

Evaluation of seven commercial assays for detecting group A rotavirus antigen.

PHLS East Virology Advisory Committee – internal document

### **Specimens**

A panel of 203 faecal specimens was used in the evaluation including 100 presumptive rotavirus-positive specimens received for rotavirus genotyping by the Cambridge laboratory and 103 specimens, negative in the IDEIA Rotavirus ELISA and supplied by the Norwich laboratory.

### **Assays**

#### **ELISAs**

Three conventional ELISAs were evaluated. The IDEIA Rotavirus assay (DAKO, Ely, UK,) the RIDASCREEN® assay (r-biopharm, Darmstadt, Germany) and the Premier Rotaclone® (Meridian Diagnostics, Cincinnati, USA). All assays were performed according to the manufacturers' instructions.

The IDEIA Rotavirus assay utilises a polyclonal antibody in a solid-phase sandwich enzyme immunoassay to capture Group A rotavirus-specific antigen. The captured antigen is detected using a polyclonal antibody conjugated to HRPO. The RIDASCREEN® and the Premier Rotaclone® assays use the same principle but the capture and conjugated antibodies are rotavirus VP6-specific monoclonal antibodies.

### **Rapid devices and test systems**

Four rapid devices or test systems were evaluated. The Murex Rotavirus Latex Test (Murex, Dartford, UK), the RotaStrip Quick Test (Coris, Namur, Belgium), the RotaStick One-Step Test (Novamed Ltd, Israel) and the ImmunoCard® STAT rotavirus assay (Meridian Diagnostics, Cincinnati, USA). All assays were performed according to the manufacturers' instructions.

The Murex Rotavirus Latex Test is a rapid slide agglutination test in which latex particles coated with rotavirus-specific antibodies agglutinate in the presence of rotavirus antigens. A control latex coated with non-immune rabbit serum is used to identify non-specific agglutination. The RotaStrip Quick test and the RotaStick One-Step Test both utilise rotavirus-specific monoclonal antibodies conjugated to colloidal gold particles to form antigen-antibody complexes which migrate along the test strip and are captured by a rotavirus-specific polyclonal antibody immobilised on the strip resulting in a visible band. A control band which detects mouse IgG is used to indicate proper migration of the sample along the strip. The ImmunoCard® STAT rotavirus assay is an immunochromatographic system utilising gold particles coated with monoclonal antibodies to rotavirus. The diluted sample is added to the test device and mobilizes the coated gold particles which migrate along the membrane through the test zone containing polyclonal anti-rotavirus antibodies and the control zone containing anti-mouse IgG antibody. The control band is used to indicate proper migration of the sample through the test device.

## **RT-PCR**

cDNA obtained through random priming and reverse transcription of extracted RNA was tested in PCRs to amplify the VP7 gene and part of the VP4 gene according to the method of Iturriza et al, 1999.

## **Results**

Table 1 lists the results of all tests performed on the faecal samples. Three of the 100 samples sent for rotavirus genotyping were negative in the RT-PCRs and 2 of the 103 samples selected as negative as a result of testing in the IDEIA assay were positive in the RT-PCRs. This gives totals of 99 positive and 104 negative specimens tested in the evaluation.

## Commercial assays

Assay	Sample preparation	Test time	Test temp	No of tests in kit	Controls
Rotaclone	100µl faeces in 1 ml kit buffer	70 min	ambient	48	Pos included in kit Neg is sample diluent
Dako IDEIA	100µl faeces in 1 ml kit buffer	70 min	ambient	98	Pos included in kit Neg is sample diluent
Ridascreen	100µl faeces in 1 ml kit buffer	70 min	ambient	95	Pos included in kit Neg is sample diluent
Immunocard	25µl faeces in 350µl kit buffer	10 min	ambient	30	Conjugate control on each device Pos included in kit
Latex	10% in buffer	2 min	ambient	25	Control latex included Pos included in kit
Rotastrip	5% in buffer	>3 < 15 min	ambient	25	Conjugate control on each strip
Rotastick	100µl in 600µl kit buffer	5 min	ambient	25	Conjugate control on each strip

Table 2 is a comparison of the results of the conventional ELISAs and Table 3 the results of the rapid devices or tests systems.

Table 4 shows the sensitivity and specificity of all assays calculated from the results of this evaluation.

Table 4 Sens. and specif

		ELISAs			Rapid Devices			
		Rotaclone	Dako IDEIA	Ridascreen	Immunocard	Latex	Rotastrip	Rotastick
True positive	No	99	99	99	99	99	99	99
False negative	No	3	2	3	3	11	5	5
True negative	No	104	104	104	79	104	104	104
False positive	No	5	3	2	3	0	0	0
Sensitivity	%	97,1	98,0	97,1	97,1	90,0	95,2	95,2
Specificity	%	95,4	97,2	98,1	98,3	100,0	100,0	100,0