

Evaluation of three rubella IgG enzyme immunoassays

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Background and Description of the Assays

The National Blood Service (NBS) currently performs 35% of the ante-natal testing for England. Testing for rubella IgG forms an important part of this service, requiring the NBS to consistently differentiate between those patients who are immune to rubella and those at risk of infection. The NBS needs to ensure that the most sensitive, specific and cost effective kits are used for rubella screening.

Three assays fulfilling the NBS specification for provision of microbiology test kits, were evaluated to determine their ability to correctly identify the rubella status of specimens from individuals with or without previous rubella infection, and to determine the level of antibody present. The three assays were Bioelisa Rubella IgG colour, Captia Rubella IgG and Mercia Rubella G. All three kits have obtained a CE Mark.

The three assays are all enzyme immunoassays based on the “sandwich” principle. The assays use either a monoclonal or polyclonal antibody selected to recognise human IgG antibodies. Upon completion of the assay, the development of colour indicates the presence of IgG antibody to rubella antigen, while no or low colour development suggests the absence of such antibody. All three kits use an in-well dilution procedure which would fit with the NBS operational requirements. An additional feature of two kits, Bioelisa Rubella IgG colour and Mercia Rubella G, is that they include Sample Addition Monitoring, meaning that a colour change may be observed on addition of samples and can be measured colorimetrically. Further assay information is shown in Table 1.

Limited sensitivity and specificity investigations were done at the Microbiological Diagnostics Assessment Service based at the Health Protection Agency’s Centre for Infection

Table 1: Assay Information

General			
Assay name	Bioelisa Rubella IgG colour	Captia Rubella IgG	Mercia Rubella IgG
Manufacturer / UK agent	Biokit / Launch	Trinity Biotech / Sterilab Services	Microgen Bioproducts
Product number	3000-1219	2325300	M5066 / M506
Number of tests in one pack	480	96	480 / 96
Specimen volume	10µL	10µL	10µL
Presentation			
Assay type	one-Step Sandwich ELISA	one-Step Sandwich ELISA	one-Step Sandwich ELISA
Solid phase	12 x 8 microtitre plate wells	12 x 8 microtitre plate wells	12 x 8 microtitre plate wells
Coating	Inactivated rubella antigen	Inactivated rubella antigen	Rubella antigen
Conjugate	Peroxidase labelled rabbit anti-Human	Peroxidase labelled goat anti-Human	Peroxidase labelled mouse monoclonal
Substrate	TMB	TMB	TMB
Controls per plate	4	3	3
Negative control	1	1	1
Antibody positive control	1 - High	1 - Calibrator	1 - Cut-off Calibrator 10IU
Antibody positive control	1 - Medium	1 - Positive	1 - Calibrator 200IU
Antibody positive control	1 - Low		1 - Validation control 20-50IU (Optional)
Reading wavelength	450 / 630	450 / 630	450 / 630
Cut-off computation	Mean [Low pos]	Mean [Calibrator]xCorr factor	Mean [Cut-off Calibrator]
Equivocal zone	=>0.9<1.0 OD/CO	=>0.9<1.0 OD/CO	=>0.9<1.0 OD/CO
Stages			
Preparation / sample well loading	30 minutes	30 minutes**	30 minutes
Prewash of reaction plate	n/a	n/a	n/a
Incubation status	Static	Static	Static
Sample incubation	60 minutes 37°C	25 +/-5 minutes 21-25°C	30 minutes 37°C
Conjugate incubation	30 minutes 37°C	25 +/-5 minutes 21-25°C	30 minutes 37°C
Number of washes	4	5	5
Substrate incubation (time/temp)	30 minutes 20-25°C	10-15 minutes 21-25°C	30 minutes 18-25°C
Reading	5 minutes	5 minutes	5 minutes
Total incubation times	120 minutes	65 minutes	90 minutes
Approximate time to completion	160 minutes	100 minutes	130 minutes
Number of optional procedures	none	none	One
Additional equipment required			
Incubator, type not specified (*Dry incubator)			
Microplate spectrophotometer (* ELx 808i)			
Micropipettes: 40 - 200µL, 200 - 1000µL & 2 - 10mL			
Multichannel pipettes: 50 - 300µL			
Disposable tips			
Reagent troughs and bottles			
Measuring cylinder			
Distilled water			

Notes:

* Equipment used in this evaluation.

** Due to the short R° incubation the samples were first aliquotted into an uncoated plate and transferred into the test plate using a multichannel pipette.

Evaluation Panel and Method

The evaluation panel consisted of 721 specimens (Table 2a). Of these, 330 specimens were from rubella antibody negative subjects, 302 from rubella antibody positive subjects and 4 from specimens whose rubella status was not determined. A further 48 specimens were from three commercial seroconversion panels and 25 specimens were from a mixed titre performance panel. Replicates of 11 quality control samples and dilutions of the NIBSC 2nd British anti-rubella standard were also tested.

Specimens (10µL) and specimen diluent (200µL) were added manually to all three assays. Due to the short incubation time at room temperature for the Captia assay, 40µl of each sample was first aliquotted into an uncoated Sterilin microtitre plate and 10µl transferred with a multichannel pipette into the Captia coated plate to reduce the pipetting time. The test procedures following this stage are given briefly, as follows:

Bioelisa assay: The ELISA plate was transferred to the Stuart SI19 incubator for continuation with the test procedure. The wells were incubated at 37°C for 60 minutes. The wells were then washed four times and 100µL of conjugate was added to each of the test and control wells which were then incubated for a further 30 minutes at 37°C. The wells were again washed four times and 100µL of TMB was added to all wells and incubated at 37°C for 30 minutes. Finally, 100µL of 1M sulphuric acid was added to all wells.

Captia assay: The wells were incubated at 21-25°C for 25 minutes and the wells then washed five times. 100µL of conjugate was added to each of the wells which were then incubated for a further 25 minutes at 21-25°C. The wells were again washed five times and 100µL of TMB was added to all wells and incubated at 21-25°C for 30 minutes. Finally, 100µL of 1M sulphuric acid was added to all wells.

Mercia assay: The wells were incubated at 37°C for 30 minutes. The wells were washed five times and 100µL of conjugate was added to all wells which were then incubated for a further 30 minutes at 37°C. The wells were again washed five times and 100µL of TMB was added to all wells and incubated at 18-25°C for 30 minutes. Finally 100µL of 1M sulphuric acid was added to all wells.

The reactions were read on an ELx808i plate reader at 450nm (with 630nm reference) using KC4 software. The OD/CO readings were calculated and converted into International Units (IU)/mL according to the procedure given in each kit insert.

Initially, the status of the 'routine' specimens was determined by using the consensus of the three assays' results. Specimens with an IU/mL value in all three assays of less than 9 were regarded as negative, 9 to 11 as equivocal and greater than 11 as positive. Where there were discordant results at this stage those specimens were re-tested in duplicate on the same aliquot of specimen to exclude the possibility of technical error. Any specimen that gave a result that still differed from the observed consensus was tested against an algorithm employed by the Virus Reference Division (VRD) at the Health Protection Agency Centre for Infections,. This included testing such specimens using the qualitative Dade Behring Enzygnost anti-Rubella IgG and, for quality control purposes only, in an independent in-house rubella IgG antibody capture enzyme immunoassay (GACELISA). The results of the Dade Behring assay were interpreted based on the PHLS guidelines on the management of rash illness¹. Further testing, where required, by Single Radial Haemolysis was conducted at the Microbiology Department, Royal Preston Hospital. The results of these tests are shown in Tables 6a and 6b. Additionally, a number of samples were tested for Rubella IgM by HPA-MiDAS (see 'Sensitivity findings of discordant samples' section, page 8).

A second batch of each of the Bioelisa Rubella IgG colour, Captia Rubella IgG and Mercia Rubella G assays were tested to examine variation on a panel of 167 specimens, Table 2b. The panel consisted of 42 rubella antibody negative, 40 rubella antibody positive, 48 samples from three commercial seroconversion panels, one mixed titre commercial panel consisting of 25 samples, replicates of 11 quality control samples and dilutions of the NIBSC 2nd Rubella British Standard, Table 2b.

Table 2a: Evaluation panel used for 1st batch of each rubella assay

Specimen Category	Number of samples
Anti-Rubella IgG negative Screened NBS Blood donations NBS Colindale (unscreened) ¹ Quality Control Reference Unit (ESL) BioClinical Partners Inc Kings College Hospital	235 13 9 45 28
Anti-Rubella IgG positive NBS Colindale (unscreened) ¹ Manchester NBS Quality Assurance Laboratory Boston Biomedical Inc.	270 25 2 5
Anti-Rubella IgG indeterminate Screened NBS Blood donations NBS Colindale (unscreened) ¹ Kings College Hospital	1 2 1
Seroconversion panels² RP001 RP011 RP014	15 members 20 members 13 members
Performance panel PTR201	25 members
Quality Control specimens HPA anti-rubella QC1 Accurun 140 rubella positive control Accurun 25 multi-marker positive control Accurun 810 multi marker negative control Accurun 800 Accurun ToRCH negative control Virotrol ToRCH positive control Virotrol-I multi-marker positive control Viroclear negative control Viroclear ToRCH positive control Bio-Rad Liquicheck ToRCH Plus negative control Bio-Rad Liquicheck ToRCH Plus positive control	1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3)
2nd British anti-Rubella Standard	1 (diln. X 4)
TOTAL No. SAMPLES	721

Notes.

¹ Previously untested for rubella IgG.² Seroconversion following rubella vaccination.

Rep = repeated

Table 2b: Evaluation panel used for 2nd batch of each rubella assay

Specimen Category	Number of samples
Anti-Rubella IgG negative Screened NBS Blood donations NBS Colindale (unscreened) ¹ Quality Control Reference Unit (ESL)	33 2 7
Anti-Rubella IgG positive NBS Colindale (unscreened) ¹	40
Seroconversion panels² RP001 RP011 RP014	15 members 20 members 13 members
Performance panel PTR201	25 members
Quality Control specimens HPA anti-rubella QC1 Accurun 140 rubella positive control Accurun 25 multi-marker positive control Accurun 810 multi marker negative control Accurun 800 Accurun ToRCH negative control Virotrol ToRCH positive control Virotrol-I multi-marker positive control Viroclear negative control Viroclear ToRCH positive control Bio-Rad Liquicheck ToRCH Plus negative control Bio-Rad Liquicheck ToRCH Plus positive control	1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3) 1 (rep x 3)
2nd British anti-Rubella Standard	1 (diln. X 4)
TOTAL No. SAMPLES	167

Specificity findings

Three hundred and thirty rubella antibody negative blood donor specimens were included in the evaluation panel. None of the 330 specimens were initially reactive or equivocal in the Bioelisa assay (initial reactive rate 0.0%, 95% confidence interval 0.0-1.1%). Two specimens were reactive (initial reactive rate 0.6%, 95% confidence interval 0.2-2.6%) and one specimen was equivocal in the Captia assay. Two specimens were reactive in the Mercia assays (initial reactive rate 0.6%, 95% confidence interval 0.2-2.6%), (Table 3a, Figure 1). After retesting, none of the specimens were repeatedly reactive in the Mercia assay (repeat reactive rate 0.0%, 95% confidence interval 0.0-1.1%). No samples were repeatedly reactive in the Captia assay; however one sample was equivocal (9.7IU), (repeat reactive rate 0.0%, 95% confidence interval 0.0-1.7%), (Table 3b, Figure 1).

Table 3a: Initial specificity

Assay Product code	Number tested	Number positive >11IU (reactive rate)	Number equivocal 10-10.9IU (reactive rate)	Number equivocal 9.1-9.9IU (reactive rate)	Number negative <9IU	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour 3000-1219	330	0 (0.0%)	0 (0.0%)	0 (0.0%)	330	<9.2 - <9.2	<9.2	<9.2
Captia Rubella IgG 2325300	330	2 (0.6%)	0 (0.0%)	1 (0.3%)	327	2.5-17.2	2.9	2.6
Mercia Rubella G M5066	330	2 (0.6%)	0 (0.0%)	0 (0.0%)	328	2.7-14.7	3.8	3.5

Table 3b: Repeat specificity

Assay Product code	Number tested	Number positive >11IU (reactive rate)	Number equivocal 10-10.9IU (reactive rate)	Number equivocal 9.1-9.9IU (reactive rate)	Number negative <9IU	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour 3000-1219	330	0 (0.0%)	0 (0.0%)	0 (0.0%)	330	<9.2 - <9.2	<9.2	<9.2
Captia Rubella IgG 2325300	330	0 (0.0%)	0 (0.0%)	1 (0.3%)	329	2.5-9.7	2.8	2.6
Mercia Rubella G M5066	330	0 (0.0%)	0 (0.0%)	0 (0.0%)	330	2.7-7.6	3.7	3.5

Sensitivity findings ('routine' positives)

Three hundred and two randomly selected rubella antibody positive specimens were included in the evaluation panel. Two hundred and ninety-three of the 302 specimens (sensitivity 97.0%, 95% confidence interval 94.4-98.6%) were positive, five (1.66%) were equivocal and four (1.3%) were negative by the Bioelisa assay. 280 (sensitivity 92.7%, 95% confidence interval 89.2-95.4%) were positive, 13 (4.3%) were equivocal and 9 (2.98%) were negative by the Captia assay. 277 (sensitivity 91.7%, 95% confidence interval 88.0-94.6%) were positive, 11 (3.6%) were equivocal and 14 (4.64%) were negative by the Mercia assay (Table 4a, Figure 1).

After retesting, the Bioelisa assay detected 297 samples (98.3%, 95% confidence interval 96.2-99.5%), three samples were equivocal (0.99%) and two were negative (0.66%). The Captia assay detected 292 (96.7%, 95% confidence interval 94.0-98.4%), four samples (1.32%) were equivocal and six were negative (1.99%). The Mercia assay detected 296 samples (98.0%, 95% confidence interval 95.7-99.3%), four samples (1.32%) were equivocal and two samples (0.66%) were negative (Tables 4b, Figure 1).

Table 4a: Initial sensitivity (302 rubella IgG positive samples)

Assay	Product code	Number tested	Number negative <9IU	Number equivocal 9.1-9.9IU	Number equivocal 10-10.9IU	Number positive >11IU (Sensitivity)	95% confidence interval %	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour	3000-1219	302	4 (1.33%)	2 (0.66%)	3 (0.99%)	293 (97.02%)	94.4-98.6%	<9.23->54.2	39.1	42.5
Captia Rubella IgG	2325300	302	9 (2.98%)	6 (1.99%)	7 (2.32%)	280 (92.72%)	89.2-95.4%	2.5-31.1	17.3	17.8
Mercia Rubella G	M5066	302	14 (4.64%)	2 (0.66%)	9 (2.98%)	277 (91.7%)	88.0-94.6%	5.4-160.0	23.9	20.3

Table 4b: Repeat sensitivity (302 rubella IgG positive samples)

Assay	Product code	Number tested	Number negative <9IU	Number equivocal 9.1-9.9IU	Number equivocal 10-10.9IU	Number positive >11IU (Sensitivity)	95% confidence interval %	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour	3000-1219	302	2 (0.66%)	2 (0.66%)	1 (0.33%)	297 (98.34%)	96.2-99.5%	<9.23->54.2	39.1	42.4
Captia Rubella IgG	2325300	302	6 (1.99%)	2 (0.66%)	2 (0.66%)	292 (96.69%)	94.0-98.4%	5.5-31.1	17.6	17.8
Mercia Rubella G	M5066	302	2 (0.66%)	3 (0.99%)	1 (0.33%)	296 (98.01%)	95.7-99.3%	7.1-160.0	24.5	20.6

Seven of the 302 rubella IgG positive specimens gave discordant results in the three assays evaluated, see section 'Sensitivity findings of discrepant samples'. A further analysis of the assays under evaluation of the results for 295 rubella IgG positive samples, which excluded the seven specimens, was undertaken. Two hundred and ninety of the 295 specimens (sensitivity 98.3%, 95% confidence interval 96.1-99.4%) were positive, three (1.02%) were equivocal and two (0.68%) were negative by the Bioelisa assay. 279 (sensitivity 94.6%, 95% confidence interval 91.3-96.9%) were positive, 10 (3.38%) were equivocal and 6 (2.03%) were negative by the Captia assay. 276 (sensitivity 93.6%, 95% confidence interval 90.1-96.1%) were positive, 9 (3.05%) were equivocal and 10 (3.38%) were negative by the Mercia assay.

After retesting, the Bioelisa assay detected 294 samples (99.7%, 95% confidence interval 98.1-100%), one sample was equivocal (0.34%) and none were negative. The Captia assay detected 291 (98.6%, 95% confidence interval 96.6-99.6%), two samples (0.68%) were equivocal and two were negative (0.68%). The Mercia assay detected 293 samples (99.32%, 95% confidence interval 97.6-99.9%) and two samples (0.68%) were negative. The initial and repeat sensitivity findings are shown in Tables 5a and 5b.

Table 5a: Initial sensitivity (295 rubella IgG positive samples)

Assay	Product code	Number tested	Number negative <9IU	Number equivocal 9.1-9.9IU	Number equivocal 10-10.9IU	Number positive >11IU (Sensitivity)	95% confidence interval %	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour	3000-1219	295	2 (0.68%)	1 (0.34%)	2 (0.68%)	290 (98.31%)	96.1-99.4%	<9.23->54.19	39.7	43.2
Captia Rubella IgG	2325300	295	6 (2.03%)	3 (1.02%)	7 (2.37%)	279 (94.58%)	91.3-96.9%	2.5-31.1	17.5	17.8
Mercia Rubella G	M5066	295	10 (3.38%)	2 (0.68%)	7 (2.37%)	276 (93.56%)	90.1-96.1%	5.4-160.0	24.3	20.5

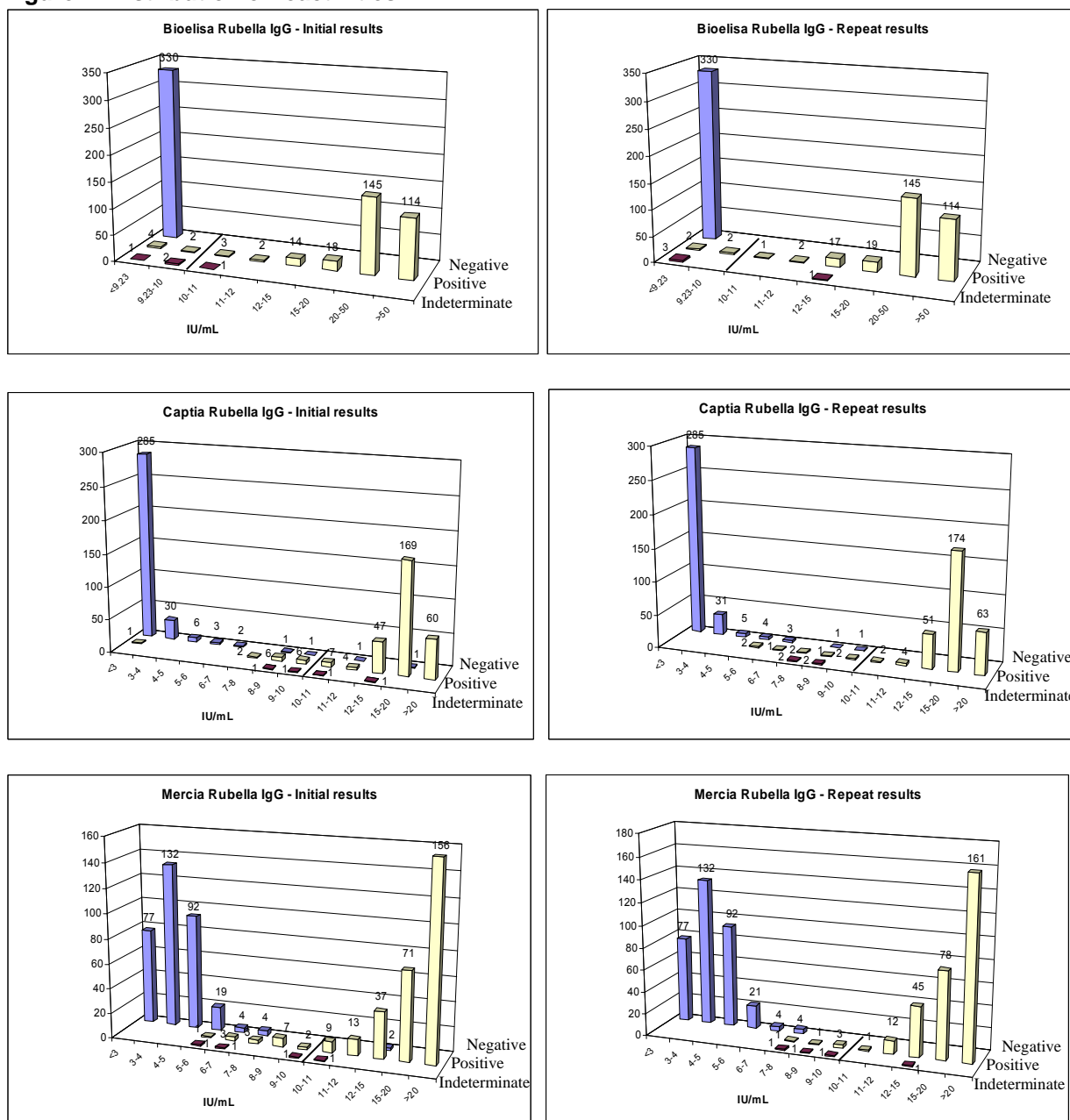
Table 5b: Repeat sensitivity (295 rubella IgG positive samples)

Assay	Product code	Number tested	Number negative <9IU	Number equivocal 9.1-9.9IU	Number equivocal 10-10.9IU	Number positive >11IU (Sensitivity)	95% confidence interval %	Range IU	Mean IU	Median IU
Bioelisa Rubella IgG colour	3000-1219	295	0 (0%)	1 (0.34%)	0 (0%)	294 (99.66%)	98.1-100%	9.71->54.19	39.7	43.2
Captia Rubella IgG	2325300	295	2 (0.68%)	1 (0.34%)	1 (0.34%)	291 (98.64%)	96.6-99.6%	6.9-31.1	17.8	17.8
Mercia Rubella G	M5066	295	2 (0.68%)	0 (0%)	0 (0%)	293 (99.32%)	97.6-99.9%	7.1-160.0	24.9	20.7

Equivocal results

On initial testing of the total of 636 blood donor/patients' samples (including those in the Indeterminate category), eight samples (1.26%) gave an equivocal result, (ie OD/CO >0.9<1.1, 9-11IU) in the Bioelisa assay of which three (0.47%) remained equivocal on repeat testing. For the Captia assay, 16 (2.52%) were initially equivocal and five (0.79%) were again equivocal on repeat testing. One further sample which was initially false positive was equivocal on repeat testing. Thirteen specimens (2.04%) were initially equivocal in the Mercia assay of which five (0.79%) were repeatedly equivocal. A breakdown of these results is shown in Tables 3, 4, 5 and 6 and may be viewed in Figure 1.

Figure 1: Distribution of reactivities



Note. Repeat results graphs: the average value of duplicate repeat test results is shown for the positive, negative and indeterminate specimens.

Sensitivity findings of discordant samples

Thirty-five specimens from 636 blood donor/patients' samples did not give a concordant result in the evaluation i.e. positive (>10IU) or negative (<10IU) status or were within the equivocal zone, according to the kit instructions, on initial testing. On repeat testing in duplicate 24 samples conformed to the majority result and were assigned a positive or negative status.

The remaining 11 samples were further tested by the Virus Reference Division (VRD), HPA in their routine testing algorithm of Dade Behring Enzygnost Rubella G assay and an in-house GACELISA. Seven samples were categorised as positive and were therefore included in the sensitivity analysis, see Table 6a. However, for the four remaining samples, the VRD report stated 'unable to confirm rubella immunity' therefore these samples were categorised as indeterminate for the purposes of this evaluation and the results are therefore presented separately, Table 6b.

The samples were tested also by single radial haemolysis at the Microbiology Department, Royal Preston Hospital. the results may be seen in Tables 6a and 6b.

HPA-MiDAS additionally tested the 11 samples for IgM using the Microimmune Rubella IgM Capture EIA. All 11 samples were rubella IgM negative.

Table 6a: Results for seven specimens that were initially equivocal or discordant by the three kits, and that were assigned positive status by the VRD algorithm.

Specimen number	Microbiological Diagnostics Assessment Service Test Results									Virus Reference Division Test Results			Preston
	Bioelisa Rubella IgG colour			Captia Rubella IgG			Mercia Rubella IgG			Dade Behring Enzygnost Rubella IgG	In-house GACELISA	Result	SRH
	Initial result IU (OD/CO)	Repeat results IU (OD/CO)		Initial result IU (OD/CO [#])	Repeat results IU (OD/CO [#])		Initial result IU (OD/CO)	Repeat results IU (OD/CO)		IU (OD*)	Qualitative result	Final status	Zone (mm) [‡]
05R0118A	<9.2 (0.73)	9.3 (0.92)	10.5 (1.05)	8.5 (1.13)	8.8 (1.17)	9.4 (1.23)	7.0 (0.77)	8.4 (0.81)	10.9 (1.10)	7 (0.20) positive	positive	Positive	7
05R0119A	12.7 (1.21)	12.1 (1.2)	11.0 (1.1)	9.8 (1.26)	7.4 (1.02)	7.4 (1.01)	10.0 (1.01)	10.4 (1.06)	8.7 (0.80)	9 (0.24) positive	positive	Positive	0†
05R0145a	12.2 (1.17)	16.2 (1.5)	17.1 (1.6)	14.4 (1.62)	8.7 (1.16)	9.6 (1.25)	6.1 (0.62)	10.9 (1.03)	9.0 (0.85)	10 (0.27) positive	positive	Positive	0†
05R0170a	<9.2 (0.79)	<9.2 (0.66)	9.9 (0.98)	8.7 (1.16)	5.0 (0.65)	6.1 (0.84)	10.6 (1.07)	10.8 (1.09)	11.6 (1.17)	9 (0.229) positive	positive	Positive	7
05R0172a	10.9 (1.08)	<9.2 (0.37)	<9.23 (0.90)	8.7 (1.15)	7.6 (1.03)	8.2 (1.11)	6.8 (0.70)	12.0 (1.20)	13.8 (1.36)	10 (0.267) positive	positive	Positive	7
05R0283a	9.7 (0.97)	<9.2 (0.91)	11.53 (1.13)	9.7 (1.25)	10.2 (1.30)	9.9 (1.27)	15.3 (1.47)	12.2 (1.23)	13.0 (1.30)	8 (0.23) positive	positive	Positive	8
05R0360a	13.2 (1.23)	14.1 (1.33)	12.8 (1.23)	9.5 (1.24)	7.6 (1.03)	8.5 (1.13)	7.3 (0.69)	11.3 (1.14)	9.7 (0.96)	8 (0.22) positive	positive	Positive	8

Notes. [#] Captia assay OD/CO value 1.28 is equivalent to 10IU for this batch number. *Enzygnost assay cut-off value = 0.200 (IU/mL value may vary but final status of sample is interpreted in conjunction with other test results¹). [‡] 15IU/mL control ≡ 8mm. †Results following absorption: in initial testing, sample test zone = sample control zone.

Table 6b: Results for four specimens that were equivocal or discordant by the three kits, and that were assigned indeterminate status by VRD algorithm.

Specimen number	Microbiological Diagnostics Assessment Service Test Results									Virus Reference Division Test Results			Preston
	Bioelisa Rubella IgG colour			Captia Rubella IgG			Mercia Rubella IgG			Dade Behring Enzygnost Rubella IgG	In-house GACELISA	Result	SRH
	Initial result IU (OD/CO)	Repeat results IU (OD/CO)		Initial result IU (OD/CO [#])	Repeat results IU (OD/CO [#])		Initial result IU (OD/CO)	Repeat results IU (OD/CO)		IU (OD*)	Qualitative result	Final status	Zone (mm) [‡]
05r0043a	9.7 (0.98)	<9.2 (0.79)	9.4 (0.95)	12.9 (1.51)	9.2 (1.21)	8.6 (1.15)	6.6 (0.68)	8.2 (0.76)	9.6 (0.95)	9 (0.23) positive	equivocal	Indeterminate	7
05R0052a	<9.2 (0.51)	<9.2 (0.84)	<9.2 (0.81)	10.3 (1.31)	7.7 (1.04)	8.1 (1.10)	5.4 (0.53)	7.2 (0.62)	7.2 (0.62)	7 (0.19) equivocal	negative	Indeterminate	4
05R0290a	9.8 (0.98)	<9.2 (0.77)	<9.2 (0.92)	8.3 (1.12)	6.6 (0.91)	5.9 (0.82)	10.5 (1.05)	8.0 (0.67)	10.4 (1.06)	8 (0.197) equivocal	positive	Indeterminate	8
06R0112a	10.3 (1.02)	11.3 (1.11)	14.0 (1.30)	9.8 (1.27)	8.0 (1.08)	7.6 (1.03)	9.2 (0.93)	12.0 (1.26)	12.9 (1.37)	11 (0.288) positive	negative	Indeterminate	7

Notes. [#] Captia assay OD/CO value 1.28 is equivalent to 10IU for this batch number. *Enzygnost assay cut-off value = 0.200 (IU/mL value may vary but final status of sample is interpreted in conjunction with other test results¹). [‡] 15IU/mL control ≡ 8mm.

Seroconversion sensitivity

The ability of the Bioelisa Rubella IgG colour, Captia Rubella IgG and Mercia Rubella G assays to detect antibody in three seroconversion panels (post rubella vaccine) was compared.. When the aggregate scores were calculated, the Captia Rubella IgG assay was ranked 1st with a score of 31 positive samples out of a total of 48 samples, Bioelisa Rubella IgG colour was ranked 2nd with a score of 29 and Mercia Rubella G was third with a total of 25 (Table 7 and Appendix Table 11)

A mixed titre performance panel consisting of 23 positive specimens and two negative specimens was also tested. Captia Rubella IgG correctly identified all of the positive and negative specimens; Bioelisa Rubella IgG colour identified 22 of the positive samples and Mercia Rubella G identified 21 samples (Table 8 and Appendix Table 12).

Table 7: Combined seroconversion scores (3 panels)

Panel	Number of specimens in panel	Bioelisa Rubella IgG colour	Captia Rubella IgG	Mercia Rubella G	Abbott* Rubazyme-M IgM S/CO	Abbott* Rubazyme IgG S/CO	Gull Labs* ELISA Rubella IgM S/CO	Gull Labs* ELISA Rubella IgG S/CO
RP001-01	15	7 (28)	8 (24)	6 (31)	9 (21)**	8 (24)	9 (21)**	7 (28)
RP011-01	20	15 (19)	15 (19)	15 (19)	15 (19)**	15 (19)	16 (16)**	15 (19)
RP014-01	13	7 (21)	8 (19)	4 (33)	8 (19)**	8 (19)	8 (19)**	8 (19)
Total**	48	29	31	25	32	31	33	30
Product Number		3000-1219	2325200	M5066	NS	NS	NS	NS
Lot Number		G-1006	525	4361	NS	NS	NS	NS

Notes:

All panels taken from male individuals following vaccination with Meruvax II Vaccine.

The total for each assay was calculated by summing the correct positive reactions for each of the panels. A higher score suggests higher sensitivity. The number in parenthesis is the number of days from the initial bleed to the first positive sample.

* Results were extracted from BBI / BCP data sheets. NS = Kit product and lot numbers were not stated.

** IgM detection assays therefore panels began positive but had one or more negative results later on in the panel.

Table 8: Scores for one mixed titre performance panel

Panel	Number of specimens in panel	Bioelisa Rubella IgG colour	Captia Rubella IgG	Mercia Rubella IgG	Abbott* EIA Rubella IgG	Abbott* IMx Rubella IgG	Murex* Rubella Total (Latex Agg.)
PTR201	25	22	23	21	23	23	23

Notes: The total for each assay was the number of reactive samples for the panel

*Data taken from BBI data sheet.

Quality Control Results

Appropriate quality control samples are those that give a value within the dynamic range of the assay and are typically 2-4 times the cut-off value. However, when considering QC samples for rubella assays, the IU value would be a better indicator of suitability. In such cases, an IU value of between 15IU and 20IU might be considered the most suitable.

For all three assays, a number of QC samples would be suitable for use, dependant upon the level of sensitivity required, see Table 9.

Table 9: Results of quality control samples

Quality control sample	Bioelisa Rubella IgG colour									
	Lot G-1006					Lot I-4806				
	IU 1	IU 2	IU 3	IU 4	Mean	IU 1	IU 2	IU 3	IU 4	Mean
HPA anti-rubella QC1	27.9	20.6	25.3	NT	24.6	25.3	27.2	27.8	NT	26.8
Accurun 140 rubella positive control	19.2	20.3	26.5	NT	22.0	21.9	22.9	22.3	NT	22.4
Accurun 25 multi-marker positive control	25.2	23	24.5	NT	24.2	25.7	21.3	22.4	NT	23.1
Accurun 810 multi marker negative control	>54.2	>54.2	>54.2	NT	>54.2	>54.2	>54.2	>54.2	NT	>54.2
Accurun 800 Accurun ToRCH negative control	<9.3	<9.3	<9.3	NT	<9.3	<9.2	<9.2	<9.2	NT	<9.2
Virotrol ToRCH positive control	17.1	18.1	21.1	NT	18.8	14.7	16.9	16.1	NT	15.9
Virotrol-I	16.4	19.9	NT	NT	18.2	14.8	16.4	17.4	NT	16.2
Viroclear negative control	<9.3	<9.3	<9.3	NT	<9.3	<9.2	<9.2	<9.2	NT	<9.2
Viroclear ToRCH positive control	32.8	33.2	43.1	NT	36.4	42.6	33.1	47.2	NT	41.0
Bio-Rad Liquicheck ToRCH Plus negative control	<9.3	<9.3	<9.3	NT	<9.3	<9.2	<9.2	<9.2	NT	<9.2
Bio-Rad Liquicheck ToRCH Plus positive control	14.9	14	16.3	NT	15.1	18.6	17.7	15.9	NT	17.4
NIBSC 2nd British Standard*	10.2	10	10.6	10.4	10.3	9.9	<9.2	<9.2	11.3	9.7 [#]

Notes. *Standard reconstituted in 1mL distilled water and an aliquot of a 1:8 dilution made to give 10IU/ml. All kits tested on the same day with the same aliquot.

[#]Approximated mean value

Quality control sample	Captia Rubella IgG									
	Lot 525					Lot 523				
	IU 1	IU 2	IU 3	IU 4	Mean	IU 1	IU 2	IU 3	IU 4	Mean
HPA anti-rubella QC1	17.6	16.8	17.4	NT	17.3	18.6	17.9	17.9	NT	18.1
Accurun 140 rubella positive control	16.1	17.2	20.8	NT	18.0	18.7	21.4	19.7	NT	19.9
Accurun 25 multi-marker positive control	18.2	17.2	16.5	NT	17.3	19.9	16.5	20.8	NT	19.0
Accurun 810 multi marker negative control	20.8	23.0	26.9	NT	23.5	30.4	30.6	30.5	NT	30.5
Accurun 800 Accurun ToRCH negative control	8.2	7.3	6.9	NT	7.5	4.9	4.4	4.4	NT	4.6
Virotrol ToRCH positive control	17.4	16.8	18.2	NT	17.5	17.6	15.8	17.9	NT	17.1
Virotrol-I	14.9	16.8	NT	NT	15.9	12.1	14.9	14.2	NT	13.7
Viroclear negative control	2.9	2.8	2.9	NT	2.9	2.5	2.5	2.5	NT	2.5
Viroclear ToRCH positive control	17.4	16.8	18.2	NT	17.5	20.4	19.5	22.4	NT	20.8
Bio-Rad Liquicheck ToRCH Plus negative control	2.6	2.6	2.6	NT	2.6	2.4	2.4	2.4	NT	2.4
Bio-Rad Liquicheck ToRCH Plus positive control	12.3	9.3	12.9	NT	11.5	10.5	9.6	10.6	NT	10.2
NIBSC 2nd British Standard*	8.6	8.4	8.6	9.0	8.6	9.3	8.2	8.8	8.5	8.7

Notes. *Standard reconstituted in 1mL distilled water and an aliquot of a 1:8 dilution made to give 10IU/ml. All kits tested on the same day with the same aliquot.

Quality control sample	Mercia Rubella G									
	Lot 4361					Lot 4261				
	IU 1	IU 2	IU 3	IU 4	Mean	IU 1	IU 2	IU 3	IU 4	Mean
HPA anti-rubella QC1	26.9	30.4	41.3	NT	32.9	23.3	30.8	37.6	NT	30.6
Accurun 140 rubella positive control	34.6	37.3	46.0	NT	39.3	22.6	23.4	24.2	NT	23.4
Accurun 25 multi-marker positive control	33.8	34.5	34.8	NT	34.4	32.8	26.6	25.6	NT	28.3
Accurun 810 multi marker negative control	155.6	99.9	188.8	NT	148.1	245.6	191.2	175.3	NT	204.0
Accurun 800 Accurun ToRCH negative control	4.1	4.4	4.4	NT	4.3	6.1	6.0	6.0	NT	6.0
Virotrol ToRCH positive control	18.1	16.3	18.8	NT	17.7	20.5	18.2	24.9	NT	21.2
Virotrol-I	12.2	12.7	14.8	NT	13.2	15.8	14.8	14.0	NT	14.9
Viroclear negative control	3.3	3.3	3.3	NT	3.3	4.7	4.7	4.7	NT	4.7
Viroclear ToRCH positive control	37.1	37.5	49.7	NT	41.4	50.6	51.8	56.4	NT	52.9
Bio-Rad Liquicheck ToRCH Plus negative control	3.3	3.3	3.3	NT	3.3	5.0	4.9	4.9	NT	4.9
Bio-Rad Liquicheck ToRCH Plus positive control	16.7	14.4	13.5	NT	14.9	14.2	13.2	20.3	NT	15.9
NIBSC 2nd British Standard*	9.2	9.1	9.3	9.8	9.4	7.8	8.8	8.5	8.6	8.4

Notes. *Standard reconstituted in 1mL distilled water and an aliquot of a 1:8 dilution made to give 10IU/ml. All kits tested on the same day with the same aliquot.

Batch comparison

Two batches of each of the Bioelisa Rubella IgG colour, Captia Rubella IgG and Mercia Rubella G assays were tested to examine variation against a panel of 167 specimens, Table 2b. The comparative results are shown in Table 10 and *Appendix* tables 11 and 12.

The Bioelisa Rubella IgG colour lot I-4806 detected one sample less than lot G-1006 for panel RP001. All other samples gave consistent results.

The Captia Rubella IgG lot 523 did not detect three anti-rubella positive specimens on initial testing; however the samples were reactive on repeat testing with this batch. All other samples gave consistent results.

The Mercia Rubella G assay lot 4361 detected one sample less for panel RP001, and two samples less than lot 4261 for panel RP014. All other samples gave consistent results.

Table 10: Comparison of two batches of each assay

Specimen category	Number of specimens	Number of initial reactive specimens (>10IU)					
		Bioelisa Rubella IgG		Captia Rubella IgG		Mercia Rubella IgG	
		G-1006	I-4806	lot 525	lot 523	4361	4261
Rubella IgG negative	42	0	0	0	0	0	0
Rubella IgG positive	40	40	40	40	37* (40)	40	40
RP001	15	7	6	8	9	6	7
RP011	20	15	15	15	15	15	15
RP014	13	7	7	8	8	4	6
PTR201	25	22	22	23	23	21	21
Total	153	91	90	94	92* (95)	86	89

Note. * The three positive samples that were <10IU on initial testing were reactive on repeat testing

Conclusion

All three assays (Bioelisa, Captia and Mercia) performed well in terms of specificity. Bioelisa Rubella IgG colour assay gave no reactive samples; Captia Rubella IgG and Mercia Rubella G both gave 2/330 initial reactive results (0.6%) with known rubella IgG negative samples. All three assays gave 100% specificity when repeat tested (0/330 repeat reactive). For ante-natal testing, it is more acceptable for a rubella kit to have false negatives rather than false positives.

The sensitivity rates for all three assays were also acceptable for use by the National Blood Service.

Reference

1. Morgan-Capner P, Crowcroft NS. Guidelines on the management of, and exposure to, rash illness in pregnancy (including consideration of relevant antibody screening programmes in pregnancy). *Comm Dis Public Health* 2002; 5(1): 59-71.

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Appendix

Table 11: Comparison of reactivities of three commercial seroconversion panels for two batches of each assay

Panel	International units					
	Bioelisa Rubella IgG		Captia Rubella IgG		Mercia Rubella IgG	
	G-1006	I-4806	lot 525	lot 523	4361	4261
RP001-01	<9.23	<9.23	2.55	2.427	4.12	4.71
RP001-02	<9.23	<9.23	2.53	2.438	4.12	4.67
RP001-03	<9.23	<9.23	2.53	2.445	4.12	4.69
RP001-04	<9.23	<9.23	2.52	2.416	4.09	4.64
RP001-05	<9.23	<9.23	2.62	2.514	4.11	4.69
RP001-06	<9.23	<9.23	4.62	4.476	4.53	5.12
RP001-07	<9.23	<9.23	9.74	10.414	6.05	7.13
RP001-08	<9.23	<9.23	13.00	14.482	8.11	9.38
RP001-09	11.09	<9.23	13.95	15.943	9.32	10.98
RP001-10	28.43	22.32	20.85	22.238	14.89	16.99
RP001-11	22.17	30.4	20.39	21.879	12.24	16.08
RP001-12	16.43	24.61	18.20	20.773	12.05	14.74
RP001-13	18.38	23.24	20.00	19.843	12.27	14.70
RP001-14	19.05	20.46	19.94	18.927	13.17	14.38
RP001-15	21.15	24.28	20.11	21.239	14.17	17.03
RP011-01	<9.23	<9.23	2.76	2.605	4.16	4.71
RP011-02	<9.23	<9.23	2.81	2.629	4.16	4.81
RP011-03	<9.23	<9.23	2.76	2.629	4.17	4.76
RP011-04	<9.23	<9.23	2.88	2.644	4.17	4.76
RP011-05	<9.23	<9.23	7.30	6.996	5.71	6.57
RP011-06	25.42	25.45	26.13	19.351	13.38	13.92
RP011-07	34.55	39.39	25.31	23.007	17.34	19.15
RP011-08	39.76	40.73	26.20	24.736	24.12	31.62
RP011-09	39.62	42.70	24.93	23.384	26.10	26.49
RP011-10	>54.19	42.70	23.88	25.514	24.06	33.74
RP011-11	>54.19	53.30	9.37	26.476	22.01	26.49
RP011-12	>54.19	53.84	25.21	25.178	24.51	25.77
RP011-13	52.12	>54.19	23.68	23.348	27.14	32.02
RP011-14	>54.19	>54.19	23.91	23.557	34.19	36.18
RP011-15	45.28	53.84	27.02	26.241	32.27	35.64
RP011-16	>54.19	>54.19	27.13	27.922	34.91	44.61
RP011-17	54.03	44.30	23.52	30.199	42.49	45.06
RP011-18	>54.19	51.88	27.77	29.362	46.45	44.61
RP011-19	>54.19	>54.19	24.96	32.661	37.47	44.28
RP011-20	>54.19	>54.19	28.36	29.277	45.61	50.16
RP014-01	<9.23	<9.23	2.62	2.65	4.09	4.71
RP014-02	<9.23	<9.23	2.63	2.67	4.11	4.70
RP014-03	<9.23	<9.23	2.64	2.56	4.12	4.69
RP014-04	<9.23	<9.23	2.76	2.82	4.16	4.80
RP014-05	<9.23	<9.23	3.65	3.59	4.29	5.05
RP014-06	<9.23	<9.23	11.43	12.27	6.11	7.90
RP014-07	12.77	12.87	13.65	15.21	7.46	8.84
RP014-08	17.92	16.78	14.70	18.40	8.43	10.58
RP014-09	17.60	16.50	15.54	17.02	9.39	10.95
RP014-10	20.18	17.23	17.53	18.46	12.27	13.31
RP014-11	20.48	19.45	16.89	19.52	12.11	14.27
RP014-12	28.95	18.19	20.00	20.59	15.09	17.12
RP014-13	30.78	22.4	17.65	23.45	16.85	16.20

Table 12: Comparison of reactivities of a commercial mixed titre performance panel for two batches of each assay

Panel	International units					
	Bioelisa Rubella IgG		Captia Rubella IgG		Mercia Rubella IgG	
	G-1006	I-4806	lot 525	lot 523	4361	4261
PTR201-01	15.64	13.95	15.80	14.61	26.31	13.34
PTR201-02	38.96	44.16	28.07	25.63	71.07	37.37
PTR201-03	52.37	49.67	29.70	28.93	37.63	31.30
PTR201-04	>54.19	>54.19	29.40	28.05	109.83	58.84
PTR201-05	<9.23	<9.23	2.77	2.51	4.34	4.70
PTR201-06	28.46	16.28	16.92	15.27	28.56	24.82
PTR201-07	22.74	17.23	21.87	21.21	13.28	13.44
PTR201-08	21.78	16.28	18.31	18.57	27.60	22.98
PTR201-09	>54.19	>54.19	31.39	33.20	89.26	55.01
PTR201-10	>54.19	>54.19	27.24	28.63	65.62	35.91
PTR201-11	35.63	30.51	16.65	16.30	38.41	23.21
PTR201-12	>54.19	>54.19	29.79	28.51	114.69	79.77
PTR201-13	>54.19	>54.19	31.63	30.97	36.12	28.12
PTR201-14	24.07	16.11	17.94	15.92	23.59	20.43
PTR201-15	>54.19	>54.19	26.56	30.92	10.45	88.36
PTR201-16	<9.23	<9.23	2.78	2.67	4.44	4.86
PTR201-17	48.42	36.95	21.61	22.87	60.31	13.51
PTR201-18	27.47	28.34	21.39	21.18	33.73	14.56
PTR201-19	13.41	13.53	14.21	14.16	9.85	9.08
PTR201-20	>54.19	>54.19	31.82	26.51	9.06	50.28
PTR201-21	>54.19	49.33	25.06	26.36	62.27	22.58
PTR201-22	15.97	10.48	13.35	12.83	14.88	11.60
PTR201-23	53.7	36.46	25.02	27.76	68.06	52.20
PTR201-24	<9.23	<9.23	12.88	12.55	8.54	8.08
PTR201-25	>54.19	>54.19	26.13	27.92	70.91	41.81