DRG® Anti-H. Pylori IgM (EIA-4101)

CE

Revised 23 June 2011 rm (Vers. 2.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Intended Use: Determination of Anti-H. Pylori Specific Antibodies of the IgG, IgA or IgM type in Human Serum or Plasma by Microplate Enzyme Immunoassay

EXPLANATION OF THE TEST

DRG's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted specimen, or control is first added to a microplate well. Biotinylated *H. Pylori* is added, and then the reactants are mixed. A reaction result between the autoantibodies to *H.Pylori* and the biotinylated *H.Pylori* to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG, M or A conjugate is then added to permit quantitation of reaction through interacting with human IgG, M, or A of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

PRINCIPLE

A Sequential ELISA Method

The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenous added biotinylated H. Pylori antigen.

Upon mixing biotinylated antigen, and a serum containing the antibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the followed equation:

$$\text{h-Ab}_{(\text{X-H.Pylori})} + {}^{\text{Btn}}\text{Ag}_{(\text{H.Pylori})} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \text{h-Ab}_{(\text{X-H.Pylori})} - {}^{\text{Btn}}\text{Ag}_{(\text{H.Pylori})}$$

$^{\text{Btn}}\text{Ag}_{(\text{H.Pylori})}$ = Biotinylated Antigen (Constant Quantity)

$\text{h-Ab}_{(\text{X-H.Pylori})}$= Human Auto-Antibody (Variable Quantity)

$\text{Ab}_{(\text{X-H.Pylori})}$-$^{\text{Btn}}\text{Ag}_{(\text{H.Pylori})}$ = Immune Complex (Variable Quantity)

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

$h\text{-}Ab_{(X\text{-}H.Pylori)}\text{-}{}^{Btn}Ag_{(H.Pylori)}+Streptavidin_{C.W.} \Rightarrow$ immobilized complex (IC)

$Streptavidin_{C.W.}$ = Streptavidin immobolized on well

Immobilized complex = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG, M or A) is then added to the microwells. This conjugates binds to the immune complex that formed.

$IC_{(h\text{-}IgG,M\ or\ A)} + {}^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)} \Rightarrow {}^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)} - IC_{(h\text{-}IgG,\ M\ or\ A)}$

$IC_{(h\text{-}IgG,\ M\ or\ A)}$ = Immobilized Immune complex (Variable Quantity)

${}^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)}$ = Enzyme-antibody Conjugate (Constant Quantity)

${}^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)} - I.C._{(h\text{-}IgG,\ M\ or\ A)}$ = Ag-Ab Complex (Variable)

The anti-h-IgG, IgM or IgA enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained

REAGENTS

Materials provided:

A. Anti-H.Pylori Calibrators -- 1ml/vial - Icons A-E

Five (5) vials of references for anti-H.Pylori at levels of 0**(A)**, 10**(B)**, 25**(C)**, 50**(D)**, and 100**(E)** U/ml* of the IgG, IgM or IgA type. Store at 2-8°C. A preservative has been added. *Manufacturers' Reference Value

B. H.Pylori Biotin Conjugate – 13ml/vial - Icon

One (1) vial of biotinylated inactivated H.Pylori in a buffering matrix. A preservative has been added. Store at 2-8°C**.**

C. Enzyme-antigen Conjugate – 13ml/vial - Icon

One (1) vial of anti-human IgG, IgM or IgA-horseradish peroxidases (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C**.**

D. Streptavidin Coated Microplate -- 96 wells - Icon

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent Concentrate - - 20ml

One (1) vial of serum diluent containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate -- 20ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C

G. Substrate A --7ml/vial - Icon S^A

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

H. Substrate B -- 7ml/vial - Icon S^B

One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

I. Stop Solution -- 8ml/vial - Icon STOP

One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

J. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.
Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:

1. Pipette capable of delivering 10, 25, & 50 µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

PRECAUTIONS

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395**.**

SPECIMEN COLLECTION AND PREPARATION

The Specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tubes containing EDTA or heparin. Allow the blood clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 ºC for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (IgM & IgA) or 0.050ml (IgG) of the diluted specimen is required.

REAGENT PREPARATION:

1. **Serum Diluent:** Dilute the serum diluent concentrate to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer:** Dilute contents of Wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.
3. **Working Substrate Solution:** Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

Note: Do not use the working substrate if it looks blue.

4. **Serum Sample Dilution (1/100):** Dispense 0.010ml (10µl) of each specimen into 1 ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8ºC for up to forty-eight (48) hours.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. Format the microplates' wells for each serum reference, control and specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or diluted specimen into the assigned well for IgG determination. **For IgM or IgA, pipette 0.050ml (50µl) of the appropriate serum reference, control or diluted sample specimen into the assigned well.**
3. Add 0.100 ml (100µl) of H.Pylori Biotinylated Conjugate Solution.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature**.**
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of Enzyme anti-h-IgG, IgM or IgA Conjugate Solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Cover and incubate for thirty (30) minutes at room temperature.
10. Repeat steps (6 & 7) as explained above.
11. Add 0.100 ml (100µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

12. Incubate at room temperature for fifteen (15) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**

14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 100 U/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material in the serum diluent. Multiply by the dilution factor to obtain the concentration of the specimen.

CALCULATIONS OF RESULTS

A reference curve is used to ascertain the concentration of anti-*H. Pylori* in unknown specimens.

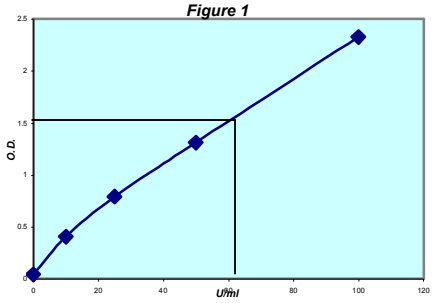
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-*H.Pylori* activity in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-*H.Pylori* activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.603 (intersects the dose response curve) at 64.0 U/ml anti-H. Pylori concentration (See Figure 1). *

EXAMPLE 1 (Typical results for IgG, M or A)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.042	0.044	0
	B1	0.046		
Cal B	C1	0.424	0.406	10
	D1	0.388		
Cal C	E1	0.810	0.791	25
	F1	0.772		
Cal D	G1	1.351	1.312	50
	H1	1.273		
Cal E	A2	2.377	2.328	100
	B2	2.279		
Sample Specimen	C2	0.163	0.172	***5.2***
	D2	0.182		
Sample Specimen	A3	1.534	1.603	***64.0***
	B3	1.671		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



Assay Performance

1) It is important that the time of reaction in each well is held constant for reproducible results.
2) Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3) Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4) If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5) The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
6) Plate readers measure vertically. Do not touch the bottom of the wells.
7) Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8) Use components from the same lot. No intermixing of reagents from different batches.
9) Very high concentration of anti-*H.Pylori* in sample specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any sample specimen with over 3.0 units of absorbance.
10) Samples, which are contaminated microbiologically, should not be used.
11) Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DRG's IFU may yield inaccurate results.
12) All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
13) It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

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