DRG® H. Pylori Rapid Screen Test (RAP-2734)

Revised 9 Sept. 2008 (Vers. 2.0) **RUO in the USA**

INDICATION

The DRG H. Pylori Combo Rapid Test Device is a rapid chromatographic immunoassay for qualitative detection of H. Pylori Specific Antibodies in serum and/or whole blood.
For in vitro use only.

SUMMARY

The infection of Helicobacter pylori (H. pylori) is associated with a variety of gastro-intestinal diseases, such as stomach ulcers, chronic active gastritis and gastrointestinal adenocarcinoma. Antibodies to H. pylori are developed in individuals infected with H. pylori as a serological response. Detecting specific antibodies to H. pylori can be used as a qualitative assay in the diagnosis of H. pylori infection, either as an adjunct to endoscopy or as an alternative measure in symptomatic patients. The H. Pylori Rapid Test is a chromatographic immunoassay (CIA) for the rapid determination of antibodies to H. pylori in serum and/or whole blood specimens. The test is to be used as an aid in the diagnosis of infection due to H. pylori.

The DRG H. pylori Combo Rapid Test is an immuno-chromatography rapid test. It is designed to detect human H. pylori antibodies concentration in sample as low as 20 U/ml within 10 minutes.

TEST PRINCIPLE

The DRG H. pylori Combo Rapid Test utilizes a lateral flow, immunochromatographic assay technology and specific antigens to H. pylori for the qualitative detection of H. pylori antibodies concentration in whole blood and/or serum. H. pylori specific antigens are precoated onto membrane as a capture reagent on the test band region. During the assay the specimen is first allowed to react with H. pylori specific antigen-gold conjugate complexes. The mixture then moves laterally on the membrane to the test region which is coated with immobilized antibodies to H. pylori. If H. pylori antibodies are present in the specimen, a color band is formed on the test (T) region. Absence of the colored band in the test region indicates a negative result. To serve as a procedural control, a colored band in the control (C) region will always appear regardless the presence of H. pylori antibodies in the whole blood and/or serum specimen.

REAGENTS PROVIDED

1. The DRG H. pylori Combo Rapid Test test device contains H. pylori antigens coated on the membrane and H. pylori antigens conjugated with colloidal gold.
   A test device is packed in a protective pouch.
2. Test Running Buffer is ready for use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Lancets (for fingerstick whole blood only)
2. Disposable heparinised capillary tubes and dispensing bulb (for fingerstick whole blood only)

ACTIVE INGREDIENTS

Coated Antibodies:
Control region: Goat anti-mouse(Ig G) polyclonal antibody
Test region: Specific H. pylori antigens
Labeled Antibodies:
Colloidal gold conjugate of specific H. pylori antigens

METHOD OF MANUFACTURE

Nitrocellulose Membrane Manufacture:
- The purified specific H. pylori antigens, diluted in phosphate buffer saline, is coated on the test region. Simultaneously, goat anti-mouse IgG polyclonal antibody, diluted in phosphate buffer saline, is coated on the control region.
- The coated membrane is dried for a minimum of 24 hours then sealed in an aluminum bag which contains silica gel desiccant.

H. Pylori colloidal gold conjugate pad manufacture:
- A buffer solution containing specific H. pylori antigens/colloidal gold conjugate is coated onto non-woven cloth sheets.
- The gold conjugate pad is dried for minimum 24 hours then sealed in an aluminum bag which contains desiccant.

Test Device Assembly:
- The coated membrane, the conjugate pad, and an absorbent pad is applied to an adhesive-coated backing.
- Waterproof label is applied over the conjugate pad and the absorbent pad, the assembled sheet of material is cut into strips. The test strips are then vacuum-dried for a minimum of 4 hours.
- The assembled test strip is sealed in an aluminum pouch along with a desiccant packet.

STORAGE AND STABILITY

The DRG H. Pylori Combo Rapid Test device is to be stored refrigerated or at room temperature (2°C to 25 °C) under dry condition for the duration of the shelf life.
The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. DO NOT FREEZE.

PRECAUTION

1. For *in vitro* use.
2. Do not use after expiration date.
3. Test device should remain sealed in pouch until ready for use.
4. Do not eat, drink or smoke in the area where the specimens or kits are handled.
5. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
6. Wear protective clothing such as laboratory coats, disposable gloves or eye protection when specimens are being tested.

7. Humidity and temperature can adversely affect results.
8. As with all screening tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a physician after all clinical findings have been evaluated.

SPECIMEN COLLECTION AND STORAGE

The DRG H. Pylori Combo Rapid Test is designed for the use of both whole blood and serum as specimen. Collect blood and coagulate blood specimen following standard clinical procedure for proper collection of specimens. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

Whole blood specimen collection:

1. Collect fresh blood specimen just prior to using the assay. Specimens must be fresh.
2. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
3. 50 µL of blood is enough for assay.

Serum specimen collection:

1. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolysed specimens.
2. Testing should be performed immediately after specimen collection. Remove serum by centrifugation. Do not leave the specimens at room temperature for prolonged periods. Specimens can be stored refrigerated at 2-8 °C up to 3 days. Freeze specimen at -20 °C or lower for long term storage.
3. Avoid repeated freezing and thawing of specimens.

Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

ASSAY PROCEDURE

Procedural Note

1. Read the package insert carefully before starting the assay.
2. Do not open foil pouch until ready to begin testing. If specimens or test device have been stored in the refrigerator, allow all specimens and device to warm to room temperature (18-25°) before testing.
3. All drops must be free falling with the reagent bottles held vertically. In order to avoid contamination of reagents, do not allow the tips of the bottles to come in contact with other surfaces.

Assay

1. Remove the device from the protective pouch and label the device with specimen identification.
2. Add 50 µL of fresh blood or 25 µL of serum to the Sample Well (for Card) or Sample Pad (for Dipstick). Than add 3 drops (150 µL) of test running buffer into the sample well or sample pad.
3. Read the result within **10 minutes**. Observe the colored band developed over the control region indicating the assay is complete.

Note: *In order to prevent an incorrect reading, do not read the test results after 15 minutes. If the test is read after 15 minutes, the intensity of the colored lines may change or a new line may appear. To avoid confusion, discard all test devices after interpreting results.*

AFTER TESTING

The specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all test devices in an approved biohazard container. Residual sample should be disposed of in a medically approve manner after the completion of all testing.

INTERPRETATION OF RESULT

Positive: Presence of two pink colored and visible bands within the test. One in control region (C) and another in test region (T), indicates presence of H. pylori antibodies with 20 U/ml or higher in whole blood and/or serum.

Negative: Presence of a single colored band in the control region (C) indicates absence of H. Pylori antibodies or the concentration of H. Pylori antibodies in whole blood and/or serum is below the detection level (20 U/ml).

Invalid: If after 10 minutes no band appears in control region, or a band appears in the test region only. An invalid result may be due to improper assay procedures or damage of the device. The assay is inconclusive and the specimen should be repeated using a new test device.

Note: Do not interpret result after **15 minutes**. The test band intensity may be weaker or stronger than that of the control band, but a very faint band in the test region indicates that the concentration of H. Pylori antibodies is near the detection level in the sample. The sample should be re-tested or confirmed with a more specific method before a positive determination is made.

QUALITY CONTROL

The DRG H. Pylori Combo Rapid Test devices have a built-in process Control Region (C). A pink band should always appear in this region regardless of the presence of any H. Pylori in sample. This pink process Control band verifies: 1) that sufficient serum volume was added, 2) that proper flow is obtained, and 3) that the reagents were working. If this band is missing, the test was not performed correctly or failed to function correctly. Such a test is inconclusive and the specimen should be repeated using a new test device.
Good laboratory practice requires use of control materials to ensure proper test device performance and reliability. DRG H. Pylori Combo Rapid Test has been standardized to EIA data. Quality control standards are available for the validation of device functionality from commercial sources such as BBI, BioAmerica and Sigma. Negative and positive controls containing H. Pylori at various concentrations are available commercially. When testing the quality control standards, use the same assay procedure as with a serum sample. It is recommended that control specimens be used for each new lot of kits.

- **Appearance:**
  All material is visually inspected before their use in manufacture.

- **Specificity:**
  10 test kits are randomly selected and tested with negative control panel. All test results must be negative.
- **Sensitivity:**
  10 test kits are randomly selected and tested with positive control panel. All test results must be positive.
- **Internal Control:**
  A colored band must appear in the control region of the membrane with each tested kit, which indicating proper performance and reagent reactivity.
- **Reproducibility:**
  Randomly selected test kits from different lots must give the same results when assaying the same sample.

PERFORMANCE CHARCATERISTICS

Sensitivity

The DRG H. Pylori Combo Rapid Test has been designed for the detection of H. Pylori antibodies in whole blood and/or serum at the detection sensitivity of 20 U/ml as indicated by the appearance of a colored band on the test region of the test device. Additionally, samples containing less than 20 U/ml may also produce a faint positive result. These sensitivity studies were performed. A H. Pylori standard panel was prepared by spiking fresh normal human pooled serum specimens with H. Pylori standard (calibrated according to Stanford H. Pylori Standard) to concentrations of 0, 5, 10, 15, 20, 25, 30 and 35 U/ml. In all, 50 test strip and 50 test card devices per standard level were tested in this procedure.

Table 1: The result of the sensitivity study for the test device

DRG H. Pylori	Concentration (U/ml)							
	0	5	10	15	20	25	30	35
Test No.	50	50	50	50	50	50	50	50
Positive	0	0	0	7	50	50	50	50
Negative	50	50	50	43	0	0	0	0

Summary of Sensitivity Results:

Concentrations of H. Pylori antibodies equal to or lower than 10 U/ml were identified as negative results for all samples. Concentrations of H. Pylori antibodies equal to or higher than 20 U/ml were identified as positive results for all samples. Therefore, the sensitivity of the DRG H. Pylori Combo Rapid Test was determined to be 20 U/ml. Occasionally, specimens containing less than 20 U/ml may also yield positive results. Specimens containing high levels of H. Pylori antibodies consistently gave positive results.

Interference Testing

The following substances were added to serum which had H. Pylori levels of 0 and 30 U/ml. None of the substances at concentration tested interfered in the DRG H. Pylori Combo Rapid Test.

Acetaminophen	20 mg/dl
Acetylsalicyclic Acid	20 mg/dl
Ampicillin	20 mg/dl
Ascorbic Acid	20 mg/dl
Atropine	20 mg/dl

Caffeine	20 mg/dl
Gentesic Acid	20 mg/dl
Glucose	2,000 mg/dl
Hemoglobin	1 mg/dl
Human Serum Protein	2,000 mg/dl
Tetracycline	20 mg/dl
Uric Acid	10 mg/dl

Precision

Summary of Within-Lot Reproducibly

Three batches of normal human serum demonstrated to be negative for H. Pylori were spiked with H. Pylori antibodies to levels of 0 and 30 U/ml. Single lots of the DRG H. Pylori Combo Test devices were used to test for reproducibility of results. Five devices in each of three batches were tested per concentration. Results of within-lot reproducibility analytical studies clearly showed excellent repeatability for all 3 batches of positive and negative serum samples, using one lot of the DRG H. Pylori Combo Rapid Test devices. The negative and positive values were correctly identified in 100% of cases.

Summary of Inter-Lot Reproducibility

To test inter-lot reproducibility, normal human serum known to be negative for H. PYLORI was spiked with H. PYLORI to levels of 0, and 30 U/ml. The samples were blinded by dispensing the mixed solutions into letter coded labeled vials, and were used to test three (3) lots of Test devices. Twenty (20) devices per concentration for each lot were tested. The results of these tests clearly demonstrate that there is no appreciable inter-lot variation when testing both positive and negative spiked samples across three (3) different lots of DRG H. Pylori Combo Rapid Test devices. The negative and positive values were correct in 100% of cases.

Kit Comparisons

Assay Comparisons & Equivalency

Accuracy and equivalency comparisons of both the DRG H. Pylori Combo Rapid Test Card and Test Strip were evaluated against 133 individual in-house laboratory samples, as well as against 120 individual external EIA-certified clinical laboratory samples. The results have been tabulated below.

Table 2. DRG® H. Pylori strip vs. BioAmerica EIA

Strip	EIA (+)	EIA (-)	Row Totals
(+)	83	5	88
(-)	3	162	165
Col. Totals	86	167	253

When compared to EIA Assay, the percent agreement with DRG H. Pylori Combo Test Strip positive samples and 83/86 or 96.5%. Negative samples recovered at 162/167 or 97.0%, while the overall relative accuracy obtained was 245/253 or 96.8%.

Table 3. DRG® H. Pylori Card vs. BioAmerica EIA

Card	EIA (+)	EIA (-)	Row Totals
(+)	83	6	89
(-)	2	162	164
Col. Totals	85	168	253

When compared to EIA Assay, the percent agreement with DRG H. Pylori Combo Rapid Test Card positive samples and 83/85 or 97.6%. Negative samples recovered at 162/168 or 96.4%, while the overall relative accuracy obtained was 245/253 or 96.8%.

Limitations

- The H. Pylori Rapid Test is not reusable. The test works only if the instructions are followed precisely. Do not use the test after the expiration date shown on the package or if the moisture absorbent pack is wet.
- The result should be used only as an aid in diagnosis and should not be interpreted as a final diagnoses. To confirm diagnosis of gastritis and/or peptic ulcers, clinical findings should be considered.
- Serum from patients infected with C. jejuni may have a low cross-reactivity with this test.
- A test result read after 15 minutes may not be consistent with the original reading obtained within the 10 minutes test period.

Expected Values

The cut off value of DRG H. Pylori Combo Rapid Test is generally agreed at 20 U/ml. It has been compared with a leading commercial H. Pylori EIA test. The correlation between these two systems is over 96.8% of both test formats.

BIBLIOGRAPHY

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- Evans D. J., et al. A sensitive and specific serologic test for detection of Campylobacter pylori infection. Gastroenterology 96: 1004-1008, 1989.
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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..