

Plasma
Whole blood is collected in potassium EDTA (EDTA-2K or EDTA-3K) or sodium heparin anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. If the assay cannot be performed immediately, then the sample should be capped and frozen at -20°C or below. Avoid more than 2 freeze-thaw cycles.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Solution 1 x 60 mL
Reagent 2 (R-2) Latex Suspension 1 x 12 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** H. pylori Calibrator, Cat. No. KAI-241C.

Controls: **K-*ASSAY*®** H. pylori Control, Cat. No. K242C-2M or K242-4M.
2 levels: Level 1 = Negative, Level 2 = Positive

Saline (0.9% w/v NaCl)

Two Reagent Clinical Chemistry Analyzer Capable of:
Accurate absorbance readings at approx. 570 nm
Accurately dispensing the required volumes
Maintaining 37°C

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 917):

Sample 3.5 µL
↓
• ← R1 (Buffer Reagent) 200 µL
↓ 37° 5 min.
• ← R2 (Reacting Reagent) 40 µL
↓ 37° 5 min.
Endpoint, 570 nm (main) / 800 nm (secondary)

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(HPY)
ASSAY CODE / ASSAY POINT	(2 POINT END) (10) (19) (32) (0) (0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(3.5) (0.0) (0)
R-1 VOLUME (R1)	(200) (0)
R-2 VOLUME (R3)	(40) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (0) (LOWER)
CALIB. TYPE	(SPLINE)
POINT	(6)
SPAN POINT	(6)
SD LIMIT	(999.9)
DUPLICATE LIMIT	(32000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(U/mL)
STD.(1) Conc.-POS.	(0.0) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	(*6) - (6)

Use isotonic saline as STD (1)
* 2-6: Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

A six-point calibration curve should be made using the **K-*ASSAY*®** H. pylori Calibrator and saline (0 U/mL). It is recommended that each laboratory determine calibration frequency, as this would depend on the analyzer in use as well as the types and number of other assays being run. A new calibration curve should be made at least once every two weeks or when a new lot of reagent is used.

QUALITY CONTROL

A quality control program is recommended for all clinical testing laboratories. It is recommended the **K-*ASSAY*®** H. pylori Control containing both negative and positive controls be run with each batch of samples to monitor the procedure. QC intervals and limits should be adapted to each laboratory's individual requirements. Each laboratory should establish corrective measures if values fall outside the limits.

LIMITATIONS OF PROCEDURE

The measurable range for this *H. pylori* test kit is between 3 to 100 U/mL. If the *H. pylori* antibody concentrations are greater than the highest calibrator value, dilute the sample with isotonic saline and re-assay. Multiply the result by the dilution factor to compensate for the dilution.

PERFORMANCE

Precision

(Within Run)
Serum control samples were assayed 10 times on the same day.

Control (Negative)	Control (Positive)
N = 10	N = 10
Mean = 5.1	Mean = 20.6
SD = 0.1	SD = 0.3
CV = 2 %	CV = 1 %

Accuracy / Correlation

A comparison of the **K-*ASSAY*®** H. pylori Test Reagent and another *H. pylori* antibody test was performed with the following results.

		Other *H. pylori* Test		
		Positive	Negative	Overall
K-*ASSAY*® H. pylori Test Reagent	Positive	59	6	65
	Negative	3	54	57
	Overall	62	60	122

Positive agreement:	59 / 62	=	95.2%
Negative agreement:	54 / 60	=	90.0%
Overall agreement:	113 / 122	=	92.6%

The 6 samples judged as positive by the **K-*ASSAY*®** H. pylori Test Reagent and negative by the other H. pylori Test were analyzed by a rapid urease test and endoscopy. The results show that 4 of these 6 samples were actually positive. 2 of these 6 samples were indeterminable.

The 3 samples judged as negative by the **K-*ASSAY*®** H. pylori Test Reagent and positive by the other *H. pylori* Test were analyzed by a rapid urease test and endoscopy. The results show that 2 of these 3 samples were actually negative. 1 of these 3 samples was indeterminable.

MATRIX COMPARISON

Paired samples (39 pairs of serum and heparin plasma samples and 56 pairs of serum and EDTA plasma samples) were tested. Overall agreement with the serum samples was 100% for the heparin plasma and 98.2% for the EDTA plasma.

Assay Range

3-100 U/mL

INTERFERENCE

Bilirubin:	No interference up to 30 mg/dL
Hemoglobin:	No interference up to 500 mg/dL
Lipid:	No interference up to 5% Intra-fat (which contains 10% soybean oil)

INTERPRETATION OF RESULTS

10 U/mL should be used as the cutoff value.

If the test result is ≥ 10 U/mL, the result is judged as positive.

If the test result is < 10 U/mL, the result is judged as negative.

U = unit determined by internal specifications

PRECAUTIONS CONCERNING INTERPRETATIONS

1. Samples from the patients having early infection, long severe atrophic gastritis or low immunity are sometimes judged as negative due to the insufficient increase of antibodies.

2. Test results should be interpreted with a complete clinical assessment.

REFERENCES

1. Chang, A. H.; Parsonnet, J. (2010). "Role of Bacteria in Oncogenesis". Clinical Microbiology Reviews. 23 (4): 837–857.
2. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of Helicobacter pylori Infection". Clin Microbiol Rev. 19 (3): 449–90.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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