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ProSpecT Astrovirus EN

Microplate Assay

REF R240196 Σ 96

1. INTENDED USE

The ProSpecT™ Astrovirus test is a qualitative amplified enzyme immunoassay for the detection of astrovirus in human faeces.

2. SUMMARY

Astroviruses (family Astroviridae) are small (28nm), round, positive-stranded RNA viruses which are characterised by their distinct star like appearance[1,2]. At least eight human serotypes have now been identified[3].

Astroviruses are now recognised as a common cause of viral gastroenteritis in young children worldwide[3,4]. The incubation period is between 3-4 days and symptoms usually last 2-3 days but may persist for up to 12 days in immunocompetent individuals and significantly longer in immunocompromised patients. Recent reports indicate that the incidence of astrovirus infection may have been greatly underestimated due to limitations of available diagnostic methods. These studies suggest that after rotavirus, astrovirus may be the second most common cause of infantile viral gastroenteritis and the third most common pathogen behind Salmonella sp and rotavirus in community acquired diarrhoea[5,6,7]. Astroviruses have been associated with outbreaks of gastroenteritis in hospitals, families, communities and adult institutes. Large food borne outbreaks have also been reported in Japan[8,9].

Until recently astrovirus infection has been traditionally diagnosed by electron microscopy (EM). EM relies on good morphological preservation of the specimen and the skill of the operator. However, because only 10% of astrovirus particles possess the characteristic star like morphology, detection can often be difficult leading to mis-diagnosis[10,11]. In addition because of the small size of astroviruses they are not as easily observed as rotaviruses and can be confused with the appearance of other small round structured viruses. Therefore the use of EM morphology alone as a diagnostic criterion has resulted in an underestimation of the incidence of these viruses. Although improvements have been made in culture techniques[12,13] this is rarely the method of choice for routine diagnosis because of lack of sensitivity. Recent developments in diagnostic procedures include dot blot hybridisation, PCR and EIA[11,14,15]. The availability of specific antiserum raised against group, genus or type specific epitopes has resulted in the development of immunoassays for direct antigen detection[15]. These assays have been shown to have increased sensitivity compared with EM and offer a more economical, reliable and rapid screening method[7].

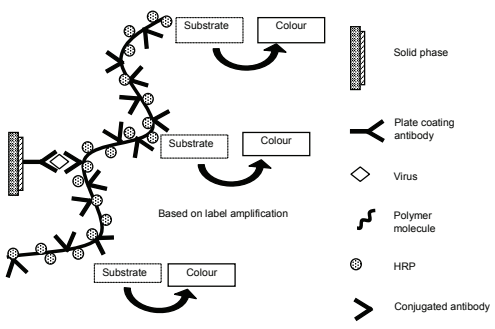
Recently new generation amplified immunoassays incorporating label and signal amplification technology have demonstrated improved sensitivity comparable with molecular amplification methods[16,17].

ProSpecT Astrovirus is a qualitative amplified enzyme immunoassay for the rapid detection of astroviruses in human faecal specimens. The test utilises a combination of genus specific monoclonal and polyclonal antibodies in conjunction with label amplification to detect all known strains of human astroviruses in a microplate based immunoassay.

3. PRINCIPLE OF THE TEST

The ProSpecT Astrovirus Microplate Assay utilises a polyclonal antibody and a dextran polymer conjugate with a high incorporation of enzyme and antibody molecules in a solid phase immunoassay to detect astrovirus antigen. Breakapart microwells are coated with genus specific astrovirus polyclonal antibody. Faecal suspension is added to the microwell and astrovirus antigen present in the specimen binds to the solid phase. The dextran conjugated genus specific monoclonal antibody binds to astrovirus antigen captured on the solid phase, thereby linking the conjugate polymer complex incorporating the multiple enzyme molecules (label amplification). A chromogen is used to detect bound enzyme and results in a colour change which is stopped by the addition of acid. Colour intensity significantly above background levels is indicative of the presence of astrovirus antigen in the specimen.

Schematic diagram of the ProSpecT Astrovirus assay principle



4. SYMBOL DEFINITIONS

The following symbols have been used throughout the product information.

REF	Product code and catalogue number
	Consult the instructions for use
Σ N	Contains sufficient for 'N' tests
	Manufactured by
IVD	*In vitro* diagnostic medical device
	Use by
LOT	Batch Code
	Storage temperature limitations

5. KIT CONTENTS

Σ 96 - Each kit contains sufficient materials for 96 determinations. - The shelf life of the kit is as indicated on the outer box label.

Store all components at 2-8°C.

Before use bring all reagents to room temperature (20-25°C) and mix gently. Store all unused reagents at 2-8°C after use.

All reagents except the Wash Buffer are supplied ready to use. If reagents are poured out for use with multichannel pipettes do not pour excess reagent back into the bottle.

Instructions for use
Transfer pipettes
Microplate cover
Certificate of contents
Procedure card

MICROTITRATION PLATE One 96 well microtitration plate of twelve, 8 microwell break-apart strips coated with an Astrovirus specific rabbit polyclonal antibody.

A resealable foil pouch containing desiccant is provided for storage of unused microwells. Microwells may be used for up to 16 weeks after initial opening, provided they are stored correctly in the pouch.

One bottle of each of the following:

SAMPLE DILUENT 120ml Sample Diluent: tris buffered saline containing antimicrobial agent and red dye

CONTROL + 4ml Positive Control: inactivated Astrovirus type 1 (non-infectious recombinant protein) in buffer containing antimicrobial agent

CONTROL – 4ml Negative Control: tris buffered saline containing antimicrobial agent and red dye

CONJUGATE 12ml polymer Conjugate: Astrovirus genus specific monoclonal antibody conjugated to a dextran polymer backbone linked to multiple horseradish peroxidase (HRP) molecules in a buffered protein solution containing antimicrobial agent and blue dye

WASH BUFFER (x10) 120ml Wash Buffer concentrate (x10): phosphate buffered solution containing antimicrobial agent and detergent

Dilute 10x Wash Buffer concentrate by adding 1 part concentrate to 9 parts distilled or deionised water. Diluted Wash Buffer is stable for up to 30 days when stored at 2-8oC

SUBSTRATE TMB 12ml Substrate: 3,3'-5,5'-tetramethylbenzidine in a mildly acidic buffer

STOP SOLUTION 12ml Stop Solution: 0.46mol/L sulphuric acid

6. PRECAUTIONS

IVD - For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

6.1. The Positive Control contains Astrovirus type 1 recombinant protein which must be handled and disposed of as though potentially infectious.

6.2. Stop Solution contains sulphuric acid (0.46mol/L).

6.3. Wash Buffer contains potential skin sensitiser (<1% v/v). Avoid skin contact. Wear disposable vinyl or nitrile gloves.

6.4. Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.

6.5. Do not pipette materials by mouth.

6.6. Wear disposable gloves while handling clinical specimens and reagents. Always wash hands after working with infectious materials.

6.7. Dispose of all clinical specimens in accordance with local legislation.

6.8. ProSpecT Astrovirus reagents contain a proprietary anti-microbial agent which presents no hazard to the user if normal laboratory safety precautions are followed.

ANALYTICAL PRECAUTIONS

6.9. Components must not be used after the expiry date printed on the labels. Do not mix or interchange the following reagents as performance may be compromised:- Plate, Conjugate and Controls.

6.10. The following common reagents may be used across the ProSpecT product range:- Wash Buffer, TMB Substrate and Stop Solution.

6.11. Avoid contamination of reagents.

6.12. When using the dropper bottle method ensure all controls and reagents are added in the same way. (Performance of the kit may be adversely affected if a combination of pipette and dropper methods are used).

6.13. Use separate disposable pipettes or pipette tips for each sample, control or reagent (if not using dropper bottles) in order to avoid cross contamination of either samples, controls or reagents which could cause erroneous results.

6.14. Store deionised or distilled water for dilution of concentrated reagent in clean containers to prevent microbial contamination.

6.15. Avoid contamination with metal ions and oxidising agents.

6.16. Do not use substrate showing a blue colour prior to its addition to the microwells.

6.17. Protect Conjugate and Substrate from light.

6.18. Microwells cannot be re used.

6.19. Unused working strength Wash Buffer can be stored for up to 30 days at 2-8°C for subsequent use. When not in use Wash Buffer reservoirs should be rinsed in deionised or distilled water and left to dry.

6.20. Manual or automated washing equipment must be free of microbial contamination, be correctly calibrated and maintained according to the manufacturer's instructions.

6.21. When using reagent dropper bottles, hold the bottles vertically with the nozzle approximately 5mm above the microwell. Squeeze the bottle gently and ensure that the drops fall freely into the microwells without touching the sides of the well. Avoid contamination of all the dropper nozzles.

7. COLLECTION OF FAECAL SPECIMENS

Faecal specimens should be collected as soon as possible following the onset of symptoms.

Faecal specimens for direct testing should be collected into containers that do not contain media, preservatives, animal sera, metal ions, oxidising agents or detergents, as all of these additives may interfere with the ProSpecT Astrovirus test.

If rectal swabs are collected they must contain sufficient faecal material to obtain a 10% suspension of faeces (see section 8).

Specimens may be stored for 8 days at 2-8°C prior to testing. For long term storage of faecal specimens, store at -20°C.

8. PROCEDURE

REQUIRED MATERIALS PROVIDED

See Kit Contents, section 5

MATERIALS REQUIRED BUT NOT PROVIDED

Faecal specimen collection containers

Clean screw-capped disposable containers (minimum 3ml capacity) for preparation of faecal specimen

Clean absorbent paper (onto which microwells can be tapped dry)

Precision micropipettes and disposable tips to deliver 50µl, 100µl and 1000µl

Waste discard container with suitable fresh disinfectant

Timer

Wash bottle for Wash Buffer

Distilled or deionised water

OPTIONAL MATERIALS NOT PROVIDED

Microplate reader capable of reading 450nm (with 620-650nm reference optional)

Vortex mixer with plate adapter or plate shaker incubator

Automated plate washer or suitable equipment for washing 8 microwell strips

PROCEDURE

8.1. Open the foil pouch, remove the required number of microplate strips and place into a microplate strip holder. Use one well for the Negative Control and one well for the Positive Control. If using less than 8 wells break off the required number of wells from a strip and return the unused wells to the foil pouch with desiccant. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO STORAGE AT 2-8°C.

DILUTON OF FAECAL SAMPLES

Add 1ml of Sample Diluent to a suitable labelled container and use to prepare a 10% suspension or dilution of faecal specimen by addition of approximately 0.1g of solid faeces (small pea-sized portion) or approximately 100µl of liquid faeces using transfer pipettes. Mix thoroughly and leave transfer pipette in container for later use.

Rotate rectal swabs in 1ml of Sample Diluent whilst squeezing swab against the side of the container to release faecal material. Mix thoroughly.

Faecal suspensions previously preserved in formalin should be further diluted in ProSpecT Astrovirus Sample Diluent to prepare a 10% suspension of faeces before testing.

Specimens suspended/diluted in ProSpecT Astrovirus Sample Diluent may be stored at 2-8°C for up to 8 days prior to testing.

NOTE: Faecal specimens prepared in ProSpecT Adenovirus, ProSpecT Rotavirus and ProSpecT Norovirus Sample Diluent can also be tested in the ProSpecT Astrovirus test. Alternative Sample Diluents have not been validated for use.

8.2. Add 2 drops (or 100µl) of each diluted specimen, Negative Control or Positive Control to the separate microwells. At least one Negative Control and one Positive Control should be included in each batch of tests.

8.3. After addition of all specimens and controls, use a micropipette to add 100µl of Conjugate to each microwell and mix gently for 20-30 seconds

8.4. Cover the plate and incubate at 20-30°C for 60 +/- 5 minutes

8.5. Shake out or aspirate the contents of the wells. Wash by completely filling each well with diluted Wash Buffer (~350-400µl per well). Shake out or aspirate all fluid from wells after each wash. Wash a total of 5 times. After the last wash remove contents and strike plate on clean paper towels or aspirate. If using an automated washer, this should be programmed to complete 5 wash cycles. Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer.

8.6. Add 2 drops (or 100µl) of Substrate to each microwell.

8.7. Cover the plate and incubate at 20-30oC for 10 minutes

8.8. Microwells can be read visually immediately after the second incubation (See sections 9 and 10).

8.9. Alternatively, stop the Substrate reaction by adding 2 drops (or 100µl) of Stop Solution to each microwell. Ensure thorough mixing of the microwells before reading the results. The coloured product is stable for up to 30 minutes after addition of Stop Solution.

8.10. Read spectrophotometrically at 450nm (see sections 9 and 10).

9. QUALITY CONTROL

At least one Positive and one Negative Control must be included each time the test is performed.

VISUAL DETERMINATION

All Negative Control microwells should be colourless. If this is not the case the test results should not be determined visually.

The Positive Control microwell should show a distinct blue colour clearly distinguishable from the Negative Control.

SPECTROPHOTOMETRIC DETERMINATION

The Negative Control value, or mean of the Negative Control values, should be less than 0.150 absorbance units.

The Positive Control value must be greater than 0.500 absorbance units

10. RESULTS

VISUAL DETERMINATION

Any specimens giving a blue colour more intense than that of the Negative Control is positive. Any specimen giving colour equal to or less than the Negative Control is negative. Microwells in which the colour intensity is difficult to interpret when compared to the Negative Control should be read photometrically after addition of Stop Solution or retested.

SPECTROPHOTOMETRIC DETERMINATION

10.1. The microwells should be read photometrically within 30 minutes of addition of the Stop Solution.

10.2. Mix the contents of the microwells and read the absorbance of each microwell using a spectrophotometer set at 450nm. Ensure the bottoms of the microwells are clean before reading. The reader should be blanked on air before the plate is scanned.

10.3. If the spectrophotometer allows for the use of a reference wavelength (at 620 to 650nm), dual wavelength reading should be performed.

10.4. Calculate the cut-off value by adding 0.100 absorbance units to the Negative Control value, or mean value when more than one Negative Control is included.

10.5. Interpret the test results:

Positive: clinical sample absorbance value > the cut-off value

Negative: clinical sample absorbance value < the cut-off value

Equivocal: clinical sample absorbance value within 0.010 absorbance units of the cut-off value. These samples should be retested or the patient resampled

11. PERFORMANCE LIMITATIONS

11.1. The validity of results with the ProSpecT Astrovirus Microplate Assay depends on the control reactions performing as expected. See Quality Control section 9.

11.2. A negative result does not exclude the possibility of astrovirus infection in the patient. Failure to detect astrovirus may be a result of factors such as collection of specimen at an improper time in the disease when too few virions are present, improper sampling, handling of the specimen.

11.3. ProSpecT Astrovirus test detects genus specific viral proteins present in human serotypes of astrovirus. The test cannot be used to differentiate between serotypes of astrovirus, or to detect non-human astrovirus serotypes.

11.4. The reagents are provided at fixed working concentrations. Test performance will be adversely affected if reagents are modified or stored under conditions other than those detailed in section 5.

11.5. The use of ProSpecT Astrovirus Microplate Assay for direct testing of specimens other than faecal specimens is not recommended as either the presence of insufficient antigen or inadequate specimen collection may cause misleading negative results. A positive result in faecal specimens, in association with diarrhoea, is highly suggestive of astrovirus gastroenteritis.

11.6. A positive result does not preclude the presence of other enteric pathogens. Whilst the relationship between astrovirus and gastroenteritis is well established,3,4 concurrent infection with other microbial pathogens is possible.

11.7. Meconium samples have not been validated for use with the ProSpecT Astrovirus Microplate Assay.

11.8. Test results should be interpreted in conjunction with information available from epidemiological studies, clinical assessment of the patient and other diagnostic procedures.

12. EXPECTED VALUES

Positivity rates may vary according to the prevalence of astrovirus in different populations, geographical location, specimen collection, handling, storage, and transportation of specimens, cell culture system used and the general health environment of the patient populations under study.

Astrovirus occurs worldwide and peaks during winter/spring in temperate zones[18,19]. Infants below the age of 1 year are commonly affected and by 5 years of age more than 80% of children show serological evidence of past infection[20].

Of the eight human serotypes, type 1 is by far the most prevalent (>60%) in the UK and evidence indicates its frequency increases in alternate years[21]. Dual infections with other enteric pathogens, particularly rotavirus, are common[22].

13. PERFORMANCE CHARACTERISTICS

CLINICAL STUDIES

ProSpecT Astrovirus test was independently evaluated in a clinical study performed at a UK reference centre.

The study was conducted on faecal specimens collected from 94 patients presenting with gastroenteritis (51 female, 42 male, 1 unknown, age range 0.1–88.4 years). The aim of the study was to compare the performance of the ProSpecT Astrovirus with the previously validated IDEIA™ Astrovirus. Electron Microscopy was used to confirm the status of positive specimens.

The results of the study are shown in table 13.1.

CLINICAL PERFORMANCE

The ProSpecT Astrovirus test showed a sensitivity of 100% and specificity of 98.3%.

Table 13.1 Sensitivity and Specificity of ProSpecT Astrovirus vs IDEIA Astrovirus

		IDEIA Astrovirus	
		+	-
ProSpecT Astrovirus	+	35	1[a]
	-	0	58
Sensitivity		100% (35/35)	
Specificity		98.3% (58/59)	

[a] Sample confirmed positive by Electron Microscopy

PRECISION

Intra-assay precision

The intra-assay precision was assessed using three faecal specimens representing low, medium and high positive specimens. Each control preparation was tested 24 times in eight assays performed by two operators. The mean absorbance and mean coefficient of variation for intra assay was determined.

Table 13.2 Mean intra-assay precision of the ProSpecT Astrovirus test

Specimen	Mean AU	Mean %CV
Low	0.454	5.3
Medium	0.973	4.4
High	1.568	4.2

Inter-assay precision

The inter-assay precision was assessed using three faecal specimens. Each dilution was tested in eight assays performed by two operators and the mean absorbance values and coefficient of variation determined.

Table 13.3 Inter-assay precision of the ProSpecT Astrovirus test

Specimen	Mean AU	Mean %CV
Low	0.454	5.7
Medium	0.973	5.1
High	1.568	3.7

CROSS REACTIVITY

The following micro-organisms were tested and shown to be negative in the ProSpecT Astrovirus test. Cross reactivity tests were performed either on clinical specimens for which the microbial status had been determined, or on laboratory cultures of known organisms containing approximately 10^7-10^8 viable organisms per ml. The source of micro-organisms is referenced in the key below:

Viruses

Adenovirus[a] Calicivirus[a]

Rotavirus[a]

Bacteria

Acinetobacter sp *Listeria monocytogenes*

Aeromonas hydrophila *Neisseria gonorrhoea*

Bacteroides fragilis *Peptococcus sp*

Campylobacter coli *Peptostreptococcus sp*

Citrobacter freundii *Proteus sp*

Clostridium difficilea *Pseudomonas aeruginosa*

Clostridium perfringens *Salmonella typhimurium*

Enterobacter cloacae *Serratia marcescens*

Enterococcus faecalis *Shigella sonnei*

Escherichia coli *Staphylococcus aureus*

Gardnerella vaginalis *Staphylococcus epidermidis*

Haemophilus influenzae *B-haemolytic streptoccocus group A*

Klebsiella sp *Veillonella sp*

Lactobacillus sp

Other micro-organisms

Candida albicans

Key:

[a]Micro-organisms present and tested in faeces

All other micro-organisms were grown and tested in broth culture.

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