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SUBJECT **NEW PRODUCT**

We announce the launching of a new member of the bioelisa family

bioelisa CMV colour

96 tests Code 3000-1245

480 tests Code 3000-1246

This assay is intended for the detection of TOTAL antibodies to CMV in human serum or plasma.

bioelisa CMV colour is suitable for blood bank purposes. It incorporates several features that facilitate the CMV screening in Elisa format:

- IgG + IgM detection**
- In plate dilution**
- Sample and reagent addition monitoring**

Enclosed you will find product information that we hope will be helpful for introducing the new assay.



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Introduction

bioKit is an expert company in Torch diagnosis. Production and purification of raw materials is fully controlled by our company. This fact allows to provide customer with a high consistency in batch to batch production and supply. As bioKit has an important presence in blood banks, world wide, we have designed a new product, specially oriented to the CMV blood bank serology screening.

Free CMV donations are required for special recipients: Those immunosuppressed patients, waiting for a transplantation or newborns with immature immune system. In both cases a CMV transmission by blood transfusion may produce fatal results.

Product description

bioelisa CMV colour is an ELISA test for detection of antibodies to *Cytomegalovirus* in human serum or plasma. The test is performed by incubating diluted test specimen in an antigen-coated well. Antibodies to CMV, if present in the specimen, will combine with the antigens attached to the well. The plate is then washed to remove residual test specimen, and enzyme-labelled antibodies to human IgG and IgM (conjugate) are added. The conjugate will bind to anti-CMV antibodies that have combined with the antigens on the well during the first incubation. After another washing to eliminate unbound material an enzyme substrate solution containing a chromogen is added. This solution will develop a blue colour if the sample contains anti-Cytomegalovirus antibodies. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of the colour is proportional to the amount of anti-CMV antibodies in the test specimens.

Protocol

- Sample dilution 1:21 In plate dilution 200 µl diluent + 10 µl sample
- Controls are ready to use.
- Sample incubation: 20 minutes at Room Temperature
- 4 wash cycles
- Conjugate addition: 100 µl
- Conjugate Incubation: 20 minutes at Room Temperature
- 4 wash cycles
- Substrate addition: 100 µl
- Substrate Incubation: 10 minutes at Room Temperature
- Stop addition: 100 µl
- Reading 450/620 nm

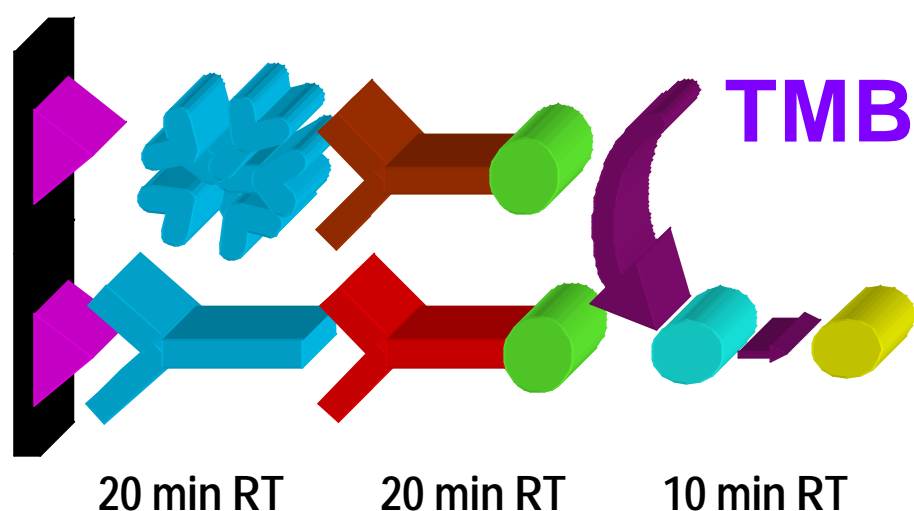
Product characteristics

Method

bioelisa CMV colour is a new assay that utilizes the indirect method format: CMV natural viral antigens are coated onto the solid phase. Conjugate is based on a mixture of anti-human IgG and anti-human IgM, labelled with peroxidase. See figure 1

To close the seroconversion window, **bioelisa CMV colour** is able to detect both IgG and IgM antibodies. There is a risk of CMV transmission by blood donors, early infected by CMV. Those samples would be missed by methods using only IgG detection.

Figure 1



Incubation time

This is a very fast assay, with less than one hour of total incubation and handling time, for obtaining the final results.

The assay is designed to be automated in Elisa instruments present in the blood bank laboratories.

The 20 minutes at room temperature incubation time makes necessary to take precautions in the elisa instruments, not to produce overincubation in the first samples dispensed.

- Large 8 probes Elisa instruments are able to dispense the full plate in less than 5 minutes.
- Standard Elisa instruments with one single probe for dispensing, needs to perform a special washing protocol to compensate the delay in dispensing a full plate.

Performing on best 2000

To overcome the problem of time to dispense the full plate, the biokit best 2000 instrument includes a synchronized washing.

The Incubation time starts at the moment that **the first sample** is dispensed. Normally time starts after the last sample is dispensed.

Five to Eight minutes after the last dispensed sample in a full plate, the washing process starts for the first strip. Seems strange, but this is because the 20 minutes incubation time for the first strip is over.

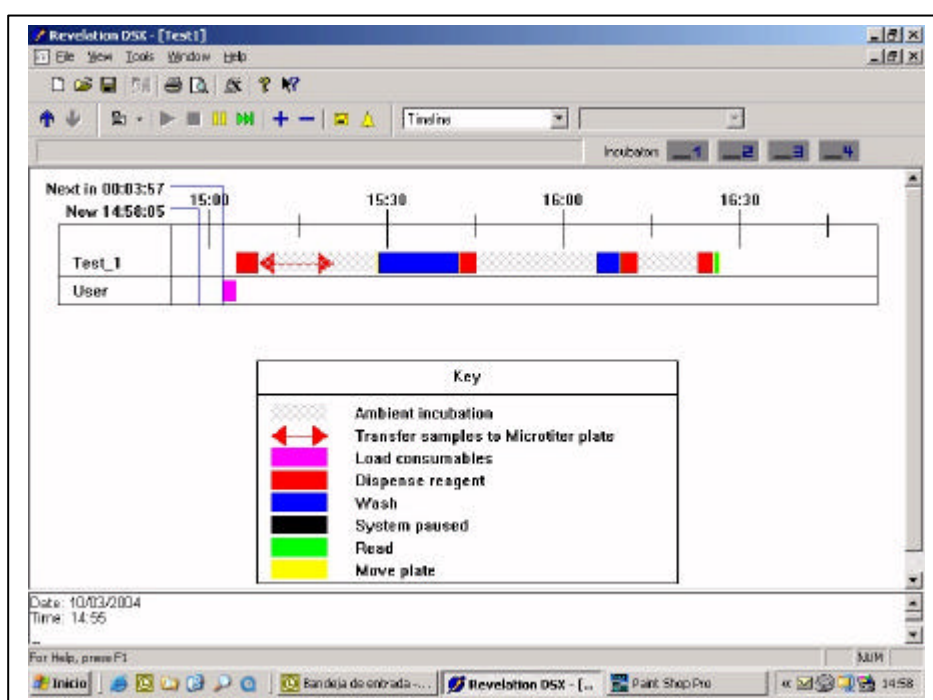
The synchronized washing automatically compensates the incubation time, to make it equal in the whole plate. It is performed by an automatic delay in the washing of every strip. Washing is focused in a strip mode: The 4 wash cycles are performed in the same strip. Wash solution remains in the well after the last aspiration. Washing of the next strip starts when incubation time of the samples in this strip reaches the correspondent 20 minutes.

Following strips are performed in the same way.

For a full plate, the initial wash takes approximately 25 minutes.

In *figure 2* it appears the best 2000 scheduler for a full plate performance. The blue rectangle indicates the time need for performing the wash. The wash after sample incubation is much longer that one after the conjugate incubation. This is the synchronized wash that allows sample and controls to be incubated exactly 20 minutes regardless its position. Washing is enlarged by strip mode washing and automatically soak times.

Figure 2



In plate sample dilution

This is one of the main advantages of the assay. Sample dilution does not need to be performed outside in a tube. The sample dilution 1/21 can be performed directly in the microplate, saving time and further manipulation.

This is a critical issue, as external sample dilution can be hardly performed in a blood bank

Sample addition monitoring

Sample diluent is able to change its colour after sample addition. This fact allows to check that sample has been really dispensed, both visual and spectrophotometrically.

Sample diluent is violet coloured. After adding sample, colour becomes blue. Manual dispensing of plates is facilitated by this feature. Optical density of the diluted sample can be read at 620 nm with single reading. Diluted samples produce optical density higher than 0.500 absorbance units. Automated Elisa instruments able to read absorbances before sample incubation, can be used for sample monitoring. Table 1 and 2 show a real example of a plate with sample

diluent before and after sample addition. First row is dedicated to the controls, while last row has not been dispensed to see the difference.

Table 1
Optical densities of 200 μ l of sample diluent at 620 nm.
Row 1 is not dispensed as controls are ready to use

	1	2	3	4	5	6	7	8	9	10	1	12
A	BL	467	466	467	468	464	467	464	464	464	462	463
B	NC	469	462	467	468	467	473	468	471	471	473	475
C	NC	460	467	459	458	458	461	468	467	464	462	464
D	LPC	454	456	457	451	461	455	450	448	455	453	452
E	LPC	465	456	455	461	455	461	455	455	454	459	459
F	LPC	454	461	461	455	459	461	459	458	457	467	462
G	HPC	460	462	460	459	459	461	457	459	456	459	458
H	HPC	455	453	453	454	453	455	454	453	454	455	456

Table 2
Optical densities of 200 μ l of sample diluent + 10 μ l of sample at 620 nm
Row 12 has not been dispensed to see the OD's difference
Ready to use controls include a yellow and green dye for negative and positive controls

	1	2	3	4	5	6	7	8	9	10	1	12
A	32	837	657	648	625	669	722	703	729	622	707	463
B	2638	636	642	677	627	622	717	783	773	684	669	475
C	2638	694	638	628	620	628	699	745	610	659	651	464
D	784	639	651	617	607	618	624	627	669	617	637	452
E	787	619	618	621	601	607	678	678	683	635	640	459
F	800	669	638	633	619	648	648	606	596	624	649	462
G	569	684	638	650	636	616	644	803	714	653	658	458
H	561	670	603	645	653	616	617	704	669	662	651	456

Conjugate and substrate have a colour code that can be read also by spectrophotometrical reading at 450 nm, (single reading) to check its addition:

Conjugate dispensed wells > 0.400 absorbance units

Substrate dispensed wells > 0.060 absorbance units

Automated elisa instruments like bests 2000 allows the sample and reagent monitoring in its capabilities. After sample incubation and before conjugate and substrate addition, the robotic arm moves the plate the reader to monitor the addition. Optical densities are compared to a reference value to determinate if the sample or reagent has been added

In case of sample monitoring, it has to be performed after incubation time; otherwise it can not be possible to make the synchronized washing.

Reference values for samples and reagent are only indicatives. Each laboratory have to establish its own reference values, according to their populations.

Cut-Off control

Cut-off value depends on a dedicated low positive control. This control produces the minimum reactivity correspondent to a clinical significant sample. Reactivity of this control, adapts itself to the day to day run conditions.

In a hypothetical example of extreme results lower or higher than expected, but under the validation criteria, the Low positive control moves its reactivity accordingly. *see table 3*

No unexpected false positive or false negative are foreseen because microplate produces too much or too less reactivity.

Table 3

	NC	Cut-Off control	PC
Normal conditions	0.044	0.400	1.650
Low reactivity RUN	0.018	0.290	1.120
High reactivity RUN	0.060	0.510	2.100

The cut-serum produces lower absorbance in the first case. Low positive samples would produce also proportional low reactivity, so no false negative will be reported.

In second situation the cut-off control would produce much higher absorbance. True negative samples, but with slightly high reactivity, will not reach the cut-off value, so no false positive will be expected.

CE mark

bioelisa CMV colour belongs to the special certified products included in the class II B products. Only companies with a demanding certified quality system are able to obtain the CE mark for such kind of products.

The number written below of the CE mark logo in the package insert, 0843, corresponds to the identification of the notified body that audits biokit for the research, development and production of the In vitro diagnostic products.

biokit SA is audited by UL Laboratories based in United Kingdom and the United States of America

See enclosed certificate in annex 4



Package insert and official biokit labels are enclosed in annexes 1 and 2 respectively.

bioelisa CMV colour vs. bioelisa CMV IgG

There are several differences between the two assays that are summarized in the table below:

Test name	bioelisa CMV IgG	bioelisa CMV colour
Code number	3000-1216	3000-1245/3000-1246
Method	EIA/HRP/TMB	EIA/HRP/TMB
Format	2 - step indirect sandwich	2 - step direct sandwich
Solid phase	12x8 Microtiter wells	12x8 Microtiter wells
Test per kit	96	96 and 480
Coating	CMV natural antigens	CMV natural antigens
Detection	IgG	IgG+IgM
Conjugate	Anti-human IgG	Anti-human IgG Anti-human IgM
Substrate	TMB	Coloured TMB
Specimen dilution	1:100 tube dilution 10 µl sample 1000 µl diluent	1:21 In well dilution 10 µl sample 200 µl diluent
SAMPLE MONITORING	No	Yes
Reagent Monitoring	No	Yes
Controls	- Negative control - Low positive calibrator - High positive calibrator	- Negative control - Low Positive control - Positive control
Sample incubation	60 min / 37°C	20 min / RT
Conjugate Incubation	30 min / 37°C	20 min / RT
Substrate incubation	30 min / RT	10 min / RT
Filter Wavelength	450 nm	450 nm
Cut-off	- Low positive calibrator	Low positive control
Quantitative assay	Yes	No, only qualitative

bioelisa CMV colour: Key features and benefits

Features	Benefits
Total antibody detection	- Suitable for blood bank - Enhanced sensitivity - Extra security for blood receptors
In plate dilution	Easy and fast performing No need of external tube dilutions
20 min incubation time	Very fast assay accepts high workload
Cut-off serum	Adapts sensitivity and specificity to the daily run conditions
Sample addition monitoring	Allows to control sample addition in both manual or automated dispensing
Performance	Excellent results with internal and external evaluations.
Official approvals	CE mark in the class II B products

Automated performance:

The CE mark is a new European In vitro Diagnostic regulation, that requires that all the ELISA assays must be validated in the automated Elisa instruments. End users running elisa in automated instruments may be asked, by national health authorities, to have the validated protocol on the type of instrument they are using in the laboratory.

It has to be proved that the automated performance in the laboratory instrument, produces a comparable result versus the manual performance, which is considered as reference.

bioelisa is in process to validate the whole bioelisa product range in the best 2000 and the Biomaster junior

In annex 3 it is included the validation of the bioelisa CMV colour used in combination with the best 2000, where it is informed and documented the correlation between manual an automated performance.

Document is organized as a scientific article, with the introduction, material and methods, results and conclusion.

It has been run panel of about 150 samples, manually and automatically to check sensitivity, specificity and reproducibility intra-run. It includes description of the instrument protocol that has been validated. This is the official bioelisa protocol that bioelisa guarantees it works correctly.

Product performance:

bioelisa CMV colour has been evaluated before launching. Internal and external evaluations have been conducted to assess the product performance.

Internal evaluations

Sensitivity and specificity

The kit was evaluated by testing 642 serum samples from blood bank donors in parallel with another commercial EIA: Captia CMV TA.

449 samples were classified as positive and 190 as negative assuming Captia as a reference test. Three samples were classified as equivocal and were eliminated to perform calculations. After retesting discrepant samples, two were classified as false positive by bioelisa and one as false negative, giving the following figures: Sensitivity 99.8% (448/449), specificity 98.9% (188/190) and overall agreement 99.5% (636/639).

		bioelisa CMV colour	
		+	-
CAPTIA	+	448	1
	-	2	188

Sensitivity	(448/449)	99.8%
Specificity	(188/190)	98.9%
Agreement	(636/639)	99.5%

biokit internal CMV QC panel

An internal panel of 22 well characterised positive samples with different reactivity and 40 fresh unselected blood donor samples were tested. In addition, two dilutions of the CLB (WHO proposed standard) and CRB standard were also tested.

All positive and negative samples were correctly detected. Correlation obtained was 100%.

Precision

Intra-assay reproducibility:

The coefficient of variation obtained for the absorbance values of a positive sample assayed in 24 replicates were 5.03%, 3.89% and 4.01% in three lots studied.

Inter-assay reproducibility:

Three positive samples of different levels were tested in triplicate in 5 different assays. The coefficients of variation obtained for the ratios absorbance/cut-off of the 3 samples were 3,6%, 4,5% and 4,9%, respectively.

Interferences

To study possible interferences, samples with potential risk of producing cross-reactions were tested, including sera positive for Rheumatoid Factor (RF), anti-nuclear antibodies (ANA), anti-Herpes simplex (HSV), anti-Epstein Barr (EBV-VCA) and from pregnant women. No evidences of interference were observed.

External evaluation

Australian Red Cross, blood services

bioelisa CMV colour was evaluated along with Zeus, Organon and Biorad EIA assays with unselected blood donors and commercial mixed titres panels. **bioelisa CMV colour** was awarded by the national tender, because its quality, for 250.000 donations a year.

See annex 5.

Scottish National Blood Transfusion Service

After being tested by the SNBTS Microbiology Test Evaluation Group (MTEG), **bioelisa CMV colour** has been included in the Scottish National Blood Transfusion Service list of approved test.

See annex 6.

Belfast Blood Transfusion Service

The Belfast blood transfusion service is using the Abbott Prism system for most of the blood bank markers. They were using the Old Abbott commander system for total detection of antibodies to CMV. Biokit was successfully evaluated against the old Abbott system. Now, it is installed a best 2000 instrument in this laboratory for the CMV screening.

Product availability:

bioelisa CMV colour is now available in stock for its two version presentation. Product is CE marked and can be sold freely in the whole European Union and related states. For distributors out of the European Union that need to register the new product in its Health Ministry, biokit can provided the required documentation for registration.

Market opportunity:

It is well known that in western countries, most of blood bank uses the Abbott closed chemiluminescence systems: Axym or Prism. Those systems do not have a CMV assay for Total antibody detection. The only available Abbott kit is for detection of just IgG. biokit was awarded in Australia by this fact, because for the rest of serology, the user continue using the Abbott kits. Same situation is present for the syphilis detection. There is no Abbott kit for syphilis based on closed system.

It could be done a package of **bioelisa CMV colour** and **bioelisa Syphilis 3.0**, with the biokit best 2000 instrument, even in a very big blood banks.

biokit has a set of scientific bibliography supporting the need of using total antibody detection kits. It has been described that there is a significant the incidence of only IgM positive blood donors, that can be missed if only a IgG detection assay is used as a screening test. The statistic risk of transmitting a blood unit, seropositive for IgM and negative for IgG is not high, but the damage to the recipient of the blood unit could be very high if it is a in a transplanted patient or a newborn.

Conclusion:

bioelisa CMV colour is an outstanding product that includes all advantages of the new generation Elisas for blood bank serology:

- Total antibody detection
- In plate dilution
- Sample addition monitoring
- High performance.

There is an important commercial opportunity as there is no available kits for CMV Total antibodies detection in the closed Abbott system. A package with the bioelisa Syphilis 3.0 can be also offered.

Annex 1: Package insert

bioelisa CMV colour

ELISA test for detection of antibodies to Cytomegalovirus in human serum or plasma.

Summary

Human cytomegalovirus (CMV) is a member of the Herpesvirus family. It is a ubiquitous agent that commonly infects individuals from diverse geographic and economic areas. Most people become infected with this virus at some time during their life; in the United States, as many as 81% of individuals older than 35 years have been exposed to this virus.

A characteristic of CMV is that it can persist for long periods in a latent form after the primary infection and therefore reactivations of the virus can occur. In normal adults and children, CMV infection, reinfection or reactivation, is usually mild or asymptomatic. However, infections in certain immunocompromised patients and in infants congenitally infected may result in clinically severe disease. Low-birth-weight preterm neonates, transplant recipients, patients receiving immunosuppressive chemotherapy and patients with acquired immunodeficiency syndrome (AIDS), are all at increased risk for serious CMV disease.

Serological tests for detecting the presence of antibodies to CMV can provide valuable information regarding history of previous infection, diagnosis of active or recent infection, as well as in screening blood for transfusion in new-borns and immunocompromised recipients.

Principle

bioelisa CMV colour is an ELISA test for detection of antibodies to Cytomegalovirus in human serum or plasma. The test is performed by incubating diluted test specimen in an antigen-coated well. Antibodies to CMV, if present in the specimen, will combine with the antigens attached to the well. The plate is then washed to remove residual test specimen, and enzyme-labelled antibodies to human IgG and IgM (conjugate) are added. The conjugate will bind to anti-CMV antibodies that have combined with the antigens on the well during the first incubation. After another washing to eliminate unbound material an enzyme substrate solution containing a chromogen is added. This solution will develop a blue colour if the sample contains anti-Cytomegalovirus antibodies. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of the colour is proportional to the amount of anti-CMV antibodies in the test specimens.

Components

- MCPL** MICROPLATE:
12 x 8 wells coated with inactivated CMV antigen. Individually separable wells.
- CONJ 51x** CONCENTRATE CONJUGATE:
Rabbit anti-human IgG and IgM antibodies conjugated with peroxidase. Contains red dye, stabilisers protein, 0.02% thimerosal and 0.001% gentamicin sulphate. To be diluted 1/51 with the conjugate diluent before use.
- DIL CONJ** CONJUGATE DILUENT:
Tris buffer containing yellow dye, additives, 0.02% thimerosal and 0.001% gentamicin sulphate.
- DIL SAMP** SAMPLE DILUENT:
Tris buffer containing detergent, stabilisers protein, dark purple dye and 0.01% thimerosal. Ready to use.
- WASH SOLN 10x** WASHING SOLUTION:
Concentrate phosphate buffer (10x) containing 1% Tween 20 and 0.01% thimerosal. To be diluted 1/10 in distilled or deionised water before use.
- SUBS BUF** SUBSTRATE BUFFER:
Citrate-acetate buffer containing hydrogen peroxide and 0.002% gentamicin sulphate.
- SOLN TMB** CHROMOGEN:
3,3', 5,5'-Tetramethylbenzidine (TMB) dissolved in dimethylsulphoxide (DMSO). Contains red dye.
- CONTROL + H** HIGH POSITIVE CONTROL:
Diluted human serum containing anti-CMV antibodies. Contains stabilisers protein, green dye and ? 0.1% sodium azide. Ready to use.
- CONTROL + L** LOW POSITIVE CONTROL:
Diluted human serum containing anti-CMV antibodies. Contains stabilisers protein, green dye and ? 0.1% sodium azide. Ready to use.
- CONTROL -** NEGATIVE CONTROL:
Diluted human serum negative for anti-CMV antibodies. Contains stabilisers protein, blue dye and ? 0.1% sodium azide. Ready to use.
- H₂SO₄ 1N** STOPPING SOLUTION (only in 1 plate kit):
1N Sulphuric acid. Ready to use.
- SEALS** ADHESIVE SEALS:
To cover microplate during incubations.
- BAG** RESEALABLE BAG:
For storage of unused strips.

Precautions

bioelisa CMV colour is intended for IN VITRO diagnostic use.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 and HCV antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution:

- Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
- Wear gloves.
- Do not pipette by mouth.
- Do not smoke.
- Dispose all used materials in a suitable biohazardous waste container. Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1 hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).
- Certain reagents in this kit contain sodium azide as preservative. Sodium azide may react with lead or copper pipes and plumbing creating highly explosive metal azides. Flush drains with water thoroughly after disposing of the remains of reagents.

Handling instructions:

- Adjust washer to the plate used (flat bottom) in order to wash properly.
- Do not mix reagents from different lots.
- Do not use reagents after expiration date.
- Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
- Use a new pipette tip for each specimen and each reagent.
- It is very important to prepare the substrate-TMB solution just 5-10 minutes before use. Keep it in a well-sealed container and avoid light exposure.
- Soaps and/or oxidising agents remaining in containers used for preparation of substrate-TMB solution can interfere with the reaction. If glass containers are used, they should be washed with 1N sulphuric or hydrochloric acid, rinsed well with distilled water and dried before use. We recommend using disposable plastic containers.

Storage and stability

The components will remain stable through the expiration date shown on the label if stored between 2-8°C. The bag containing the microtiter plate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, microplate strips are stable for 3 months at 2-8°C in the plastic bag tightly sealed, with the silicagel. Once diluted, the washing solution is stable for two weeks if stored between 2-8°C. Once diluted, the conjugate is stable for 15 days at 2-8°C. Store the chromogen in the dark. As the TMB-substrate solution is not stable once prepared, instructions for its use should be closely followed.

Available packaging

- 1 plate kit (96 tests), **REF** 3000-1245.
Contains: 1 plate, 1 x 0.35 ml concentrate conjugate, 1 x 15 ml conjugate diluent, 1 x 30 ml sample diluent, 2 x 50 ml concentrate washing solution, 1 x 14 ml substrate buffer, 1 x 1.5 ml chromogen, 1 x 2.5 ml high positive control, 1 x 4 ml low positive control, 1 x 2.5 ml negative control, 1 x 12 ml stopping solution, 1 resealable bag and adhesive seals.
- 5 plates kit (5 x 96 tests), **REF** 3000-1246.
Contains: 5 plates, 1 x 1.3 ml concentrate conjugate, 1 x 70 ml conjugate diluent, 1 x 120 ml sample diluent, 3 x 100 ml concentrate washing solution, 5 x 14 ml substrate buffer, 1 x 1.5 ml chromogen, 1 x 5 ml high positive control, 1 x 7 ml low positive control, 1 x 5 ml negative control, 1 resealable bag and adhesive seals.

Material required not provided

- Distilled or deionised water.
- Multichannel pipettes and micropipettes (10 µl, 100 µl, 1000 µl) and disposable tips.
- Tubes / microtubes for dilutions.
- Timer.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 630 nm is advisable.
- Manual or automated wash system.
- Stopping solution (5 plates kit): 1N sulphuric acid. 2N or 4N sulphuric acid could also be used.

Sample collection

Use fresh serum or plasma (EDTA). Other anticoagulants should be evaluated before use. Samples can be stored at 2-8°C for 3 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing. Samples showing visible particulate matter should be clarified by centrifugation. Serum or plasma samples should not be heat inactivated, since that may cause incorrect results.

Automatic processing

Automated or semi-automated assay may be used only with autoanalysers adequate for rapid procedures. It is very important to validate any automated system to demonstrate that results obtained for samples are equivalent to the ones obtained using manual assay. It is recommended that the user validate periodically the instrument. If there is any difficulty in the setting of Biotik automatic processors, please contact your distributor.

PROCEDURE (See summary of protocol in the last page)

Previous operations

Allow all the reagents to reach room temperature (20-25°C) before running the assay.

Gently mix all liquid reagents before use.

Dilute the concentrated washing solution 1/10 with distilled or deionised water. For one plate, mix 50 ml of the concentrate solution with 450 ml of water. If less than a whole plate is used, prepare the proportional volume of solution.

Dilute the concentrate conjugate 1/51 with the conjugate diluent according to table 1. **Mix gently.**

TABLE 1

Strips required	1	2	4	6	8	10	12
Conjugate diluent ml	1.0	2.0	4.0	6.0	8.0	10.0	12.0
Concentrate conjugate µl	20	40	80	120	160	200	240

Sample and reagent addition monitoring

The sample diluent contains a colour indicator and all the reagents and controls are coloured. This allows monitoring of sample and reagent addition to the microplate either visually or spectrophotometrically.

The sample diluent is violet colour. After the addition of serum or plasma it will change to blue. The colour change appears after a few seconds. The intensity will vary from sample to sample, but some change should always be visible. Diluted samples or other laboratory preparations may not induce a colour change.

To check the addition of sample, the wells may be read with a filter of 620 nm without reference filter.

The working conjugate is orange. To check its addition, the wells may be read at 450 nm without reference filter.

The working substrate is pink. To check its addition, the wells may be read at 450 nm without reference filter.

Monitoring at other wavelengths should be validated.

NOTE: Each laboratory should establish its own reference ranges.

In the case that a well doesn't meet the established specifications, it indicates that some problem in the dispensing may have occurred, and should be investigated further.

Assay procedure

1. Use only the number of strips required for the test. Reserve 8 wells for blank and controls. Use two wells for the negative and high positive control and three wells for the low positive control. Pipette 200 µl of sample diluent and 10 µl of each sample to the designated wells. Pipette 200 µl of each control to the corresponding wells. **DO NOT DILUTE CONTROLS. THEY ARE READY TO USE.** Leave a well empty for the substrate blank.

NOTE: All samples and controls must be added to the microplate in less than 5 minutes. For this reason, in manual assay we recommend to use microtubes for the samples and multichannel pipette to transfer 10 µl to the wells. For automatic assay, only autoanalysers adequate for rapid procedures can be used.

2. Cover the microplate with an adhesive seal, **mix gently**, and incubate for 20 minutes at room temperature (20-25°C).
3. Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soak for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.
4. Transfer 100 µl of diluted conjugate to each well of the microplate, except the one for the substrate blank. Avoid bubbles upon addition.
5. Cover the plate with an adhesive seal and incubate for 20 minutes at room temperature (20-25°C).
6. During the last 5-10 minutes of this incubation prepare the substrate-chromogen solution. If the entire plate is used add 280 µl of chromogen (TMB) to the bottle containing the substrate buffer (14 ml) and **mix well.** THE WORKING SUBSTRATE SOLUTION HAS A PINK COLOUR; discard if it becomes blue. If the entire plate is not used, follow Table 2.

TABLE 2

Strips required	1	2	4	6	8	10	12
Substrate buffer ml	1.0	2.0	4.0	6.0	8.0	10.0	12.0
Chromogen (TMB) µl	20	40	80	120	160	200	240

NOTE: The TMB is dissolved in DMSO. As the melting point of the DMSO is 18°C, the chromogen solution should be allowed to reach a temperature of 20-25°C, and be **well mixed** before use.

7. Remove and discard the adhesive seal. Aspirate and wash the wells as in step 3.
8. Add 100 µl of substrate-TMB solution to each well, including the blank.
9. Incubate for 10 minutes at room temperature (20-25°C).
10. Stop the reaction by adding 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.

- Blank the reader at 450 nm with the blank well and read the absorbance of each well, within 30 minutes. It is recommended to read in bichromatic mode using a 620 - 630 nm reference filter.

Quality control

Results of an assay are valid if the following criteria are accomplished:

- Substrate blank: absorbance value must be less than or equal to 0.100.
- Negative control: absorbance value must be less than 0.150 after subtracting the blank.
- Low positive control: absorbance greater than or equal to 0.150 after subtracting the blank.
- High positive control: absorbance equal to or greater than 0.600 after subtracting the blank.
- Ratio high positive control/low positive control: greater than or equal to 2.0.
- Ratio negative control/low positive control: less than or equal to 0.5.

Results

- Calculate the mean absorbance of the low positive control. This is the cut-off value.

Cut-off = LPCx

- Divide the sample absorbance by the cut-off value.

Positive: ratio absorbance/cut-off \geq 1.0

Negative: ratio absorbance/cut-off \leq 0.9

Equivocal: ratio absorbance/cut-off \geq 0.9 \wedge 1.0

Interpretation of results

A positive reaction should be interpreted as presence of CMV antibodies and infection by Cytomegalovirus, either recent or not.

A rise in the titre of anti-CMV antibodies by a factor of 4 or more in paired samples taken at 3-4 weeks interval, tested at the same time in adjacent wells may be indicative of recent infection. However, for diagnosis purpose it is recommended to associate this procedure with the detection of specific CMV IgM antibodies, and detection of virus DNA in white blood cells or in total blood by amplification techniques.

Limitations of the procedure

As with other serological tests, the results obtained with **bioelisa CMV colour** serve only as an aid to diagnosis and the patients' clinical history should be taken into consideration.

Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

It is recommended to retest samples with equivocal results and if result is again equivocal, other methods should be used.

As in all sensitive immunoassays, there is the possibility that non-repeatable positive results occur.

A negative result does not exclude the possibility of CMV infection.

Expected results

The prevalence of primary and recurrent CMV infection in adult populations varies widely in different areas of the world and even between different age and socio-economic groups within one geographic location. In an internal study, the prevalence of seropositivity found in 642 unselected blood donors' samples from Barcelona, Spain, was around 70%. In Central Europe, about 50% of young adults are infected with CMV. In the older population, seropositivity ranges from 70-80%¹⁰.

Performance characteristics

Evaluations

The performance of **bioelisa CMV colour** was evaluated in comparative studies with other commercial assays.

- The kit was evaluated by testing 642 serum samples from blood bank donors in parallel with another commercial EIA. 449 samples were classified as positive and 190 as negative by the reference test. Three samples were classified as equivocal and were eliminated to perform calculations. After retesting discrepant samples, two were classified as false positive by bioelisa and one as false negative, giving the following figures: Sensitivity 99.8% (448/449), specificity 98.9% (188/190) and overall agreement 99.5% (636/639).

Precision

Intra-assay reproducibility:

The coefficient of variation obtained for the absorbance values of a positive sample assayed in 24 replicates were 5.03%, 3.89% and 4.01% in three lots studied.

Inter-assay reproducibility:

Three positive samples of different levels were tested in triplicate in 5 different assays. The coefficients of variation obtained for the ratios absorbance/cut-off of the 3 samples were 3.6%, 4.5% and 4.9%, respectively.

Interferences

To study possible interferences, samples with potential risk of producing cross-reactions were tested, including sera positive for Rheumatoid Factor (RF), anti-nuclear antibodies (ANA), anti-Herpes simplex (HSV), anti-Epstein Barr (EBV-VCA) and from pregnant women. No evidences of interference were observed.

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Annex 2: Labels

bioelisa CMV colour

1x96 TESTS

REF 3000-1245

- 1 MCPL
- 1 x 0.35 ml CONJ 51x
- 1 x 15 ml DIL CONJ
- 1 x 30 ml DIL SAMP
- 2 x 50 ml WASH SOLN 10x
- 1 x 14 ml SUBS BUF
- 1 x 1.5 ml SOLN TMB
- 1 x 2.5 ml CONTROL +H
- 1 x 4.0 ml CONTROL +L
- 1 x 2.5 ml CONTROL -
- 1 x 12 ml H₂SO₄ 1N
- 1 BAG
- SEALS



IVD



CE
0843



bioelisa CMV colour

1x96 TESTS

REF 3000-1245

ELISA test for detection of antibodies to CYTOMEGALOVIRUS in human serum or plasma / test de ELISA para la detección de anticuerpos anti-CMV en suero o plasma humano / ELISA-Test Bestimmung von Anti-CMV-Antikörpern in Humanserum oder-plasma / test d'ELISA pour la détection d'anticorps anti-CMV dans du sérum ou du plasma humain / test ELISA per la determinazione di anticorpi anti-CMV nel siero o nel plasma umano / teste de ELISA para a detecção de anticorpos anti-CMV em soro ou plasma umano.



8 436003 074540

LOT F-2003
2005-06-26

bioelisa

bioelisa CMV colour

MCPL

IVD

LOT F-2003
2005-06-26



bioelisa

bioelisa CMV colour

CONJ 51x

0.35 ml

IVD

LOT F-2003
2005-06-26



bioelisa

bioelisa CMV colour

DIL CONJ

15 ml

IVD

LOT F-2003
2005-06-26



bioelisa

bioelisa CMV colour

DIL SAMP

30 ml

IVD

LOT F-2003
2005-06-26

RTU



bioelisa

bioelisa CMV colour

CONTROL +H

2.5 ml

IVD

LOT F-2003
2005-06-26

RTU



bioelisa

bioelisa CMV colour

CONTROL +L

4.0 ml

IVD

LOT F-2003
2005-06-26

RTU



bioelisa

bioelisa CMV colour

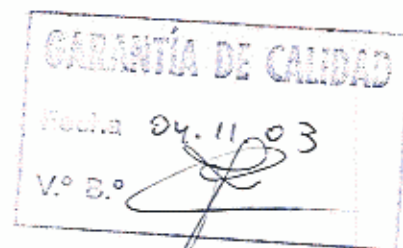
CONTROL -

2.5 ml

IVD

LOT F-2003
2005-06-26

RTU



bioelisa CMV colour

5x96 TESTS

REF 3000-1246

5	MCPL
1 x 1.3 ml	CONJ 51x
1 x 70 ml	DIL CONJ
1 x 120 ml	DIL SAMP
3 x 100 ml	WASH SOLN 10x
5 x 14 ml	SUBS BUF
1 x 1.5 ml	SOLN TMB
1 x 5.0 ml	CONTROL + H
1 x 7.0 ml	CONTROL + L
1 x 5.0 ml	CONTROL -
1	BAG
	SEALS



IVD



CE
0843

bioelisa CMV colour

bioelisa CMV colour

MCPL

LOT B-1004
2005-01-16

IVD



bioelisa CMV colour

bioelisa CMV colour

DIL SAMP

LOT B-1004
2005-01-16

RTU

120 ml

IVD



bioelisa CMV colour

bioelisa CMV colour

DIL CONJ

LOT B-1004
2005-01-16

70 ml

IVD



bioelisa CMV colour

bioelisa CMV colour

CONJ 51x

LOT B-1004
2005-01-16

1.3 ml

IVD



bioelisa CMV colour

bioelisa CMV colour

CONTROL + H

LOT B-1004
2005-01-16

5 ml

IVD



bioelisa CMV colour

bioelisa CMV colour

CONTROL + L

LOT B-1004
2005-01-16

7 ml

IVD



bioelisa CMV colour

bioelisa CMV colour

CONTROL -

LOT B-1004
2005-01-16

5 ml

IVD



bioelisa CMV colour

5x96 TESTS

REF 3000-1246

ELISA test for detection of antibodies to CYTOMEGALOVIRUS in human serum or plasma / test de ELISA para la detección de anticuerpos anti-CMV en suero o plasma humano / ELISA-Test Bestimmung von Anti-CMV-Antikörpern in Humanserum oder-plasma / test d'ELISA pour la détection d'anticorps anti-CMV dans du sérum ou du plasma humain / test ELISA per la determinazione di anticorpi anti-CMV nel siero o nel plasma umano / teste de ELISA para a detecção de anticorpos anti-CMV em soro ou plasma humano.



LOT B-1004

2005-01-16

GARANTÍA DE CALIDAD

Fecha 22-01-04

V.º B.º

Annex 3: CE mark approval, class II B

UL International (UK) Ltd

An affiliate of Underwriters Laboratories Inc.

EC Certificate - Full Quality Assurance System Approval Certificate

(Annex IV, section 3 of the Directive 98/79/EC on In Vitro Diagnostic Medical Devices)

Manufacturer:

Biokit S.A.
Can Malé
08186 Lliçà d'Amunt
Barcelona
Spain

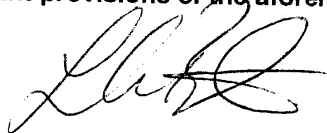
Product description: For products covered by this certificate see attachment

Scope of the quality system: The design, manufacture and distribution of in vitro diagnostic reagent kits for detection of:

1. Antibodies to rubella, toxoplasma and cytomegalovirus by enzyme immunoassay and agglutination.
2. Antibodies or antigen to hepatitis B virus by enzyme immunoassay.

We hereby declare that an examination of the full quality assurance system has been carried out following the requirements of the national legislation to which the undersigned is subject, transposing Annex IV (with the exemption of section 4 and 6) of the Directive 98/79/EC on In Vitro Diagnostic Medical Devices. We certify that the full quality assurance system conforms with the relevant provisions of the aforementioned directive.

Certificate issued by:



Certification Manager
For UL International (UK) Ltd

UL International (UK) Ltd.
Wonersh House The Guildway
Old Portsmouth Road
Guildford Surrey GU3 1LR
United Kingdom
+44 (0)1483 302130

Original certificate: 5 November 2003
Current certificate: 5 March 2004
Date of expiry: 5 November 2006



UL International (UK) Ltd

An affiliate of Underwriters Laboratories Inc.

Attachment 1 to certificate (page 1 of 2)

EC certificate number: 302

Report number: 02UKM-0625/02UKM-0626

The products covered by the above certificate are:

Product Code	Name
3000-4525	Toxocell latex 100T
300614510	Toxoplasma antigen
3000-2181	Quantex Toxo
3000-2184	Quantex Toxo Standard
3000-2185	Quantex Toxo Control
3000-1214	Bioelisa Toxo IgG 96T
3000-1210	Bioelisa Toxo IgM 96T
3000-4001	Rubagen 100T
3000-2180	Quantex Rubella
3000-2188	Quantex Rubella Standard
3000-2189	Quantex Rubella Control
3000-1215	Bioelisa Rubella IgG 96T
3000-1219	Bioelisa Rubella IgG Colour 480T
3000-1231	Bioelisa Rubella IgM 96T
3000-1216	Bioelisa CMV IgG 96T
3000-1245	Bioelisa CMV colour 96T

Issued by:



Certification Manager

For UL International (UK) Ltd

UL International (UK) Ltd.
Wonersh House The Guildway
Old Portsmouth Road
Guildford Surrey GU3 1LR
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+44 (0)1483 302130

Original certificate:

5 November 2003

Current certificate:

5 March 2004

Date of expiry:

5 November 2006



UL International (UK) Ltd

An affiliate of Underwriters Laboratories Inc.

Attachment 1 to certificate (page 2 of 2)

EC certificate number: 302

Report number: 02UKM-0625/02UKM-0626

The products covered by the above certificate are:

Product Code

3000-1102

3000-1130/1131 (96 test & 480 test)

3000-1101

Name

Bioelisa anti-HBc Test Kit

Bioelisa HBsAg Colour

Bioelisa anti-HBs

Issued by:



Certification Manager

For UL International (UK) Ltd

UL International (UK) Ltd.
Wonersh House The Guildway
Old Portsmouth Road
Guildford Surrey GU3 1LR
United Kingdom
+44 (0)1483 302130

Original certificate:

5 November 2003

Current certificate:

5 March 2004

Date of expiry:


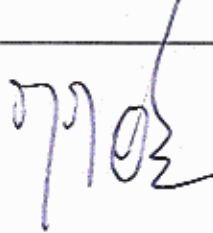


5 November 2006



Annex 4: Validation in best 2000

VALIDATION of the *bioelisa CMV colour assay* used in combination with the best 2000

Inscrita en el Registre Mercantil de Barcelona, Tomo 2.412, Llibre 1.799, fs. 96-97, Hija 25.304, 1ª Inscripció. Secció 2ª de Societats - NIF A.08330715

	Author	Reviewed	Reviewed	Approved
Name	Albert Royo	Joaquín Ortiz	Iná Camargo	Joan Guixer
Position	Collaborator	Product Manager biokit SA	Quality Control Manager biokit SA	Quality Assurance Director biokit SA
Signature				
Date	29-01-04	4/2/02	04/02/02	4-02-02

A CH-Werfen Company

VALIDATION of the bioelisa CMV Colour assay used in combination with the Best 2000

A. Introduction

The purpose of this validation is to demonstrate users and authorities, that automated performance of the **bioelisa CMV colour** assay used in combination with the best 2000 instrument, is equal to that of the performance achieved using manual techniques. Sensitivity, specificity and reproducibility intra-run data has been scrutinized. It has been also checked the effect on the assay of an over room temperature incubation.

Expected values: 100% correlation for sensitivity and specificity
 CV% less or equal 10 % reproducibility intra-run OD's
 CV% less or equal 15 % when compared to the automated-manual procedure in quantitative assays.

B. Materials and methods

1- Instruments

- Automated instrumentation:
 Biokit Best 2000; Revelation DSX version 5.14; DSX Automated Elisa System
 Serial N°: 1DXA-0401 Dynex technologies Inc. 14340 Sullyfield Circle, Chantilly, VA 20151-1683 USA.
- Manual instrumentation:
 Washer: Bioelisa Washer ELX 50; Incubator: Incubator 500 PH Electronica; Reader: Ultra
 Microplate reader ELX 808 Bio-Tek Instruments, Inc. (SN:1380009).

2- Diagnostic kit

- Bioelisa CMV Colour; Batch number: J-3303; Exp.date: 13SEP04.

3- Samples

- Samples for sensitivity: 3 Biokit Q.C. selected samples in different position in the plate. As CMV incidence is very high in adult population, it is expected to find positive samples among unselected blood donors.
- Samples for specificity: To test the specificity 147 blood bank samples (10OCT03) were tested .
- Samples for reproducibility intra-run: In this assay 24 replicates with an OD around 1.000 were tested. These replicates were dispensed in 3 full strips, everyone with a new tip.

4- Manual protocol: (see annex 1)

5- Best 2000 protocol:

Due to the very short incubations of this assay: 20' sample + 20 ' conjugate + 10' substrate, all at Room Temperature, it is included in the best 2000 protocol, a synchronized wash. Incubation time starts at the moment that the first sample is dispensed, when normally time starts after the last sample dispensed. Synchronized washing automatically compensates the incubation time, to make it equal in the whole plate. This fact makes that the automated well fill verification for sample dispensing have to be performed after sample incubation.

Incubations are performed in the best 2000 individual incubators at 23°C to control temperature in case of cold environment. An experiment has been performed to assess the **bioelisa CMV colour** performance in extreme conditions of 30°C ambient temperature. Plates results are described in annex 1.2

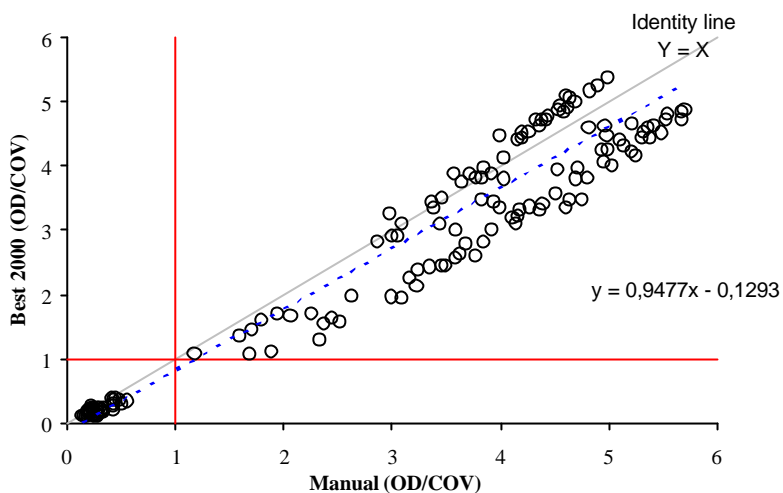
C. Results: (Plates' results are described in annex 1.1)

Manual/automated correlation: It has been tested 3 positive samples and 144 unselected blood donor samples. The chart below shows the normalized absorbances (OD/COV) for the manual technique compared to the Best 2000 automated procedure.

No discrepancies between the two methods were detected.

100% correlation for both positive and negative samples was achieved .

The correlation for this assay: **r statistic** | 0,966 96,60%

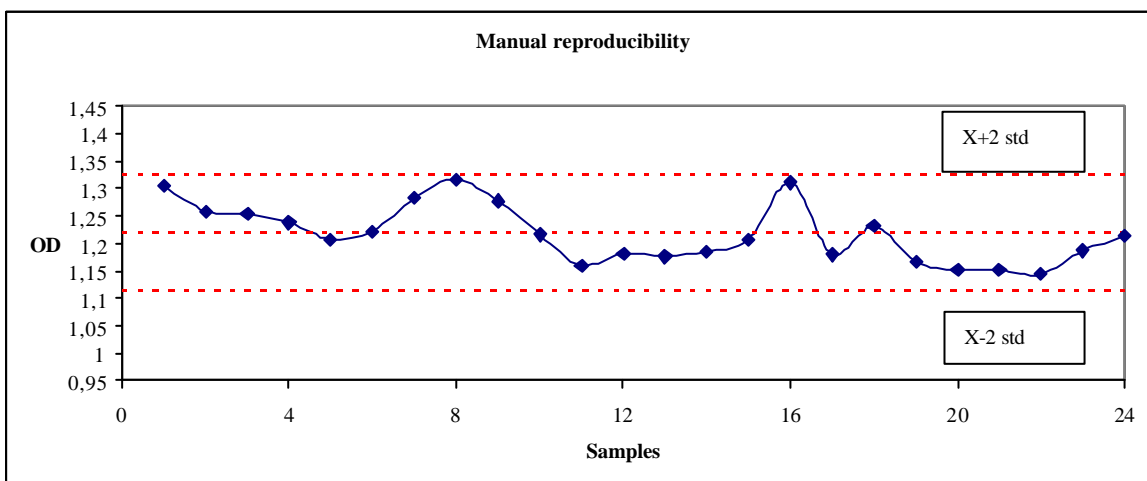


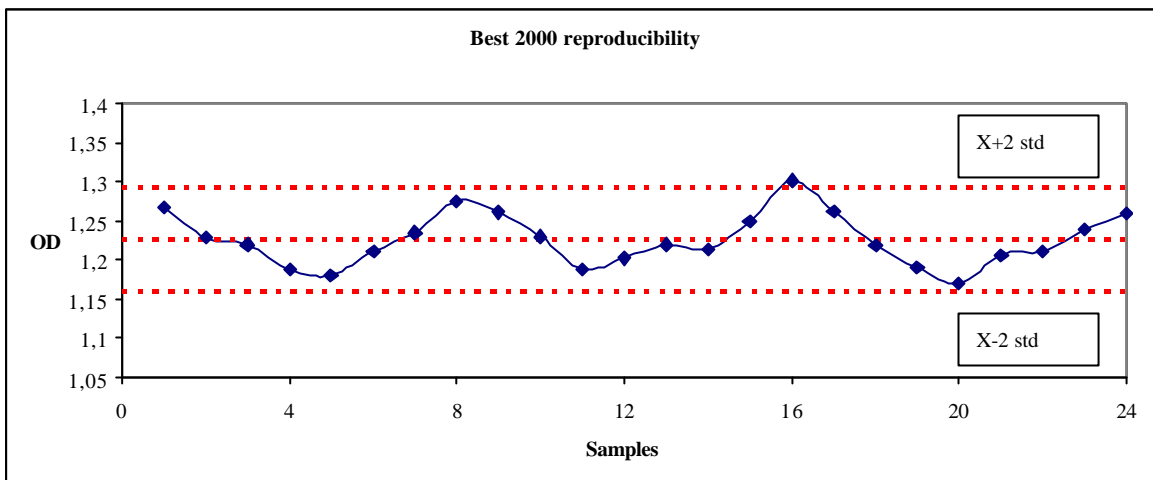
*Intermittent line shows the regression line.

*Continuous lines show the cut-off position in ratio equal to 1.

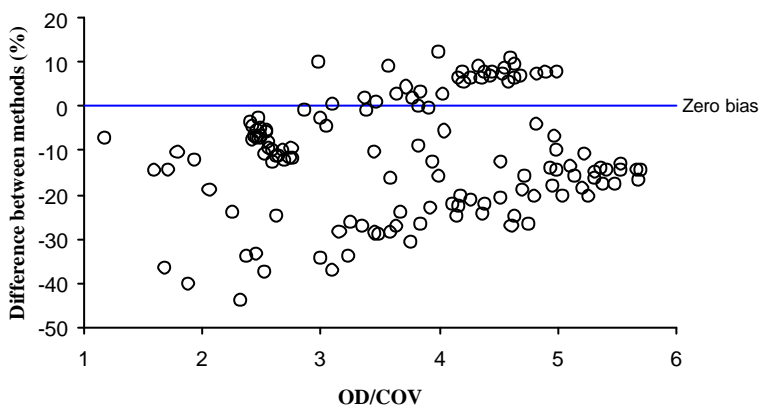
- **Reproducibility:** A total of 24 replicates of the same sample were run on the best 2000. A new tip was used each time. Results detailed below.

	Manual	Best 2000
N° samples	24	24
Mean OD	1,218	1,227
Min.	1,144	1,170
Max.	1,317	1,302
CV %	4,34%	2,71%

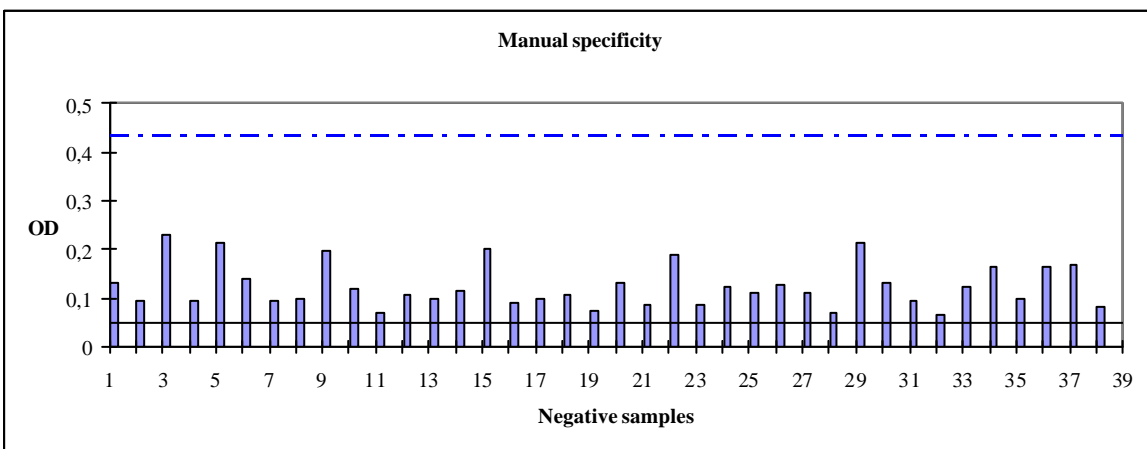


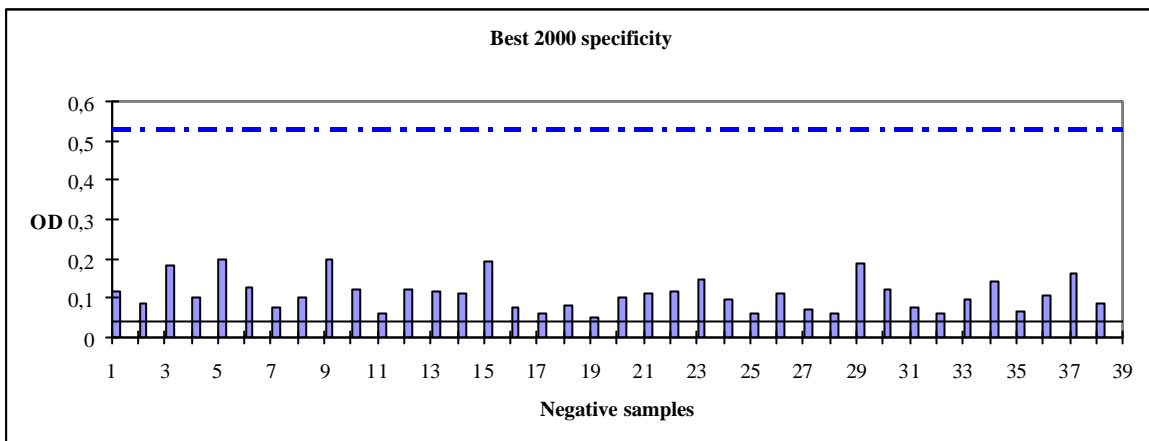


- Differences between methods, manual vs automated Best 2000 (%): Each positive sample, expressed in normalised absorbances (OD/COV), including all positive results used for reproducibility, has been plotted against the Best 2000. The variation is expressed as a percentage



- Specificity: The chart show the OD's of the negative samples.

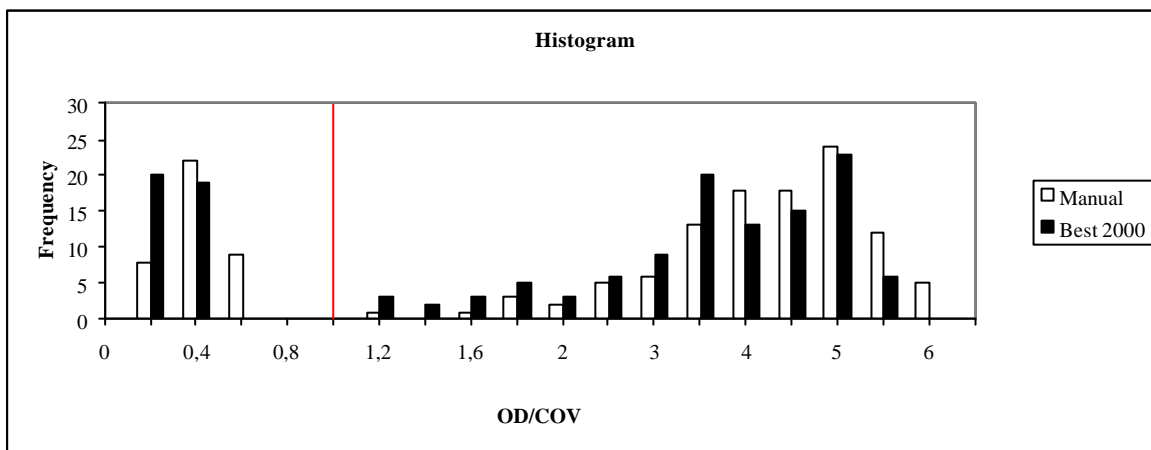




* Discontinuous line shows the COV optical density.

* Continuous line shows the NC optical density.

- Population distribution: The chart shows the distribution of samples between the two methods as a histogram.



*Single line shows the COV position.

- Effect in the assay of an over incubation temperature

As all incubations of the **bioelisa CMV colour** are performed at room temperature, it has been checked the effect of a high room temperature in the performance of the assay. A full plate, 88 samples, has been performed in the best 2000, incubating in three different situations: ambient drawer, incubator adjusted at 23°C and incubator adjusted at 30°C. Reason for configure the incubator at a 23°C temperature is to avoid problems of low reactivity in very cold laboratories. When the external laboratory temperature is low, the incubator heating system will start acting. Incubator thermostat will control the temperature not to be lower than 23°C. When the external laboratory temperature is normal or high, the incubator heating system is not working, not affecting the assay.

For the experiment, a temperature recorder was used to monitor external laboratory and internal temperatures inside the best 2000. Plates' results are described in annex 1.2

Experimental conditions:

1. External laboratory temperature was 23°C.
2. Ambient drawer temperature was 25-26°C, 2-3 °C higher than external
3. Incubator (namely 23°C) temperature was 27°C, 3-4 °C higher than external. This is because the incubator has heating and not cooling capabilities

Results: Optical densities for incubations performed in the ambient drawer and best incubator were almost the same. Optical densities for incubations performed at 30°C are higher but proportionally higher. In all cases run QC validation equations were passed

No discrepancies between the three incubation conditions were detected.

100% correlation for all the samples was achieved.

Room temperature inside the instrument is 2-4 °C higher than external Room temperature.

bioelisa CMV colour used in combination of best 2000 can be performed in a range of ambient laboratory temperature of 17-27°C.

In any case of temperatures out of this range, Quality Control equations, described in the package insert must be strictly accomplished before validate the assay.

D. Conclusion:

bioelisa CMV Colour assay, performed in combination with the Best 2000 produces comparable results to those obtained manually as reference.

Sensitivity, specificity and intra-run reproducibility results correspond to the expected values prepared for this evaluation.

bioelisa CMV colour accepts variations of incubation temperature from 20 to 30°C inside the best 2000 without affecting the performance. By incubating plates in the incubator at 23°C it is possible to control the temperature when laboratory temperature is lower.

The user should utilize this validated protocol in the Best 2000 to obtain reliable and consistent results.

Annex 1.1: Plates' results.

bioelisa CMV Colour

Lot: J-3303

Exp.date: 13SEP04

Plate:1

	Manual			Best 2000		
	OD	OD/CO	Result	OD	OD/CO	Result
Blank	0,000	0,0	-	0,000	0,0	-
NC	0,050	0,1	-	0,048	0,1	-
COV	0,477	1,0	POS	0,524	1,0	POS
PC	1,570	3,3	POS	1,834	3,5	POS
1	0,133	0,3	-	0,119	0,2	-
2	2,185	4,6	POS	2,535	4,8	POS
3	1,833	3,8	POS	2,080	4,0	POS
4	2,233	4,7	POS	2,624	5,0	POS
5	1,737	3,6	POS	1,961	3,7	POS
6	2,032	4,3	POS	2,374	4,5	POS
7	0,093	0,2	-	0,090	0,2	-
8	0,230	0,5	-	0,185	0,4	-
9	1,614	3,4	POS	1,755	3,3	POS
10 (Std)	0,983	2,1	POS	0,876	1,7	POS
11	2,109	4,4	POS	2,475	4,7	POS
12	1,703	3,6	POS	2,040	3,9	POS
13	0,092	0,2	-	0,102	0,2	-
14	1,773	3,7	POS	2,032	3,9	POS
15	1,419	3,0	POS	1,714	3,3	POS
16	1,905	4,0	POS	2,350	4,5	POS
17	2,381	5,0	POS	2,820	5,4	POS
18	2,301	4,8	POS	2,714	5,2	POS
19	0,211	0,4	-	0,198	0,4	-
20 (Std)	0,757	1,6	POS	0,711	1,4	POS
21	0,814	1,7	POS	0,767	1,5	POS
22	1,799	3,8	POS	2,011	3,8	POS
23	2,207	4,6	POS	2,578	4,9	POS
24	0,140	0,3	-	0,126	0,2	-
25	1,454	3,0	POS	1,525	2,9	POS
26	2,162	4,5	POS	2,550	4,9	POS
27	2,004	4,2	POS	2,319	4,4	POS
28	0,095	0,2	-	0,080	0,2	-
29	1,825	3,8	POS	2,003	3,8	POS
30	0,923	1,9	POS	0,891	1,7	POS
31	2,066	4,3	POS	2,473	4,7	POS
32	2,089	4,4	POS	2,468	4,7	POS
33 (Std)	0,853	1,8	POS	0,839	1,6	POS
34	0,561	1,2	POS	0,572	1,1	POS
35	1,477	3,1	POS	1,628	3,1	POS
36	2,118	4,4	POS	2,506	4,8	POS
37	1,430	3,0	POS	1,526	2,9	POS
38	2,076	4,4	POS	2,423	4,6	POS
39	2,003	4,2	POS	2,371	4,5	POS
40	0,099	0,2	-	0,102	0,2	-
41	0,196	0,4	-	0,199	0,4	-
42	2,196	4,6	POS	2,675	5,1	POS
43	0,119	0,2	-	0,122	0,2	-
44	0,068	0,1	-	0,060	0,1	-
45	1,363	2,9	POS	1,484	2,8	POS
46	2,171	4,6	POS	2,593	4,9	POS
47	1,607	3,4	POS	1,801	3,4	POS

	Manual			Best 2000		
	OD	OD/CO	Result	OD	OD/CO	Result
48	0,106	0,2	-	0,122	0,2	-
49	0,100	0,2	-	0,116	0,2	-
50	0,116	0,2	-	0,110	0,2	-
51	1,865	3,9	POS	2,041	3,9	POS
52	2,334	4,9	POS	2,759	5,3	POS
53	1,652	3,5	POS	1,832	3,5	POS
54	0,201	0,4	-	0,194	0,4	-
55	2,210	4,6	POS	2,656	5,1	POS
56	1,922	4,0	POS	2,167	4,1	POS
57	1,980	4,2	POS	2,318	4,4	POS
58	1,643	3,4	POS	1,619	3,1	POS
59	0,089	0,2	-	0,078	0,1	-
R1	1,304	2,7	POS	1,268	2,4	POS
R2	1,259	2,6	POS	1,229	2,3	POS
R3	1,253	2,6	POS	1,220	2,3	POS
R4	1,238	2,6	POS	1,188	2,3	POS
R5	1,206	2,5	POS	1,181	2,3	POS
R6	1,221	2,6	POS	1,212	2,3	POS
R7	1,283	2,7	POS	1,235	2,4	POS
R8	1,317	2,8	POS	1,276	2,4	POS
R9	1,277	2,7	POS	1,261	2,4	POS
R10	1,216	2,5	POS	1,230	2,3	POS
R11	1,160	2,4	POS	1,188	2,3	POS
R12	1,182	2,5	POS	1,203	2,3	POS
R13	1,176	2,5	POS	1,220	2,3	POS
R14	1,185	2,5	POS	1,214	2,3	POS
R15	1,208	2,5	POS	1,249	2,4	POS
R16	1,311	2,7	POS	1,302	2,5	POS
R17	1,180	2,5	POS	1,262	2,4	POS
R18	1,232	2,6	POS	1,219	2,3	POS
R19	1,166	2,4	POS	1,191	2,3	POS
R20	1,152	2,4	POS	1,170	2,2	POS
R21	1,152	2,4	POS	1,207	2,3	POS
R22	1,144	2,4	POS	1,212	2,3	POS
R23	1,187	2,5	POS	1,239	2,4	POS
R24	1,213	2,5	POS	1,260	2,4	POS
Average	1,218	2,55		1,227	2,34	
std	0,053	0,111		0,033	0,06	
CV	4,34%	4,34%		2,71%	2,71%	

Plate: 2

	Manual			Best 2000		
	OD	OD/CO	Result	OD	OD/CO	Result
Blank	0,000	0,0	-	0,000	0,0	-
NC	0,049	0,1	-	0,038	0,1	-
COV	0,392	1,0	-	0,539	1,0	-
PC	1,422	3,6	POS	1,784	3,3	POS
60	1,535	3,9	POS	1,626	3,0	POS
61	0,882	2,3	POS	0,921	1,7	POS
62	1,954	5,0	POS	2,299	4,3	POS
63	1,973	5,0	POS	2,163	4,0	POS
64	2,108	5,4	POS	2,389	4,4	POS
65	1,625	4,1	POS	1,678	3,1	POS
66	1,769	4,5	POS	1,930	3,6	POS
67	0,097	0,2	-	0,060	0,1	-
68	1,952	5,0	POS	2,416	4,5	POS
69	1,566	4,0	POS	1,810	3,4	POS
70	1,499	3,8	POS	1,878	3,5	POS
71	0,106	0,3	-	0,082	0,2	-
72	0,072	0,2	-	0,052	0,1	-
73	1,932	4,9	POS	2,284	4,2	POS
74	1,719	4,4	POS	1,838	3,4	POS
75	0,131	0,3	-	0,101	0,2	-
76	1,945	5,0	POS	2,488	4,6	POS
77	1,999	5,1	POS	2,374	4,4	POS
78	1,029	2,6	POS	1,066	2,0	POS
79	1,355	3,5	POS	1,329	2,5	POS
80	1,474	3,8	POS	1,403	2,6	POS
81	0,988	2,5	POS	0,850	1,6	POS
82	1,505	3,8	POS	1,516	2,8	POS
83	2,058	5,3	POS	2,249	4,2	POS
84	0,084	0,2	-	0,111	0,2	-
85	1,406	3,6	POS	1,615	3,0	POS
86	1,581	4,0	POS	2,053	3,8	POS
87	2,081	5,3	POS	2,392	4,4	POS
88	1,365	3,5	POS	1,329	2,5	POS
89	1,941	5,0	POS	2,192	4,1	POS
90	2,234	5,7	POS	2,630	4,9	POS
91	0,735	1,9	POS	0,604	1,1	POS
92	1,631	4,2	POS	1,735	3,2	POS
93	0,189	0,5	-	0,116	0,2	-
94	1,543	3,9	POS	1,856	3,4	POS
95	2,014	5,1	POS	2,329	4,3	POS
96	2,223	5,7	POS	2,546	4,7	POS
97	1,805	4,6	POS	1,812	3,4	POS
98	2,083	5,3	POS	2,444	4,5	POS
99	0,911	2,3	POS	0,700	1,3	POS
100	0,959	2,4	POS	0,878	1,6	POS

	Manual			Best 2000		
	OD	OD/CO	Result	OD	OD/CO	Result
101	1,272	3,2	POS	1,286	2,4	POS
102	0,930	2,4	POS	0,842	1,6	POS
103	0,086	0,2	-	0,149	0,3	-
104	1,264	3,2	POS	1,147	2,1	POS
105	1,236	3,2	POS	1,218	2,3	POS
106	1,879	4,8	POS	2,054	3,8	POS
107	0,124	0,3	-	0,097	0,2	-
108	1,839	4,7	POS	2,052	3,8	POS
109	1,422	3,6	POS	1,421	2,6	POS
110	1,771	4,5	POS	2,131	4,0	POS
111	2,121	5,4	POS	2,502	4,6	POS
112	0,109	0,3	-	0,064	0,1	-
113	1,172	3,0	POS	1,058	2,0	POS
114	0,660	1,7	POS	0,576	1,1	POS
115	1,710	4,4	POS	1,781	3,3	POS
116	0,127	0,3	-	0,113	0,2	-
117	0,111	0,3	-	0,071	0,1	-
118	0,070	0,2	-	0,060	0,1	-
119	2,045	5,2	POS	2,511	4,7	POS
120	1,612	4,1	POS	1,723	3,2	POS
121	1,815	4,6	POS	1,872	3,5	POS
122	2,103	5,4	POS	2,487	4,6	POS
123	2,149	5,5	POS	2,435	4,5	POS
124	1,31	3,3	POS	1,312	2,4	POS
125	2,22	5,7	POS	2,615	4,9	POS
126	0,215	0,5	-	0,188	0,3	-
127	1,636	4,2	POS	1,791	3,3	POS
128	0,131	0,3	-	0,123	0,2	-
129	1,673	4,3	POS	1,815	3,4	POS
130	1,211	3,1	POS	1,044	1,9	POS
131	2,039	5,2	POS	2,283	4,2	POS
132	1,864	4,8	POS	1,88	3,5	POS
133	0,094	0,2	-	0,08	0,1	-
134	0,063	0,2	-	0,062	0,1	-
135	1,404	3,6	POS	1,379	2,6	POS
136	0,121	0,3	-	0,095	0,2	-
137	0,165	0,4	-	0,144	0,3	-
138	0,097	0,2	-	0,065	0,1	-
139	0,164	0,4	-	0,108	0,2	-
140	1,44	3,7	POS	1,503	2,8	POS
141	0,168	0,4	-	0,164	0,3	-
142	1,886	4,8	POS	2,487	4,6	POS
143	1,848	4,7	POS	2,139	4,0	POS
144	0,082	0,2	-	0,089	0,2	-
145	2,168	5,5	POS	2,594	4,8	POS
146	0,108	0,3	-	0,094	0,2	-
147	2,166	5,5	POS	2,547	4,7	POS

Annex 1.2: Plates' results.

CMV Colour Incubations, best 2000

External laboratory temperature 23°C

Sample	RT Ambient drawer		23°C Incubator		30°C Incubator	
	Inside T = 25-26°C		Inside T = 26-27°C		Inside T =30°C	
	OD	OD/COV	OD	OD/COV	OD	OD/COV
Blank	0	0,00	0	0,00	0	0,00
NC	0,046	0,08	0,050	0,07	0,064	0,08
COV	0,574	1,00	0,674	1,00	0,777	1,00
PC	1,994	3,47	2,133	3,16	2,209	2,84
1	2,366	4,12	2,38	3,53	2,354	3,03
2	2,28	3,97	2,226	3,30	2,351	3,03
3	2,453	4,27	2,499	3,71	2,497	3,21
4	2,713	4,73	2,753	4,08	2,664	3,43
5	2,705	4,71	2,79	4,14	2,654	3,42
6	1,784	3,11	1,833	2,72	2,043	2,63
7	2,675	4,66	2,745	4,07	2,692	3,46
8	2,614	4,55	2,682	3,98	2,66	3,42
9	2,21	3,85	2,166	3,21	2,299	2,96
10	0,985	1,72	0,932	1,38	1,17	1,51
11	1,867	3,25	1,862	2,76	1,998	2,57
12	2,017	3,51	2,049	3,04	2,105	2,71
13	2,117	3,69	2,109	3,13	2,245	2,89
14	1,851	3,22	1,903	2,82	2,068	2,66
15	2,336	4,07	2,366	3,51	2,416	3,11
16	1,096	1,91	1,071	1,59	1,269	1,63
17	2,679	4,67	2,722	4,04	2,565	3,30
18	2,02	3,52	1,979	2,94	2,044	2,63
19	2,804	4,89	2,854	4,23	2,667	3,43
20	2,585	4,50	2,621	3,89	2,506	3,23
21	2,545	4,43	2,66	3,95	2,555	3,29
22	2,531	4,41	2,62	3,89	2,538	3,27
23	2,159	3,76	2,287	3,39	2,33	3,00
24	2,593	4,52	2,684	3,98	2,653	3,41
25	2,269	3,95	2,343	3,48	2,326	2,99
26	2,221	3,87	2,324	3,45	2,351	3,03
27	1,604	2,79	1,719	2,55	1,87	2,41
28	2,511	4,37	2,553	3,79	2,54	3,27
29	2,726	4,75	2,669	3,96	2,632	3,39
30	1,837	3,20	1,949	2,89	2,093	2,69
31	2,313	4,03	2,377	3,53	2,433	3,13
32	2,742	4,78	2,848	4,23	2,807	3,61
33	1,209	2,11	1,362	2,02	1,443	1,86
34	1,972	3,44	1,992	2,96	2,034	2,62
35	1,519	2,65	1,637	2,43	1,711	2,20
36	2,765	4,82	2,777	4,12	2,624	3,38
37	2,541	4,43	2,659	3,95	2,564	3,30
38	2,626	4,57	2,711	4,02	2,622	3,37
39	2,629	4,58	2,741	4,07	2,617	3,37

Sample	RT Ambient drawer		23°C Incubator		30°C Incubator	
	OD	OD/COV	OD	OD/COV	OD	OD/COV
40	2,686	4,68	2,722	4,04	2,761	3,55
41	0,216	0,38	0,229	0,34	0,231	0,30
42	1,737	3,03	1,833	2,72	1,894	2,44
43	2,344	4,08	2,358	3,50	2,35	3,02
44	1,262	2,20	1,423	2,11	1,416	1,82
45	1,687	2,94	1,858	2,76	1,896	2,44
46	2,298	4,00	2,373	3,52	2,334	3,00
47	2,642	4,60	2,714	4,03	2,657	3,42
48	2,121	3,70	2,306	3,42	2,296	2,95
49	2,58	4,49	2,631	3,90	2,56	3,29
50	1,037	1,81	1,173	1,74	1,344	1,73
51	2,789	4,86	2,775	4,12	2,719	3,50
52	2,55	4,44	2,542	3,77	2,484	3,20
53	2,78	4,84	2,826	4,19	2,691	3,46
54	2,367	4,12	2,456	3,64	2,411	3,10
55	2,745	4,78	2,813	4,17	2,71	3,49
56	1,392	2,43	1,662	2,47	1,811	2,33
57	2,19	3,82	2,253	3,34	2,328	3,00
58	2,389	4,16	2,513	3,73	2,435	3,13
59	2,704	4,71	2,727	4,05	2,687	3,46
60	0,16	0,28	0,192	0,28	0,19	0,24
61	2,729	4,75	2,762	4,10	2,652	3,41
62	1,826	3,18	1,868	2,77	2,057	2,65
63	1,324	2,31	1,505	2,23	1,602	2,06
64	2,689	4,68	2,823	4,19	2,727	3,51
65	1,903	3,32	1,998	2,96	2,098	2,70
66	2,685	4,68	2,691	3,99	2,644	3,40
67	2,677	4,66	2,658	3,94	2,6	3,35
68	2,84	4,95	2,862	4,25	2,766	3,56
69	2,218	3,86	2,18	3,23	2,281	2,94
70	2,558	4,46	2,624	3,89	2,611	3,36
71	2,561	4,46	2,65	3,93	2,644	3,40
72	2,302	4,01	2,472	3,67	2,498	3,21
73	2,482	4,32	2,555	3,79	2,501	3,22
74	2,581	4,50	2,657	3,94	2,559	3,29
75	2,561	4,46	2,622	3,89	2,586	3,33
76	2,477	4,32	2,534	3,76	2,535	3,26
77	2,237	3,90	2,273	3,37	2,383	3,07
78	2,736	4,77	2,781	4,13	2,701	3,48
79	2,825	4,92	2,836	4,21	2,773	3,57
80	2,417	4,21	2,468	3,66	2,482	3,19
81	2,638	4,60	2,672	3,96	2,533	3,26
82	2,426	4,23	2,493	3,70	2,462	3,17
83	1,424	2,48	1,571	2,33	1,678	2,16
84	2,697	4,70	2,793	4,14	2,739	3,53
85	2,614	4,55	2,662	3,95	2,622	3,37
86	2,813	4,90	2,86	4,24	2,795	3,60
87	2,707	4,72	2,795	4,15	2,733	3,52
88	2,688	4,68	2,878	4,27	2,75	3,54

Annex 5: Australian Red Cross blood services award

NORTH EAST REGION

NSW Head Office
153 Clarence Street
Sydney NSW 2000
Tel: 61 2 9229 4444
Fax: 61 2 9290 3316



Australian Red Cross

BLOOD SERVICE

Mr Mark Warburton
Abacus Diagnostics
5/38 Tennyson Memorial Ave
Yerongpilly QLD 4105

14th August 2003

Dear Mark

Re CMV Antibody Assay (Total)

Thank you for your assistance in providing the biokit *bioelisa CMV colour* assay for evaluation. The assay's performance against a panel of donor samples and commercial mixed titre samples was satisfactory. Having reviewed the proposal from your company, we have decided to proceed with using biokit *bioelisa CMV colour* assay for CMV antibody screening of donations. The documents associated with the establishment of the standing order will follow shortly.

Yours sincerely

Sue Ismay
Senior Scientist in Charge
Virus Serology Laboratory
ARCNS-NSW

cc: Wayne Bolton, ARCBS-NSW Laboratory Services Manager

Annex 6: Scottish National blood transfusion service



Scottish National Blood Transfusion Service
Head Office
Ellen's Glen Road
Edinburgh EH17 7QT



Telephone 0131 536 5700
Fax 0131 536 5701

www.snbits.org.uk

Mr M Hutchison
Biokit Limited
Ash House
Ash Road
New Ash Green
Longfield
KENT DA3 8JD

Date: 18 February 2004
Your Ref:
Our Ref: imf/cj/2004/MTEG029

Enquiries to: Caroline Lang
Extension: 65725
Direct Line: 0131 536 5725
Email: caroline.lang@snbits.csa.scot.nhs.uk

Dear Mr Hutchison

BIOKIT BIOELISA CMV COLOUR (3000-1245) – MTEG 030

I am writing to inform you that the above assay has been considered and signed off by the SNBTS Microbiology Test Evaluation Group (MTEG) who have produced a report sent to me this week.

I confirm that this is acceptable for use within SNBTS/NIBTS and should be added to the SNBTS list of approved tests.

Yours sincerely

Professor Ian M Franklin
National Medical & Scientific Director

cc. Dr B C Dow



Director Keith J Thompson
SNBTS is a Division of the Common Services Agency