DRG® Rotavirus Antigen (stool) ELISA (EIA-3509)

Not for Sale in the USA

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Intended Use

The Rotavirus Ag (Stool) ELISA is an *in vitro* procedure for the qualitative determination of rotavirus antigen in feces. It is a double antibody (sandwich) ELISA using a polyclonal anti-rotavirus antibody to capture the antigen from the stool supernatant. A second anti-rotavirus monoclonal antibody is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color, following the addition of the chromogen and peroxide, indicates the presence of rotavirus antigens being bound by the anti-rotavirus antibodies.

Summary

Rotavirus is one of the leading causes of gastroenteritis in children throughout the world.[2,5,7,9,11-17] Rotavirus infections are most common in infants, but repeated, asymptomatic infections are believed to occur in adults.[1,6] Rotavirus infection occurs by the fecal-oral route.[1] After an incubation period of 1 - 2 days, the onset of gastroenteritis is sudden. Symptoms can last from 4 - 5 days[6] and range from diarrhea and vomiting, to fever, and occasional abdominal pain.[1,6] Loss of fluids and electrolytes can lead to severe dehydration,[1,5,6] hospitalization, and even death.[1]
Rotavirus infection appears to peak during the winter season, except in countries with tropical or subtropical climates, where the virus is present year around.[17]
There have been many efforts to develop rapid and economical methods for detecting rotavirus antigen in stool.[3,9] Simple to perform enzyme-linked immunosorbent assays (ELISA) and latex agglutination kits have been developed.[4-8] These antigen-detection systems have become the test of choice in the clinical setting.[5,10,13]

Principle of Procedure

During the first incubation, rotavirus antigens present in the stool supernatant are captured by antibodies attached to the wells.

The second incubation adds an additional anti-rotavirus antibody that "sandwiches" the antigen.
The third incubation attaches horseradish peroxidase to the sandwich.
After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

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Reagents

	Description
Test Strips	Microwells containing anti-rotavirus polyclonal antibodies, 96 test wells in a test strip holder.
Reagent 1	One (1) bottle containing 11 mL anti-rotavirus monoclonal antibodies with blue dye and Thimerosal.
Reagent 2	One (1) bottle containing 11 mL anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.
Positive Control	One (1) vial containing 2 mL of diluted rotavirus antigen in buffer with Thimerosal.
Negative Control	One (1) vial containing 2 mL of buffer with Thimerosal.
Chromogen	One (1) bottle containing 11 mL of tetramethylbenzidine (TMB) and peroxide.
Wash Concentrate (20X)	Two (2) bottles containing 25 mL of concentrated buffer and Thimerosal.
Stop Solution	One (1) bottle containing 11 mL of 1 M phosphoric acid.

Precautions

Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
Do not add azides to the samples or any of the reagents.
Controls and some reagents contain Thimerosal as a preservative.
Treat all reagents and samples as potentially infectious materials.

Storage Conditions

Reagents, strips and bottled components:
Store between 2 – 8 °C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation

Wash/Dilution Buffer

Remove cap and add contents of one bottle of wash concentrate to 475 mL DI water. Transfer contents of diluted wash buffer into a squeeze bottle.

Test Samples

Collection of Stool (Feces)

Stools should be collected in clean containers.
Unpreserved samples should be kept at 4 °C and tested within 24 hours of collection.
Samples that cannot be tested within this time should be frozen at -20 °C until used. Freezing the specimens does not adversely affect the test.
All dilutions must be made with the diluted wash buffer.

Preparation of Sample

Fresh/Frozen Stools

Thaw frozen stools. Prepare a **1:5 dilution** of stool by adding 1 gram (approximately the size of a pea) to 4 mL of diluted wash buffer. Mix well and allow the heavy particulates to settle.
For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).
Note: Do not formalize samples prior to testing.

Performance Of Test

Materials Provided

Rotavirus Ag (Stool) ELISA Kit

Materials Required But Not Provided

Pipettes
Squeeze bottle for washing strips
Reagent grade (DI) water
Graduated cylinder

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters

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Procedure

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µL of the negative control to well #1 and 100 µL of positive control to well #2 (use both as undiluted).
3. Add 100 µL of the stool supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash.*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 5 minutes, then wash*.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 5 minutes, then wash*.
9. Add 2 drops Chromogen to each well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450 nm and 620-650 nm. Zero the reader on air.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

Interpretation of Results

Interpretation of Results – Visual

REACTIVE: Any sample well that has distinct and substantial yellow color.
NON-REACTIVE: Any sample well that does not have distinct yellow color.

NOTE: The negative control, as well as some samples, may show some slight color.

Interpretation of Results – ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

REACTIVE: Absorbance reading of 0.15 and above indicates the sample contains rotavirus antigen.
NON-REACTIVE: Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of rotavirus antigen.

Test Limitations

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

Expected Results

Normal healthy individuals should be free of rotavirus and should test negative.
A positive reaction indicates that the patient is shedding detectable amounts of rotavirus antigen. Incidence of rotavirus infection varies significantly between populations, season of the year, and geographic regions. No expected prevalence level can be assumed.

Performance Characteristics

Study #1 – vs. Commercial Lateral Flow
N=54

	Lateral Flow +	Lateral Flow -
DRG ELISA +	19	1
DRG ELISA -	0	34

Sensitivity – 19/19 = 100%
Specificity – 34/35 = 97%

Quality Control

The use of a positive and negative control allows easy validation of kit stability.
For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

Troubleshooting

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

References

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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
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
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