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

11. Avoid contamination of reagents.

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

13. Use separate disposable pipettes or pipette tips for each sample. Also use separate pipette tips for negative controls in order to avoid cross contamination of same samples, reagents or controls which could give rise to false positive results.

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

15. Avoid contamination with metal ions and oxidising agents.

16. Do not use substrate solution showing a blue colour prior to its expiry date.

17. Protect Conjugate and Substrate from light.

18. Microplates cannot be re-used.

19. Unused working strength Wash Buffer can be stored for up to 30 days at 4°C for subsequent use. When not in use Wash Buffer reservoirs should be rinsed in distilled or deionised water and left to dry.

20. Manual or automated washing equipment must be free of microbial contamination, be correctly calibrated and maintained.

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.

6. 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 preservatives, animal sera, antibiotics or antiviral agents. In addition because only 10% of astrovirus particles are present in the faeces a 10% suspension of faeces before testing is recommended.

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

Specimens may be stored for up to 24 hours prior to testing. For long term storage of faecal specimens, store at -20°C.

8. PROCEDURE

4. SYMBOL DEFINITIONS

The following symbols have been used throughout the product information.

- Product code and catalogue number
- Manufactured by
- At an diagnostic/medical device
- Use by
- Batch Code
- Storage temperature limitations

1. INTENDED USE

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

2. SUMMARY

Astroviruses (family Astroviridae) are small (28nm), round, non-enveloped viruses which are transmitted by the faecal-oral route. Many astrovirus infections are asymptomatic but significant in some cases, short term vomiting and diarrhoea may occur. Astroviruses are now recogised as a common cause of viral gastroenteritis in hospitals, families, communities and adult care facilities.

A confirmed or suspected case of gastroenteritis in a person under the age of 12 years and a known person under the age of 12 years in an epidemiologically linked household or the same household, who presents with vomiting and diarrhoea, is highly suggestive of astrovirus infection.

The ProSpecT™ ProspecT Astrovirus Microplate Assay is a sensitive and specific enzyme immunoassay for the detection of astrovirus in human faeces. The assay utilises a combination of a specific, genus, and type specific monoclonal antibodies in conjunction with a four layer amplification technology to dramatically increase sensitivity.

The ProSpecT™ ProspecT Astrovirus Microplate Assay utilizes a polyclonal antibody and a detergent polymer conjugate with a high incorporation of enzyme and antibody molecules in a solid phase immunosay to detect astrovirus antigen. Breakthrough microwells are coated with gestus specific astrovirus polyclonal antibody. fluorescent-labelled enzyme substrate and astrovirus antigen present in the specimen binds to the solid phase.

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For in vitro diagnostic use. Anyone performing an assay on 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 information for labelling for information on potentially hazardous compounds.

6. PRECAUTIONS

1. Open the foil pouch, remove the required number of twelve, 96 well microwell plates and store in the pouch.

2. Stop Solution is dispensed from a bottle calibrated to ensure complete filling and emptying of each microwell. The bottle should be inverted and tapped on absorbent paper to dislodge any air bubbles.

3. Add 2.0 µl (or 100 µl) of Substrate to each microwell.

4. Mix the contents of the microwells and read the absorbance at 450 nm (with 620 to 650 nm wavelength) using a microplate reader.

5. Ensure the bottoms of the microwells are clean before reading the absorbance.

6. The microwells should be read photometrically within 30 minutes of the Stop Solution being added.

7. When using reagent dropper bottles, hold the dropper 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 wells. Avoid contamination of all the dropper nozzles.

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

9. The Negative Control, or mean of the Negative Controls, photometric reading is equivalent or less than the Negative Control.

10. The photometric reading must be greater than 0.50 absorbance units.

11. The values, should be less than 0.150 absorbance units.

12. One 96 well microwell plate of twelve, 3.0 µl (or 30 µl) of Substrate has been used in the evaluation of EM morphology. 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 this method18,19,20,21 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 Southern Blotting. The availability of specific antisera against group, genus or type specific epitopes has resulted in the development of immunological techniques for the detection of astrovirus, which have increased sensitivity compared with EM and are more economical, reliable and rapid screening methods.

Recently new generation amplification immunosay incorporating label and signal amplification technology have demonstrated improved sensitivity compared with molecular amplification methods.

13. The Negative Control and each reagent must be tested by running the same controls or reagents which could cause erroneous results.

14. Stop Solution is dispensed from a bottle calibrated to ensure complete filling and emptying of each microwell. The bottle should be inverted and tapped on absorbent paper to dislodge any air bubbles.

15. Add 2.0 µl (or 100 µl) of Substrate to each microwell.

16. The Negative Control contains Astrovirus type 1 recombinant protein which must be handled and disposed of as though they are infectious.

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

18. Microplates cannot be re-used.

19. Mix the contents of the microwells and read the absorbance of each microwell using a microplate reader set at 450nm.

20. The results should be interpreted in conjunction with the results of other diagnostic procedures.

21. Epitope defined as a small size area on the surface of the protein which must be handled and disposed of as though they are infectious.

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

23. The Negative Control, or mean of the Negative Controls, photometric reading is equivalent or less than the Negative Control.

24. The photometric reading must be greater than 0.50 absorbance units.

25. The values, should be less than 0.150 absorbance units.

26. One 96 well microwell plate of twelve, 3.0 µl (or 30 µl) of Substrate has been used in the evaluation of EM morphology. 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 this method18,19,20,21 this is rarely the method of choice for routine diagnosis because of lack of sensitivity.

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12. EXPECTED VALUES

13. PERFORMANCE CHARACTERISTICS

14. BIBLIOGRAPHY

Table 13.1  Sensitivity and Specificity of ProSpecT Astrovirus vs IDEIA Astrovirus

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

Table 13.3  Inter-assay precision of the ProSpecT Astrovirus test

CROSS ACTIVITY

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 negative result was confirmed, or on laboratory cultures were performed either on clinical specimens for which the negative result was confirmed.

Table 13.4  Cross reactivity of the ProSpecT Astrovirus test

Other micro-organisms: Candida albicans

Key:
1. Micro-organisms present and tested in faeces
2. All other micro-organisms were grown and tested in broth culture.