



Blood and Transplant



Evaluation of six Cytomegalovirus IgG/total antibody kits suitable for use in the UK National Blood Service

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Background

Some recipients of blood donations are immunosuppressed and CMV infection in these individuals can have detrimental sequelae. Identification and exclusion of donations that are likely to transmit CMV infection to these recipients is therefore important. Blood donation screening is currently undertaken by testing for IgG/total antibodies to CMV. The proportion of UK blood donors that are CMV antibody positive is about 25-40%.

There is debate about the relative effectiveness of leucodepleted and CMV seronegative products in minimising the risk of CMV infection¹. The balance of evidence from clinical studies suggests that acceptable CMV safety for blood products can be achieved by pre-storage leucodepletion. However, this cannot be applied to other, non-blood products. Currently there is insufficient evidence to recommend the discontinuation of CMV testing. It is unlikely that this position will change until suitable randomised, prospective, controlled trials are conducted. Tests to detect CMV DNA in donors do not appear to add an increased level of detection beyond that provided by current serological screening assays¹

CMV kits to be evaluated for NBS need to fulfil their specification for provision of microbiology test kits² and should have the following features:

- CE marked
- Suitable for testing donor and patient serum and EDTA samples
- Include systems to verify correct addition of sample
- Sample volumes at a level to achieve accurate dispensing, between 10 and 200µL
- All kit components must be easily identifiable
- Microplates in 12 strips of 8 wells
- Must allow testing of samples up to 7 days post-collection
- The kit insert must be in English with full version control and have any changes highlighted It must have full details of all reagents.

A market review of commercially available IgG/total CMV kits was undertaken³ and six kits that fulfilled the NBS requirements were put forward for performance evaluation. The kits to be evaluated are Bioelisa CMV Colour (Biokit), CMV IgG ELA (Medac Diagnostika), EIAgen CMV screen (Adaltis), Mercia CMV TA (Microgen), Vironostika Anti CMV III (bioMérieux), and MastazymeTM-CMV (Mast Diagnostics). For ease of presentation, the kits will be referred to by their manufacturer's name in this report.

Description of Assays

Four of the assays evaluated are based on a competitive EIA format (EIAgen CMV screen [Adaltis], Mercia CMV TA [Microgen Bioproducts], Vironostika anti CMV III [bioMérieux] and MastazymeTM-CMV [Mast Diagnostics]). These assays have a solid phase that is coated with CMV antigens. The test specimen and the conjugate (HRP-labelled monoclonal antibodies to CMV) are co-incubated. The antibodies present in the specimen compete with the conjugate for binding sites on the CMV antigen coated solid phase. Any unbound conjugate and specimen are removed by washing. The second incubation results in the visualisation of bound conjugate using the substrate. This produces a coloured product

which is inversely proportional to the amount of CMV antibodies in the specimen. The reaction is stopped and the results read using a spectrophotometer.

The Bioelisa CMV colour assay is based on a capture method. The specimen is incubated in a microplate well which is coated with CMV antigen. If antibodies to CMV are present in the specimen they will bind to the antigen. After washing, the conjugate (enzyme-labelled antibodies to human IgM and IgG) is added and will bind to any CMV antibodies bound to the CMV antigen coated microplate. A substrate is added to this which will develop a blue colour if antibodies to CMV are present in the specimen.

The final EIA evaluated was the CMV-IgG-ELA (Medac Diagnostika). The microplate is coated with human IgM rheumatoid factor (anti-human IgG). The specimen is then added to the wells with CMV-IgG-ELA (Enzyme Labelled Antigen). If antibody to CMV is present in the specimen, it forms an immune complex with the CMV-IgG-ELA. The immune complex binds to the IgM rheumatoid factor and is detected by addition of a substrate which forms a blue colour if antibodies are present in the specimen. The reaction is then stopped and the resulting yellow colour is measured using a spectrophotometer.

A summary of the assays is given in Table 1.

Table 1: Details of kits evaluated.

Assay	EIAgen CMV Screen Kit (Adaltis)	Bioelisa CMV colour (Biokit SA/Launch Diagnostics)	Vironostika anti-CMV III (bioMérieux)	Mastazyme™ - CMV (Mast Diagnostics)	Mercia CMV TA (Microgen Bioproducts)	CMV - IgG - ELA (Medac Diagnostika/Cosmos Biomedical)
Product code	81025	3000-1245	284124	EIA802	M502	115-Q-PKS
Sample Volume (µL)	30	10	30	30	50	10
Principle of test	Competitive	Non-Competitive	Competitive	Competitive	Competitive	Non-Competitive
Solid phase	CMV antigens	CMV antigens	CMV antigens	CMV antigens	CMV antigens	human IgM rheumatoid factor
Conjugate	anti-CMV monoclonal AB with HRP	Rabbit anti-human IgG and IgM antibodies conjugated with HRP	anti-CMV monoclonal AB with HRP	anti-CMV monoclonal AB with HRP	monoclonal anti-human CMV with HRP	N/A
Antigen	N/A	N/A	N/A	N/A	N/A	CMV - IgG ELA (Enzyme Labelled Antigen)
Substrate	TMB / H ₂ O ₂	TMB / H ₂ O ₂	TMB / H ₂ O ₂	TMB / H ₂ O ₂	TMB / H ₂ O ₂	TMB
Stop solution	0.3 M Sulphuric acid	1N Sulphuric acid	0.3 M Sulphuric acid	0.3 M Sulphuric acid	0.5 M Sulphuric acid	0.5 M Sulphuric acid
Controls per run (see Table 3)	2 Calibrator control 2 PC	2 NC 2 HPC 3 LPC	3 NC 1 PC	2 Calibrator control 1 NC 1 PC	2 Cut-off control 1 NC 1 PC	2 NC 2 PC
Cut off computation	(mean[Calibrator control])*0.6	mean[LPC]	mean[NC]	(mean[calibrator control])*calibrator factor	mean[cut off control]	(mean[NC])+0.140
Equivocal zone	Equivocal zone is +/- 10% round cut-off value	OD/CO of between 0.9 and 1.0	N/A	Equivocal zone is +/- 10% round cut-off value	OD/CO value of between 0.9 and 1.1	Equivocal zone is +/- 10% round cut-off value
Stages:						
Preparation/ sample loading	Approx 30 mins	Approx 30 mins	Approx 30 mins	Approx 30 mins	Approx 30 mins	Approx 30 mins
Sample incubation	37°C / 60 mins	20 mins at RT	37°C / 60 mins	37°C / 60 mins	37°C / 90 mins	RT / 60 mins
Conjugate Incubation	N/A*	20 mins at RT	N/A*	N/A*	N/A*	N/A
Substrate Incubation	15 mins	10 mins	15 mins	15 mins	30 mins	10 mins
No of washes (washes (number of wash cycles))	4 (1)	4 (2)	4 (1)	4 (1)	5 (1)	3 (1)
Reading (Plate Reader)	Zero reader on air, read absorbance at 450 nm or 450/620 nm	Blank reader using blank well and read absorbance at 450/620 - 630 nm	Blank reader on air, read absorbance at 450 nm or 450/620 - 700 nm	Read absorbance at 450 nm	Blank reader on air, read absorbance at 450/620 - 650 nm	Blank reader using blank well and read absorbance at 450/620 - 650 nm
Duration of all incubations	75 mins	50 mins	75 mins	75 mins	120 mins	70 mins
Time taken to complete 1 plate (up to 96 samples)	105 mins	80 mins	105 mins	105 mins	150 mins	100 mins
Notes:						
* Incubated with sample		TMB = Tetramethylbenzidine		HPC = High positive control		
AB = Antibodies		NC = Negative Control		RT = Room temperature		
HRP = Horseradish peroxidase		LPC = Low positive control		N/A = not applicable		

All assays required extra equipment, this included pipettes, plate reader, plate washer, measuring cylinder, mixer and timer. In addition, the CMV-IgG-ELA (Medac) kit required 'water for injection' rather than the distilled water used by all other kits. Bioelisa CMV colour (Biokit) did not include the stop solution in the 480 test kits however it is provided in the 96 test kits.

Evaluation panel and method

The evaluation was performed at the Microbiological Diagnostics Assessment Service in accordance with a protocol that was agreed by all participating companies. The manufacturers' kit insert instructions were strictly followed.

The manufacturers were offered the opportunity to train the evaluator and Mast Diagnostics, Cosmos Biomedical (on behalf of Medac Diagnostika) and Microgen Bioproducts accepted the invitation.

The specimen panel included 514 blood donor specimens that had not been previously screened for CMV antibodies. Commercially available seroconversion panels, performance panels and quality control samples were also included.

All the panel samples were tested by one lot of each assay. All blood donor specimens, seroconversion panels and performance panels were tested once by each kit and the commercially available quality control specimens were tested at least three times. The constituents of the panel may be seen in Table 2a. A smaller subset of the panel was tested on a second lot of each assay, see Table 2b.

Initially the status, i.e. positive or negative for CMV antibody, of the blood donor specimens was determined by using an algorithm in which all six assays were in accordance. For specimens in which one assay was discordant, the status was determined to be the one shown by the majority and the sample was retested in duplicate.

For samples where two kits gave results discordant to the remaining four kits, these incompatible samples were retested in duplicate. If after retesting these samples continued to be discordant, the sample was tested by an IgG Western blot. The result of the Western blot test determined the status of the specimen. The Western blot used was the CE marked Mikrogen recomBlot CMV IgG.

In cases where three kits showed a positive and three kits showed a negative result, the sample was tested by the Western blot. Any specimens giving a result discordant with the Western blot were retested in duplicate.

After all retests were done, it was not possible to confidently characterise 23 of the 514 blood donor specimens. The results of these samples were not included in the analysis of the kits under evaluation; the results obtained, including Western blot results, can be seen in *Appendix Table 13*.

Table 2a: Specimen panel to assess the performance of CMV IgG/total antibody kits

Specimen Category	Number of Specimens
Blood donor specimens (serum & plasma) Anti-CMV Negative Anti-CMV Positive Anti-CMV indeterminate	289 202 23*
Seroconversion panels : PD – RP 003 BBI – PTC 901	15 members 9 members
Performance panels : BBI – PTC 201 BBI – PTC 202	25 members 25 members
Quality Control specimens : Positive QCs: HPA Anti CMV QC 1 BBI Accurun 145 (CMV IgG) BBI Accurun 146 (CMV IgM) BBI Accurun 25 (ToRCH IgG) BBI Accurun 26 (ToRC IgM) BR Liquichek ToRCH Plus BBL Virotrol ToRCH BBL Virotrol ToRCH-M BBL Virotrol I Negative QCs: BR Liquichek Plus BBI Accurun 800 BBL Viroclear ToRCH BBL Viroclear BBI Accurun 810	1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3) 1 (x3)
TOTAL number of specimens	602
Notes: * See <i>Appendix</i> Table 13 BBI= Boston Biomedica Inc; BBL= Blackhawk Biosystems Ltd; BR= BioRad; HPA= Health Protection Agency, Colindale UK; PD= Profile Diagnostics Inc.	

Table 2b: Specimen panel sub-set to assess the performance of a second kit lot for CMV IgG/total antibody kits

Specimen Category	Number of Specimens
Blood donor specimens (serum and plasma)	
Anti-CMV Positive	57
Anti-CMV Negative	61
Seroconversion panels	
RP-003	15 members
PTC901	9 members
Performance panels	
PTC201	25 members
PTC202	25 members
Quality Control specimens :	
Positive QCs:	
HPA Anti CMV QC 1	1 (x3)
BBI Accurun 145 (CMV IgG)	1 (x3)
BBI Accurun 146 (CMV IgM)	1 (x3)
BBI Accurun 25 (ToRCH IgG)	1 (x3)
BBI Accurun 26 (ToRC IgM)	1 (x3)
BR Liquichek ToRCH Plus	1 (x3)
BBL Virotrol ToRCH	1 (x3)
BBL Virotrol ToRCH-M	1 (x3)
BBL Virotrol I	1 (x3)
Negative QCs:	1 (x3)
BR Liquichek Plus	1 (x3)
BBI Accurun 800	1 (x3)
BBL Viroclear ToRCH	1 (x3)
BBL Viroclear	1 (x3)
BBI Accurun 810	
TOTAL number of specimens	206
Notes: BBI= Boston Biomedica Inc; BBL= Blackhawk Biosystems Ltd; BR= BioRad; HPA= Health Protection Agency, Colindale UK; PD= Profile Diagnostics Inc.	

Specificity Findings

Two hundred and eighty-nine anti-CMV negative specimens were tested by the six kits under evaluation. Initial specificities ranged from 88.6% to 100% and repeat specificities from 93.4 to 100% (Table 3).

At the end of the evaluation Microgen Bioproducts requested that the 33 initially false positive specimens be retested with a new cut off formulation. The results of the second repeat testing with the new cut off are also shown in Table 3. Although only 33 samples were tested, the results indicate that a substantially higher specificity would be achieved for Mercia CMV TA with the new cut off.

The Adaltis, bioMérieux, Mast and Microgen are competitive assays. To allow direct comparison of results of all the assays evaluated, in the tables below their reading values are expressed as CO/OD, whereas the Bioelisa and Medac are expressed as OD/CO (ie $<1.0 \equiv$ negative, $>1.0 \equiv$ positive). Equivocal results were considered a positive reaction because in the laboratory setting these would have to be investigated further.

Table 3: Specificity of six CMV kits under evaluation

Assay	Number reactive	Number reactive	Range CO/OD	Mean CO/OD	Median CO/OD	Specificity	95% Confidence Interval
EIAgen CMV Screen Kit (Adaltis)	Initial	0	0.27 – 0.71	0.52	0.52	100%	98.7-100%
	Repeat	N/A	N/A	N/A	N/A	N/A	N/A
Vironostika anti-CMV III (bioMérieux)	Initial	2	0.33 – 1.10	0.63	0.62	99.31%	97.5-99.9%
	Repeat	0	0.33 – 0.93	0.63	0.62	100%	98.7-100%
Mastazyme-CMV (Mast)	Initial	4	0.33 – 1.17	0.64	0.62	98.62%	96.5-99.6%
	Repeat	4	0.33 – 10.24	0.70	0.62	98.61%	96.5-99.6%
Mercia CMV TA (Microgen)	Initial	33	0.52 – 2.94	0.74	0.69	88.58%	84.9-92.2%
	Repeat	19	0.53 – 2.94	0.72	0.69	93.38%	89.9-96.0%
	2 nd Repeat with new cut off	2 (n=33)	0.34 – 1.14	0.57	0.55	N/A	N/A
Assay	Number reactive	Number reactive	Range OD/CO	Mean OD/CO	Median OD/CO	Specificity	95% Confidence Interval
Bioelisa CMV colour (Biokit)	Initial	9	0.04 – 1.76	0.24	0.17	96.89%	94.2-98.6%
	Repeat	6	0.04 – 1.76	0.23	0.17	97.92%	95.5-99.2%
CMV-IgG-ELA Test PCS (Medac)	Initial	15	0.11 – 3.89	0.30	0.39	94.81%	91.6-97.1%
	Repeat	8	0.11 – 9.90	0.40	0.30	97.23%	94.6-98.8%

Sensitivity Findings

Two hundred and two anti-CMV positive specimens were tested by the six assays under evaluation. Initial sensitivities ranged from 93.4% to 100% and repeat sensitivities from 99.5 to 100% (Table 4).

Table 4: Sensitivity of six CMV kits under evaluation

Assay	Number negative	Number negative	Range CO/OD	Mean CO/OD	Median CO/OD	Sensitivity	95% Confidence Interval
EIAgen CMV Screen Kit (Adaltis)	Initial	1	0.39 -8.61	4.25	4.40	99.50%	97.3-100%
	Repeat	0	1.15 -8.61	4.26	4.37	100%	98.2-100%
Vironostika anti-CMV III (bioMérieux)	Initial	2	0.56 – 10.23	5.56	5.64	99.01%	96.5-99.9%
	Repeat	0	1.02 – 10.23	5.63	5.65	100%	98.2-100%
Mastazyme-CMV (Mast)	Initial	1	0.43 – 7.46	4.67	4.81	99.50%	97.3-100%
	Repeat	0	1.66 – 7.46	4.69	4.83	100%	98.2-100%
Mercia CMV TA (Microgen)	Initial	1	0.87 – 184.88	34.45	22.44	99.50%	97.3-100%
	Repeat	0	1.20 – 184.88	34.45	22.44	100%	98.2-100%
Assay	Number negative	Number negative	Range OD/CO	Mean OD/CO	Median OD/CO	Sensitivity	95% Confidence Interval
Bioelisa CMV colour (Biokit)	Initial	3	0.43 – 4.20	2.55	2.59	98.51%	95.7-99.7%
	Repeat	1	0.77 – 4.20	2.55	2.59	99.50%	97.3-100%
CMV-IgG-ELA Test PCS (Medac)	Initial	0	1.71 – 15.55	10.29	10.63	100%	98.2-100%
	Repeat	N/A	N/A	N/A	N/A	N/A	N/A

Distribution of initial reactivities

The distribution of reactivities for the 289 CMV IgG negative and 202 CMV IgG positive specimens is shown in Figures 1 to 6. Assays with good discrimination have few or no samples wrongly classified and few reactions close to the cut-off.

For the anti-CMV negative specimens, only the Adaltis assay had no false positive results and no results close to the cut-off. For the anti-CMV positive specimens, only the Medac assay had no false negative results and no results close to the cut-off.

Figure 1: Distribution of initial reactivities for EIAgen CMV Screen Kit (Adaltis).

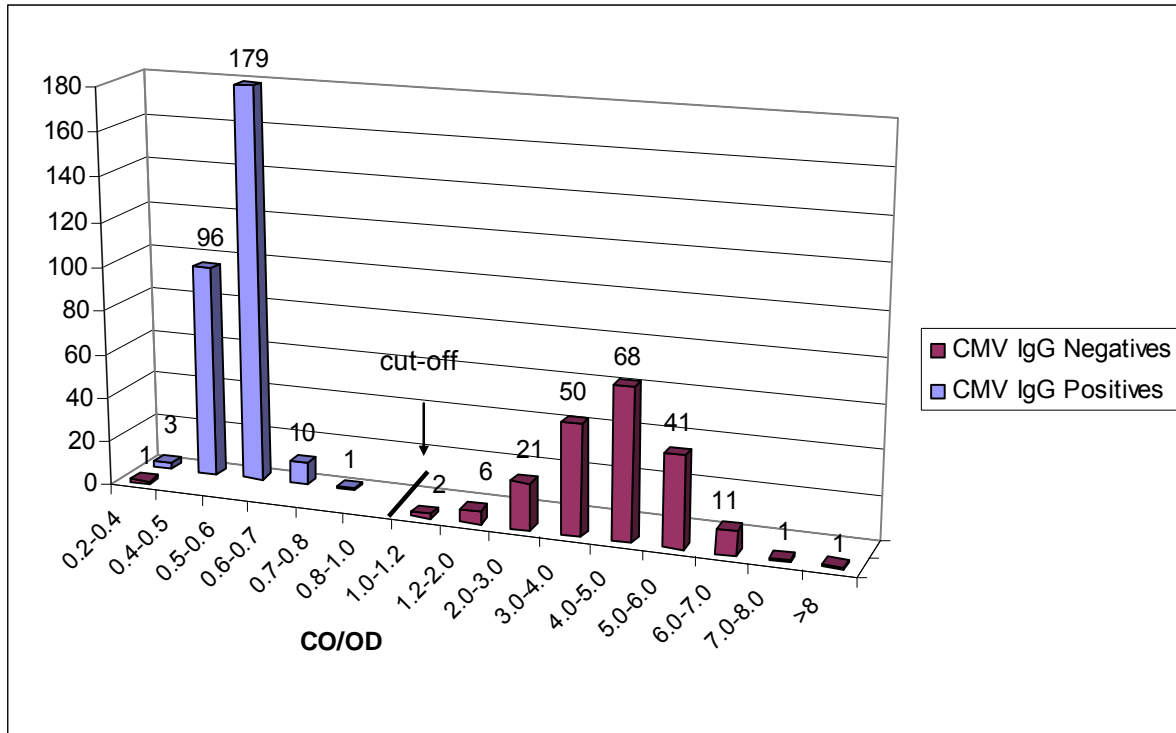


Figure 2: Distribution of initial reactivities for Vironostika anti-CMV III (bioMérieux).

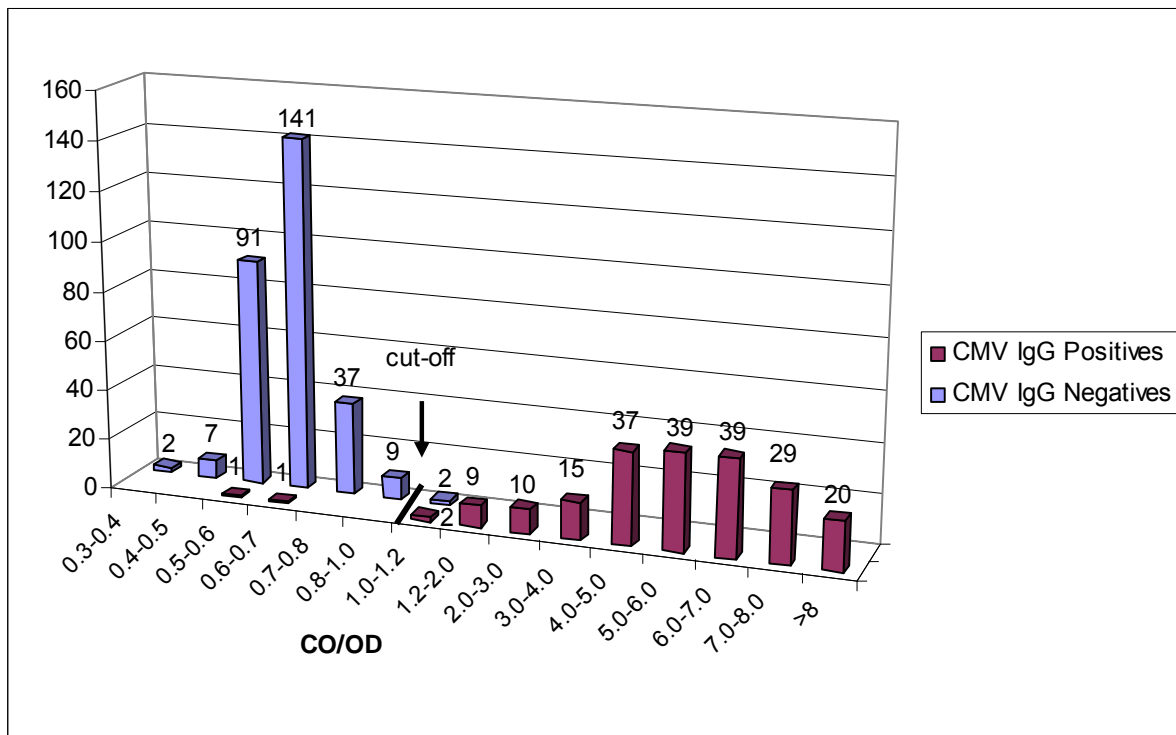


Figure 3: Distribution of initial reactivities for Mastazyme-CMV (Mast).

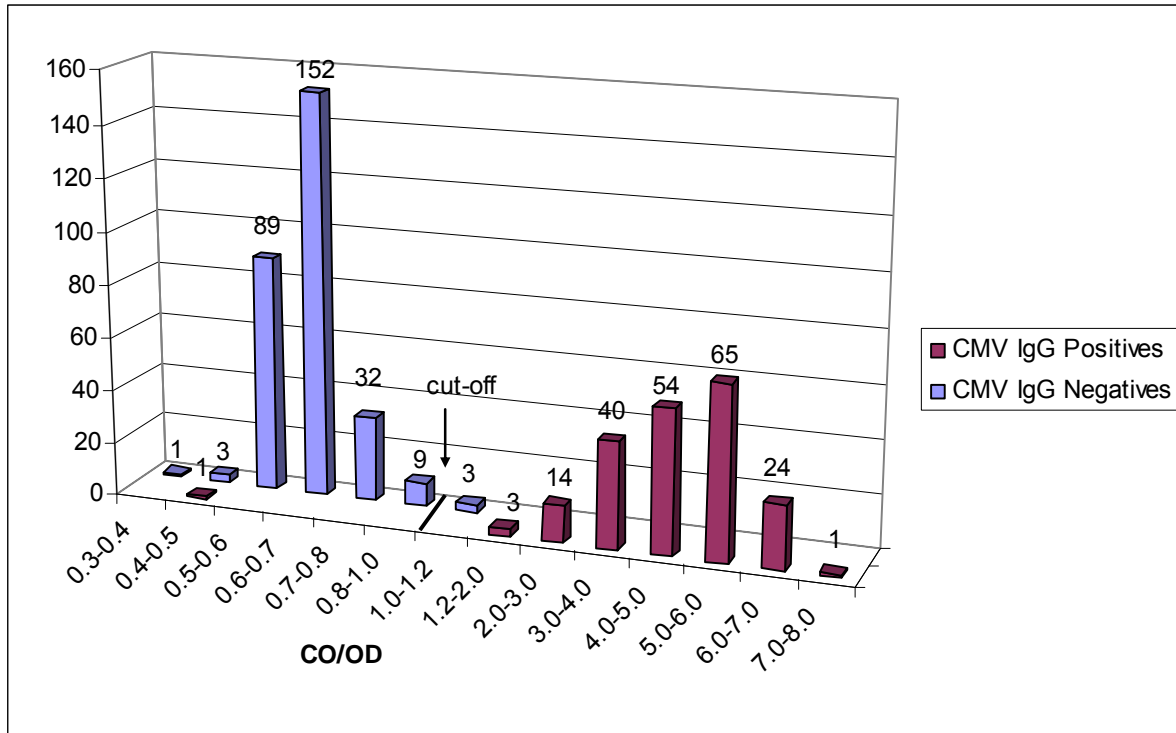


Figure 4: Distribution of initial reactivities for Bioelisa CMV colour (Biokit).

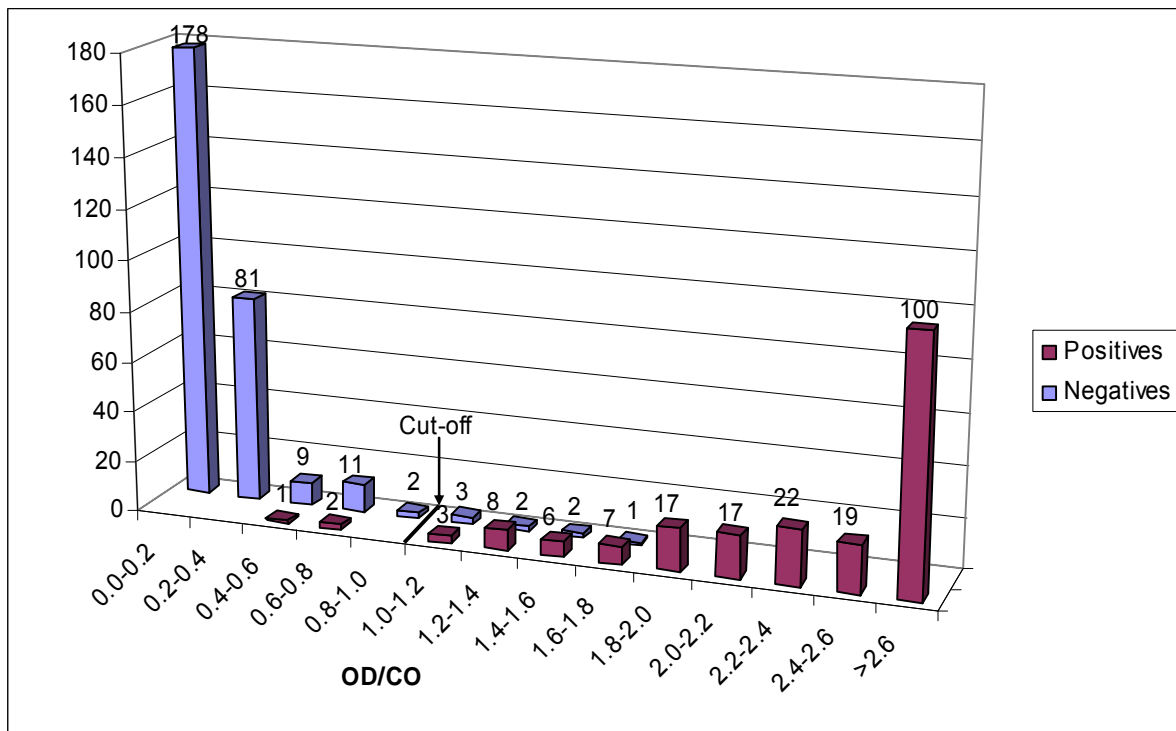


Figure 5: Distribution of initial reactivities for Mercia CMV TA (Microgen).

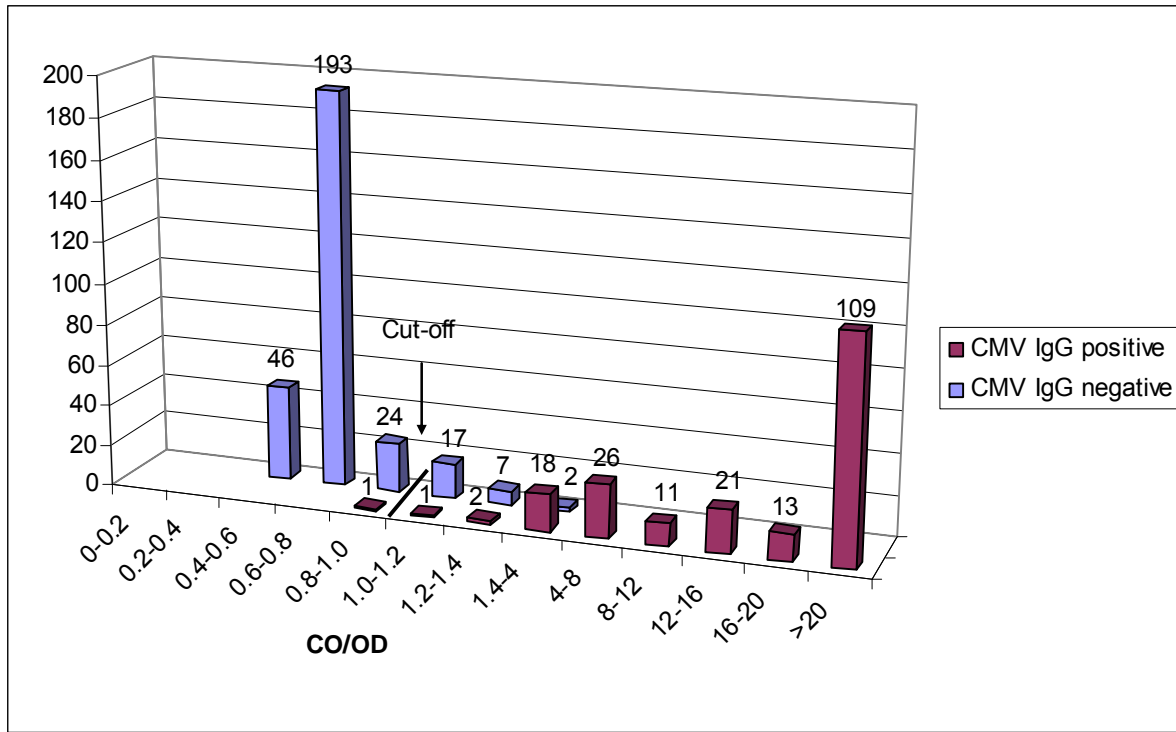
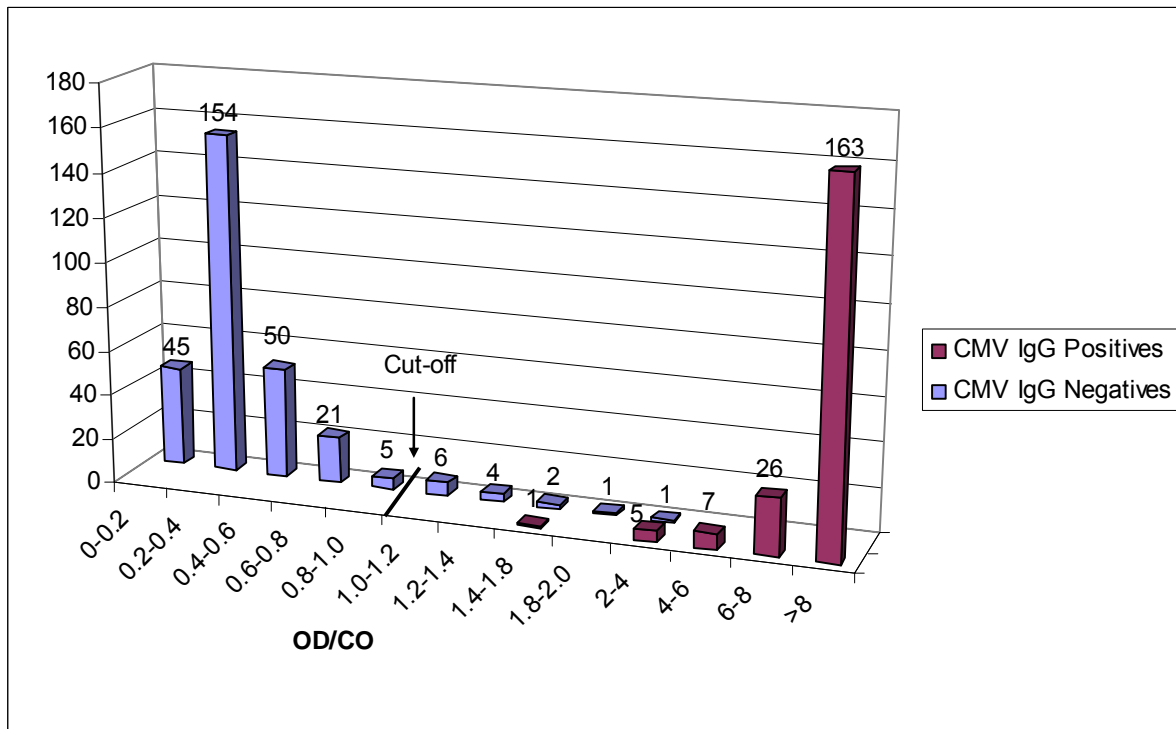


Figure 6: Distribution of initial reactivities for CMV-IgG-ELA Test PCS (Medac).



Indeterminate specimens

Five hundred and fourteen blood donor serum/plasma specimens were initially included in the evaluation panel. It was not possible to confidently assign a status of anti-CMV negative or positive to 23 (4.5%) of the 514 specimens. The result of all testing on the specimens is shown in *Appendix Table 13*.

Seroconversion Sensitivity: Aggregate scores

The ability of the six assays to CMV antibody in two commercial seroconversion panels (RP-003 and PTC901) was assessed. The highest scoring kit was the Microgen, giving a score of 19 out of 24. The bioMérieux assay scored 18, while the Adaltis, Mast, Bioelisa and Medac scored 17 (Table 5 and *Appendix Table 14*). The seroconversion scores should be considered together with the specificity scores, as an increased false positive rate may also increase the seroconversion aggregate score.

Table 5: Combined seroconversion scores (2 panels)

Kit Name	Product Number	Lot Number	Aggregate score (2 panels, n= 24)
Adaltis	81025	206	17
BioMérieux	284124	D27CA	18
Mast	EIA 802	204828	17
Bioelisa	3000-1246	G-4606	17
Microgen	M502	561	19
Medac	115-PKS	CGP34	17

Performance Panel Sensitivity: Aggregate scores

The six assays were also tested for their ability to detect CMV IgG antibody in two performance panels (PTC201 and PTC202). Each panel consisted of 23 anti-CMV positive samples and two anti-CMV negative samples. The bioMérieux, Mast and Medac kits detected all 46 of the 46 true positives, the Adaltis detected 45 and the Bioelisa and Microgen detected 43. The Bioelisa, Microgen and Medac assays each gave a reactive result in one of the four anti-CMV negative samples, while the Adaltis, bioMérieux and Mast assays detected none of the four. All scores have been calculated using the results from the first kit lot tested (Table 6 and *Appendix Table 15*).

Table 6: Combined performance panel scores (2 panels)

Kit Name	Product Number	Lot Number	Aggregate scores* (2 panels)	
			pos = 46	neg = 4
Adaltis	81025	206	45	0
bioMérieux Mast	284124 EIA 802	D27CA 204828	46 46	0 0
Bioelisa	3000-1246	G-4606	43	1
Microgen	M502	561	43	1
Medac	115-PKS	CGP34	46	1

* This refers to the number of samples for which a reactive result was obtained

Batch comparison

In order to assess the variation between different lot numbers of the same kits, 57 of the previously tested CMV IgG positive specimens and 61 previously tested CMV IgG negative specimens were retested (Tables 7 & 8 and *Appendix Table 14*). The seroconversion and performance panels were also retested with the second lot of kits (Tables 9 & 10 and *Appendix Table 15*).

Table 7: Batch comparison of CMV antibody positive specimens

Assay	Lot Number	Number negative	Mean	Range*
EIAgen CMV Screen Kit (Adaltis)	206	0	4.44	1.47 - 8.61
	198-E	0	4.34	2.37 - 6.02
Vironostika anti-CMV III (bioMérieux)	D27CA	0	5.57	1.76 - 10.23
	D27GA	0	8.82	4.46 - 13.47
Mastazyme-CMV (Mast)	204828	0	4.82	2.35 - 7.46
	208550	0	5.55	2.50 - 8.09
Bioelisa CMV colour (Biokit)	G-4606	0	2.46	1.42 - 3.22
	I-1006	0	5.93	2.58 - 8.34
Mercia CMV TA (Microgen)	671	0	34.47	2.18 - 115.08
	561	0	31.54	1.94 - 91.82
CMV-IgG-ELA Test PCS (Medac)	CGP640	0	8.37	2.48 - 13.74
	CGP34	0	10.04	1.37 - 15.65

Note. * CO/OD – Adaltis, bioMérieux, Mast and Microgen; OD/CO – Bioelisa and Medac.

Table 8: Batch comparison of CMV antibody negative specimens

Assay	Lot Number	Number reactive	Mean	Range*
EIAgen CMV Screen Kit (Adaltis)	206	0	0.51	0.27 – 0.61
	198-E	2	0.71	0.48 - 0.94
Vironostika anti-CMV III (bioMérieux)	D27CA	0	0.66	0.51 – 0.86
	D27GA	2	0.71	0.46 - 2.10
Mastazyme-CMV (Mast)	204828	0	0.64	0.33 – 0.87
	208550	2	0.58	0.25 - 0.92
Bioelisa CMV colour (Biokit)	G-4606	0	0.17	0.05 – 0.37
	I-1006	0	0.41	0.15 – 0.79
Mercia CMV TA (Microgen)	671	2	0.73	0.56 – 1.10
	561	1	0.66	0.48 – 0.94
CMV-IgG-ELA Test PCS (Medac)	CGP640	0	0.38	0.15 – 0.86
	CGP34	8	0.46	0.09 - 1.58

Note. * CO/OD – Adaltis, bioMérieux, Mast and Microgen; OD/CO – Bioelisa and Medac.

Table 9: Batch comparison: Combined seroconversion panels (2 panels)

Kit Name	Product Number	Lot Number	Aggregate score (2 panels, n= 24)
EIAgen CMV Screen Kit (Adaltis)	81025	206	17
		198-E	18
Vironostika anti-CMV III (bioMérieux)	284124	D27CA	18
		D27GA	16
Mastazyme-CMV (Mast)	EIA 802	204828	17
		208550	17
Bioelisa CMV colour (Biokit)	3000-1246	G-4606	17
		I-1006	17
Mercia CMV TA (Microgen)	M502	671	19
		561	19
CMV-IgG-ELA Test PCS (Medac)	115- PKS	CGP34	17
		CGP640	18

Table 10: Batch comparison: Combined performance panel scores (2 panels)

Kit Name	Product Number	Lot Number	Aggregate score (2 panels, n= 50)
EIAgen CMV Screen Kit (Adaltis)	81025	206	45
		198-E	46
Vironostika anti-CMV III (bioMérieux)	284124	D27CA	46
		D27GA	46
Mastazyme-CMV (Mast)	EIA 802	204828	46
		208550	46
Bioelisa CMV colour (Biokit)	3000-1246	G-4606	43
		I-1006	46
Mercia CMV TA (Microgen)	M502	671	43
		561	42
CMV-IgG-ELA Test PCS (Medac)	115-PKS	CGP34	46
		CGP640	46

Quality Control Results

Nine anti-CMV positive and five negative commercially available quality control reagents were tested in triplicate using both kit lot numbers. 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.

Table 11: Results for anti-CMV positive QCs (mean of three tests)

Assay	Lot Number	Anti CMV QC1	145 (CMV IgG)	Accurun 146 (CMV IgM)	Accurun 25 (ToRCH IgG)	Accurun 26 (ToRC IgM)	ToRCH Plus Control	Virotrol ToRCH	Virotrol ToRCH M	Virotrol I
EIAgen CMV Screen (Adaltis) CO/OD	206	3.073	3.223	3.548	2.851	3.425	2.903	1.635	2.214	2.410
	198-E	3.293	3.221	4.134	3.561	4.332	4.407	3.518	3.156	2.159
Vironostika anti CMV III (bioMérieux) CO/OD	D27CA	5.785	4.342	6.508	4.864	5.401	4.695	4.604	4.184	2.922
	D27GA	6.068	3.827	6.089	4.560	5.465	8.565	4.872	4.063	2.862
Mastazyme CMV (Mast Diagnostics) CO/OD	204828	4.648	3.482	3.825	3.624	4.462	4.274	3.588	3.245	2.442
	208550	4.583	3.351	4.573	3.826	4.910	5.131	2.608	3.578	2.576
Bioelisa CMV Colour (biokit) OD/CO	G-4606	2.7	1.79	2.608	2.064	2.436	2.342	1.332	1.096	0.382
	I-1006	3.491	3.426	3.532	2.441	3.994	2.721	1.674	1.457	0.473
Mercia CMV TA (Microgen Bioproducts) CO/OD	561	15.953	3.501	9.633	4.806	4.701	9.191	6.335	5.288	1.193
	671	2.775	13.547	4.289	8.631	5.023	4.357	8.842	5.532	5.393
CMV-IgG-ELA (Medac Diagnostika) OD/CO	CGP34	12.688	11.888	13.353	13.998	13.73	14.627	15.171	16.018	4.153
	CGP640	7.871	6.484	7.65	8.867	8.84	8.795	9.904	11.277	4.506

Note. * CO/OD – Adaltis, bioMérieux, Mast and Microgen; OD/CO – Bioelisa and Medac.

Table 12: Results for anti-CMV negative QCs (mean of three tests)

Assay	Lot Number	Liquichek Plus Control	Accurun 800 ToRCH	Viroclear ToRCH	Viroclear	Accurun 810	Accurun 810
EIAgen CMV Screen (Adaltis) CO/OD	206	0.481	0.457	0.359	0.466	2.148	0.467
	198-E	0.499	0.490	0.447	0.477	2.06	0.486
Vironostika anti CMV III (bioMérieux) CO/OD	D27CA	0.505	0.515	0.556	0.514	1.893	0.530
	D27GA	0.573	0.607	0.567	0.568	1.666	0.600
Mastazyme CMV (Mast Diagnostics) CO/OD	204828	0.487	0.488	0.454	0.478	1.895	0.531
	208550	0.550	0.542	0.507	0.524	1.739	0.575
Bioelisa CMV Colour (biokit) OD/CO	G-4606	0.018	0.103	0.29	0.295	0.197	0.197
	I-1006	0.052	0.167	0.374	0.395	0.358	0.358
Mercia CMV TA (Microgen Bioproducts) CO/OD	561	0.563	0.771	0.595	0.638	0.665	0.665
	671	1.184	0.517	0.909	0.603	0.709	0.709
CMV-IgG-ELA (Medac Diagnostika) OD/CO	CGP34	0.354	1.281	0.602	0.482	0.424	0.424
	CGP640	0.376	0.024	0.212	0.21	0.014	0.014

Note. * CO/OD – Adaltis, bioMérieux, Mast and Microgen; OD/CO – Bioelisa and Medac.

Conclusion

All six kits gave good sensitivities which ranged from 98.51% to 100%. However, the specificities varied significantly; from 100% for the Adaltis assay to 88.6% for the Microgen assay (initial specificities). At the end of the evaluation Microgen Bioproducts requested that the 33 initially false positive specimens be retested with a new cut off formulation. Retesting of the 33 samples indicated that a higher specificity, estimated at approximately 99.3%, would be achieved for Microgen with the new cut off formulation.

The aggregate scores of the seroconversion and performance panels for all kits were similar. The seroconversion aggregate scores ranged from 17 to 19, while the performance panel scores ranged from 44 to 46. These scores did not vary significantly when tested with a second lot of the kit.

There was also good correlation of detection of positive and negative samples between different kit lots except for the Medac. This kit detected 8 new false positives with a second kit lot number.

References

1. Joint UKBTS / NIBSC Professional Advisory Committee: CMV Seronegative vs. Leucodepleted blood components for at risk recipients. Position Statement confirmed July 2005 available from www.transfusionguidelines.org.uk)
2. National Blood Service statement of need for the provision and support of microbiology test kits with or without associated equipment, operating software, maintenance and associated consumables, NBS480
3. Curtis, J: Market Review of commercially available CMV screening kits Submitted to the NBS Kit Evaluation Group, May 2006

Appendix

Table 13: Uncharacterised specimens

Sample ID	Vidas	Vidas	1st	IE1	p150	CM2	p65	gB1	gB2	2nd	IE1	p150	CM2	p65	gB1	gB2	Adaltis		bioMérieux		Mast		Bioelisa		Microgen		Medac	
	IgG	IgM	W. blot							W. blot							Initial	repeat	Initial	repeat	Initial	repeat	Initial	repeat	Initial	repeat	Initial	repeat
06M0020a	Neg	Neg	pos	-	-	2+	2+	-	-	border	-	+/-	+	+	+/-	-	Neg		Neg		Neg		Neg		Pos	pos	Neg	
06M0026a	Neg	Neg	neg	-	-	-	-	-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Pos	n/t	Equiv	equiv	Neg	
06M0031a	-	-	neg	-	-	-	+/-	-	-	n/t	-	-	-	-	-	-	Neg	Pos	n/t	Pos	pos	Neg		Neg		Neg		
06M0040a	Neg	Neg	neg	-	-	-	-	-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Pos	n/t	Pos	pos	Neg	
06M0104a	Neg	Neg	neg	+/-	-	-	-	-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Equiv	Equiv	Pos	pos	Neg	
06m0142a	Neg	Neg	neg	-	-	-	+	+	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Pos	n/t	Pos	pos	Neg	
06M0143a	Neg	Neg	neg	-	-	-	+	+	-	neg	-	-	-	+/-	+/-	-	Neg		Neg		Neg		Equiv	Equiv	Pos	pos	Neg	
06M0201a	POS	Neg	neg	-	-	-	+	+/-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Pos	Pos	Equiv	pos	Pos	neg
06M0202a	Neg	Neg	neg	-	-	-	-	-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Equiv	n/t	Pos	pos	Neg	
06M0257a	Neg	Neg	neg	-	-	-	+/-	+/-	-	n/t	-	-	-	-	-	-	Neg		Neg		Neg		Equiv	Pos	Pos	pos	Neg	
06m0270a	Neg	Neg	pos	-	+	-	3+	-	-	border	-	-	-	2+	-	-	Neg		Neg		Neg		Neg		Neg		Neg	
06M0300a	Neg	Neg	pos	-	+	+/-	-	-	-	neg	-	-	-	-	-	-	Neg		Neg		Neg		Neg		Pos	equiv	Neg	
06m0305a	Neg	Neg	border	-	-	-	3+	-	-	neg	-	-	-	+	+/-	-	Neg		Neg		Neg		Pos	n/t	Equiv	n/t	Pos	n/t
06M0307a	-	-	n/t	-	-	-	-	-	-	neg	-	-	-	-	-	-	Pos		Pos		Pos		Neg	n/t	Neg	n/t	Pos	
06M0342a	Neg	Neg	pos	-	+	-	+	-	-	border	-	+/-	-	-	-	-	Neg		Neg		Neg		Neg		Neg		Pos	pos
06M0363a	-	-	neg	-	-	-	+	-	-	neg	-	-	-	-	-	-	Neg		Neg	Pos	Equiv	pos	Neg		Neg		Neg	
06M0394A	-	-	neg	-	-	-	+/-	-	-	neg	-	-	-	-	-	-	Neg		Neg	Neg	Equiv	Equiv	Neg		Neg		Neg	
06M0417A	-	-	neg	-	-	-	+/-	+/-	-	neg	-	-	-	+/-	+/-	-	Neg		Neg	Equiv	Neg		Neg		Neg		Pos	equiv
06M0425A	Neg	Neg	border	-	-	-	2+	3+	-	border	-	-	-	2+	2+	-	Neg		Neg		Neg		Equiv	neg	Pos	pos	Neg	
06M0479A	Neg	Neg	neg	-	-	-	-	-	-	n/t	-	-	-	-	-	-	Equiv	Neg	Pos	Pos	Pos	pos	Neg		Neg		Neg	
06M0484A	-	-	neg	+	-	-	-	-	-	border	+	-	-	+/-	+/-	-	Neg		Neg	Neg	Equiv	equiv	Neg		Neg		Neg	
06M0487A	Neg	Neg	neg	-	-	-	2+	-	-	n/t	-	-	-	-	-	-	Equiv	Pos	Pos	Pos	Pos	pos	Neg		Neg		Neg	
06M0510A	Neg	Neg	neg	-	-	-	+/-	+/-	-	n/t	-	-	-	-	-	-	Neg		Pos	Pos	Equiv	pos	Neg		Neg		Neg	

Note. n/t = not tested. Border = borderline result

Table 14: Seroconversion panel scores

Panel number	EIAgen CMV Screen (Adaltis) CO/OD		Vironostika anti CMV III (bioMerieux) CO/OD		Mastazyme CMV (Mast Diagnostics) CO/OD		Bioelisa CMV Colour (biokit) OD/CO		Mercia CMV TA (Microgen Bioproducts) CO/OD		CMV-IgG-ELA (Medac Diagnostika) OD/CO	
	Lot 1 206	Lot 2 198-E	Lot 1 D27CA	Lot 2 D27GA	Lot 1 204828	Lot 2 208550	Lot 1 G-4606	Lot 2 I-1006	Lot 1 671	Lot 2 561	Lot 1 CGP34	Lot 2 CGP640
PTC901-01	0.491	0.538	0.613	0.528	0.722	0.663	0.288	0.388	0.707	0.619	0.219	1.531
PTC901-02	0.508	0.556	0.617	0.506	0.734	0.652	0.292	0.358	0.660	0.632	0.237	0.520
PTC901-03	0.431	0.565	0.596	0.503	0.722	0.671	0.251	0.341	0.683	0.730	0.237	0.429
PTC901-04	0.333	0.530	0.629	0.500	0.720	0.610	0.240	0.324	0.741	0.693	0.329	3.870
PTC901-05	0.508	0.656	0.625	0.555	0.577	0.738	0.727	1.011	1.202	1.049	1.242	1.181
PTC901-06	1.061	1.534	1.731	0.938	1.922	1.264	2.747	4.559	2.983	2.496	2.822	4.441
PTC901-07	1.809	2.359	3.306	1.888	3.881	2.795	3.207	5.857	3.874	4.017	7.306	8.119
PTC901-08	2.509	2.934	4.536	2.514	2.808	3.408	3.373	6.649	4.865	6.151	8.320	8.322
PTC901-09	2.857	3.817	4.772	3.887	5.642	4.302	3.412	6.312	8.136	6.396	8.735	13.734
RP003-01	0.573	0.755	0.845	0.611	0.677	0.719	0.273	0.460	0.820	0.754	0.306	0.661
RP003-02	0.779	1.010	1.064	0.760	0.945	0.843	0.520	0.716	1.691	1.020	0.289	0.326
RP003-03	1.698	1.827	2.583	1.612	1.977	1.819	1.582	2.145	2.367	1.156	0.403	0.208
RP003-04	3.765	4.942	3.522	5.931	3.612	5.642	2.354	4.514	2.754	1.734	8.922	1.098
RP003-05	3.839	5.275	8.610	7.009	3.807	5.474	2.561	4.184	2.934	2.404	9.480	8.466
RP003-06	3.338	4.514	4.717	6.337	5.122	5.820	2.625	3.997	3.805	3.071	10.314	7.292
RP003-07	4.196	5.103	6.780	6.704	4.815	5.759	2.499	4.241	3.563	3.160	10.453	7.242
RP003-08	4.079	4.558	7.137	7.117	4.301	6.076	2.431	3.598	3.233	3.280	10.260	8.517
RP003-09	3.996	4.791	7.044	7.009	6.402	5.882	2.288	3.976	2.655	2.980	10.071	8.475
RP003-10	12.770	4.429	9.193	7.228	5.315	5.882	2.149	3.569	2.665	3.185	9.644	7.759
RP003-11	4.257	3.557	8.892	8.116	4.334	6.212	2.116	3.154	2.404	3.151	10.335	8.233
RP003-12	4.484	4.840	9.516	7.710	4.268	6.212	2.128	3.329	3.685	3.176	9.715	8.606
RP003-13	4.518	5.589	9.516	8.261	3.859	6.826	2.406	3.891	3.774	2.788	9.438	8.386
RP003-14	3.059	4.649	6.164	7.976	3.682	6.212	2.041	3.081	2.481	3.647	10.243	7.381
RP003-15	3.996	5.103	6.866	8.261	4.436	6.999	2.097	3.341	2.476	3.944	11.019	7.945

Table 15: Performance panel scores

Panel number	EIAgen CMV Screen (Adaltis) CO/OD		Vironostika anti CMV III (bioMerieux) CO/OD		Mastzyme CMV (Mast Diagnostics) CO/OD		Bioelisa CMV Colour (biokit) OD/CO		Mercia CMV TA (Microgen Bioproducts) CO/OD		CMV-IgG-ELA (Medac Diagnostika) OD/CO	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
	206	198-E	D27CA	D27GA	204828	208550	G-4606	I-1006	671	561	CGP34	CGP640
PTC201-01	2.443	4.891	7.622	5.711	2.987	6.190	1.650	2.537	1.713	1.803	6.863	5.689
PTC201-02	3.277	6.019	8.071	8.116	2.745	7.308	2.952	5.507	20.062	18.904	12.489	11.446
PTC201-03	2.228	2.497	3.015	2.542	3.062	3.048	2.232	4.399	4.270	3.330	10.178	12.175
PTC201-04	1.921	3.817	7.518	7.976	5.916	6.066	2.766	4.605	5.175	4.925	6.123	9.390
PTC201-05	2.544	6.178	7.954	9.441	5.253	7.308	2.458	4.256	5.465	5.638	9.900	7.915
PTC201-06	0.558	0.603	0.603	0.522	0.685	0.598	1.008	0.817	0.658	0.585	0.470	0.339
PTC201-07	2.265	5.524	6.166	8.411	4.581	6.816	1.420	2.271	3.306	2.724	6.676	6.073
PTC201-08	3.504	5.217	6.381	9.638	1.830	8.088	3.779	7.926	56.327	35.708	13.740	11.186
PTC201-09	3.277	5.459	7.730	8.411	2.795	7.678	2.313	3.725	6.657	6.559	8.849	6.938
PTC201-10	3.239	5.524	4.426	5.442	5.974	5.229	1.126	1.041	0.997	1.016	2.101	1.057
PTC201-11	3.618	3.378	6.775	5.257	6.033	5.947	2.253	4.707	3.926	3.098	10.064	12.011
PTC201-12	1.494	5.796	9.147	7.343	4.173	7.398	2.687	4.875	39.581	29.895	9.543	11.706
PTC201-13	2.465	4.649	5.434	6.609	3.931	7.053	1.099	1.715	2.569	2.624	2.132	1.486
PTC201-14	2.718	5.943	5.227	9.071	4.447	7.777	3.522	7.091	43.074	40.172	8.863	8.220
PTC201-15	1.556	4.995	5.965	6.515	4.415	6.066	3.050	5.195	16.642	14.608	11.498	10.181
PTC201-16	2.744	3.283	3.247	4.058	3.224	6.453	0.928	1.159	0.779	0.762	4.219	3.305
PTC201-17	3.044	4.429	4.772	4.244	5.298	4.932	1.236	3.223	2.672	2.300	8.680	10.356
PTC201-18	0.478	0.446	0.478	0.462	0.576	0.494	0.429	0.670	0.624	0.625	0.219	0.181
PTC201-19	5.207	4.514	9.800	7.584	7.430	8.666	3.930	7.947	47.242	38.955	10.457	12.853
PTC201-20	1.365	4.230	6.098	3.235	3.584	4.428	4.416	4.416	20.921	17.610	6.516	5.746
PTC201-21	3.714	5.103	8.071	9.441	4.513	7.489	3.868	7.382	40.681	18.364	13.781	12.209
PTC201-22	1.791	4.155	3.683	4.323	1.991	4.776	1.211	1.972	0.931	0.918	8.877	6.023
PTC201-23	1.869	5.275	3.247	9.843	4.009	7.489	3.789	7.214	40.681	47.611	10.758	12.723
PTC201-24	2.185	3.194	3.759	2.397	3.482	3.408	2.909	5.090	5.882	5.022	13.064	11.977
PTC201-25	2.917	5.657	6.775	8.896	2.649	6.385	2.652	4.277	4.221	4.542	12.717	11.712
PTC202-01	2.917	4.471	4.498	3.761	6.033	4.272	3.528	7.344	12.307	11.903	7.242	8.158
PTC202-02	3.764	3.880	6.860	5.783	6.696	6.190	1.669	2.166	5.287	4.133	10.922	10.407
PTC202-03	1.574	4.558	5.965	3.855	5.120	4.776	3.025	5.886	5.766	5.379	10.662	8.977
PTC202-04	3.842	5.275	6.693	8.567	4.117	6.453	2.338	2.490	12.960	14.948	11.863	8.034
PTC202-05	3.336	4.840	6.031	5.442	3.627	5.565	3.578	6.560	13.816	11.082	10.708	13.220
PTC202-06	0.518	0.611	0.584	0.535	0.647	0.647	0.261	0.333	1.013	0.972	0.224	0.124
PTC202-07	1.407	5.217	8.191	7.343	3.761	6.816	0.996	1.226	0.923	0.947	4.078	3.175
PTC202-08	3.526	5.459	8.575	8.411	7.085	7.308	2.070	2.587	2.095	2.157	9.995	9.266
PTC202-09	4.096	5.657	3.326	5.573	6.552	7.398	3.217	5.600	34.058	33.829	13.365	13.706
PTC202-10	3.869	4.558	8.191	5.198	6.770	5.777	3.542	6.038	48.817	51.420	13.438	14.237
PTC202-11	0.930	1.714	1.805	1.094	1.841	1.605	2.052	3.510	4.034	3.803	2.361	1.661
PTC202-12	2.979	4.268	7.954	7.117	3.881	6.740	0.932	1.327	0.791	0.795	3.201	2.079
PTC202-13	3.316	1.792	5.965	1.821	5.642	4.127	0.944	4.058	37.551	35.708	8.521	6.740
PTC202-14	1.526	4.307	6.308	6.609	3.348	6.254	1.625	2.166	3.689	3.246	5.781	4.475
PTC202-15	3.482	4.742	8.315	8.411	5.590	7.136	1.965	2.461	10.848	10.202	13.594	11.288
PTC202-16	3.594	4.388	8.997	9.638	7.085	7.878	2.884	4.437	2.816	2.546	10.521	10.542
PTC202-17	4.643	4.791	4.288	8.261	6.347	8.197	3.692	7.247	31.160	30.607	13.087	13.011
PTC202-18	2.786	3.504	6.031	3.855	5.642	3.839	3.497	6.236	13.948	10.803	13.059	15.350
PTC202-19	2.995	4.471	8.443	9.441	7.522	7.221	2.760	4.117	7.286	5.663	10.370	9.542
PTC202-20	3.376	5.217	8.071	8.728	6.552	8.310	2.501	4.226	10.241	8.570	8.676	5.576
PTC202-21	3.594	5.524	6.236	8.896	6.347	7.053	3.466	5.962	7.959	6.949	12.950	12.661
PTC202-22	2.979	5.217	4.498	7.584	6.281	7.053	3.147	5.861	3.390	3.068	8.425	11.316
PTC202-23	3.397	5.217	6.236	8.261	5.208	4.972	3.478	5.933	41.843	47.611	15.489	16.768
PTC202-24	0.522	0.585	0.609	0.494	0.720	0.613	0.226	0.295	0.668	0.669	1.023	0.203
PTC202-25	3.923	4.649	7.036	5.931	6.924	6.319	2.841	4.138	10.170	8.188	14.425	11.401



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Ref: Evaluation of six Cytomegalovirus IgG/Total antibody kits suitable for use in the UK National Blood service

Dear Ms Burgess,

Thank you for evaluating the bioelisa CMV colour in your review of CMV IgG/Total for the UK National Blood Service. We would also like to thank you for giving us the opportunity to review the data and comment on it.

We are pleased with the results of this very comprehensive study, and are delighted to see that obtained figures correspond to the expected product performance already established for the bioelisa CMV colour. Reported sensitivity of 99.50% corresponds to a first line screening assay that promotes sensitivity as the main priority.

The very good results in the sensitivity studies in both samples characterized as positive and the seroconversion panels, demonstrates the assay as a useful tool for blood screening. As the authors comment, the inability of the assay to detect the Commercial quality control material Virotrol I, is due to the fact that the reactivity does not match the dynamic range of the assay, 2-4 ratio OD/COV. The matrix type used in this material and also the fact that it is a diluted positive sample, may explain the lower reactivity. The correct detection of all other commercial QC material, including those for IgM suggest that this may be a particular problem with the Virotrol I control material.

Sensitivity of ELISAs are more influenced by antibody affinity and epitope density than by antibody quantity. Potential confusion can arise when laboratories use diluted controls to evaluate sensitivity of different manufacturer's ELISA tests.

Yours sincerely

Joan Guixer
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2nd April 2008

Dear Christine,

Thank you for giving us the opportunity to comment on the results of the NBS
Evaluation of Six CMV Assays.

I am pleased to note that the report was consistent with the high level of performance
that was expected and mirrors feedback received from customers over the years.

Best regards,

Howard Rose
Managing Director, Mast Diagnostics Division.

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