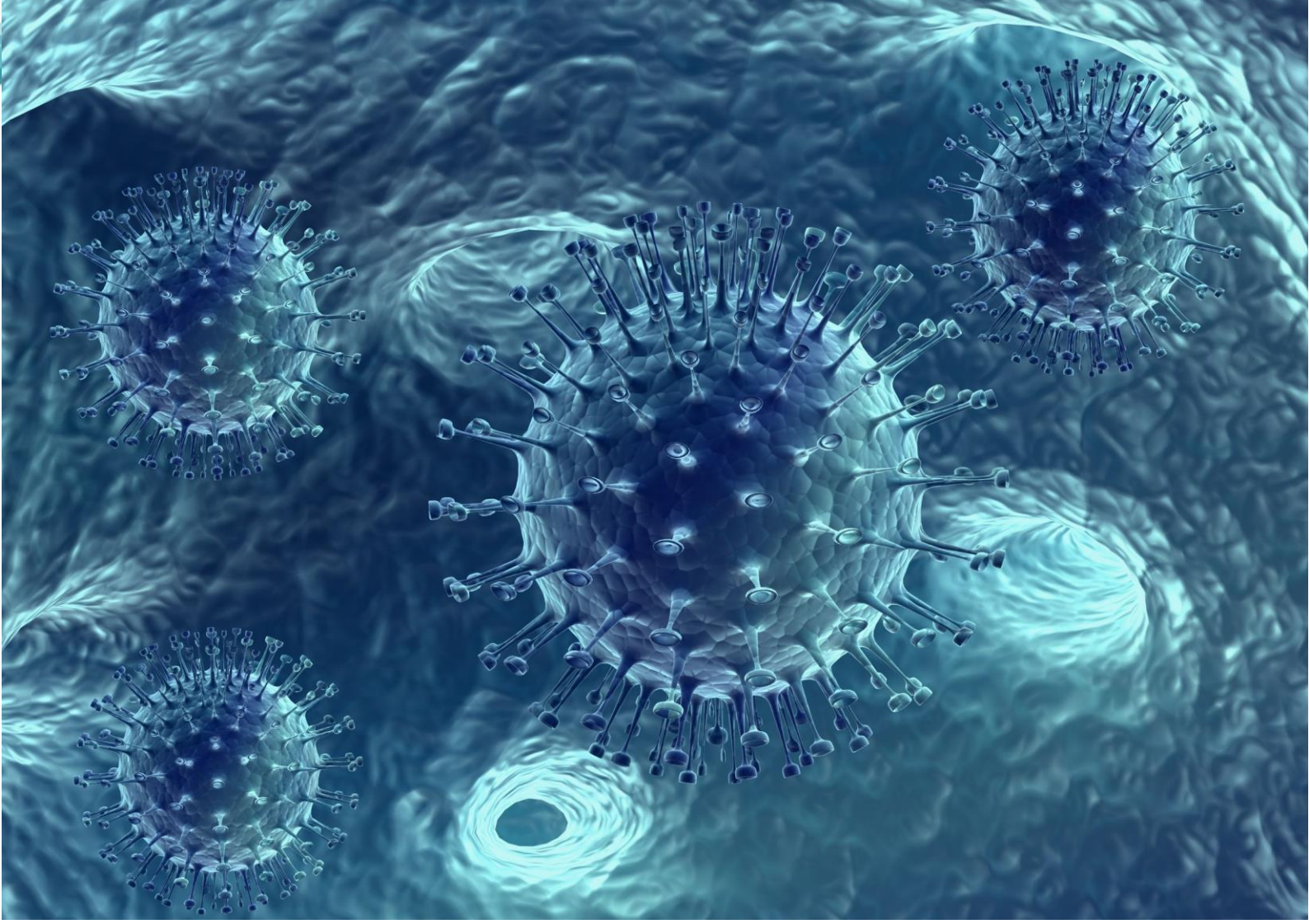

Technical Reports

rekom biotech

EVALUATION OF A CMV RECOMBINANT POLYEPITOPE CHIMERIC ANTIGEN, CHIMCMV1, BY INDIRECT AND CAPTURE ELISA ASSAYS. COMPARISON WITH OTHER CMV BIOMARKERS.

THE AIM OF THE RESEARCH PRESENTED IN THIS TECHNICAL REPORT IS THE EVALUATION OF OUR NEW CHIMERIC RECOMBINANT ANTIGEN FOR CMV, CHIMCMV1 (RAG0109), WHICH CONTAINS IN A SAME PROTEIN SELECTED ANTIGENIC REGIONS OF DIFFERENT CMV PROTEINS. WE HAVE PERFORMED INDIRECT ELISA WITH THE NAKED PROTEIN AND CAPTURE ELISA WITH THE HRP-CONJUGATED PROTEIN IN ORDER TO IMPROVE SPECIFICITY IN THE IgM DETECTION.

Human cytomegalovirus (HCMV) is endemic to populations throughout the world, and 50-80% of the adults in developed nations are sero-positive for this virus. The virus is transmitted from person to person, primarily via oropharyngeal secretions under conditions of close personal contact, but can also be transmitted vertically, by intrauterine transmission, by blood transfusion, and by bone marrow and solid organ transplantation.

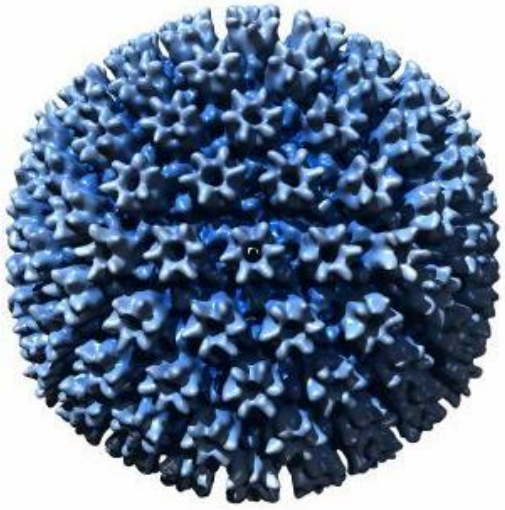

Figure 1. Three-dimensional image which correspond to the cytomegalovirus

Serological assays for IgG, IgM or total (IgG, IgM and IgA) antibody to CMV are useful in screening, determining susceptibility to primary infection, providing serological evidence of recent infection, and in certain situations, differentiating between primary and reactivated infection or reinfection.

Commercial ELISAs for detecting IgG are very sensitive and specific. Nevertheless, there are several problems regarding IgM detection. One of the difficulties that the diagnostic laboratory has faced over the past 10 years is the lack of agreement between commercial tests for the detection of CMV-specific IgM. This lack of agreement has its roots in the different viral preparations used to detect IgM antibodies to CMV. Making use of a good recombinant antigen as raw material in this kind of test can circumvent the issue, as this kind of preparations are significantly more reproducible.

There are two different types of IgM ELISA assays: indirect ELISA and immunocapture ELISA. This second one is preferable due to elude false positive results thanks to there is no possible interference with rheumatoid factor. In indirect ELISA assays, it is necessary a previous absorption of positive sera containing rheumatoid factor, by using a sorbent (anti-IgG), in order to eliminate this specific interference. Capture ELISA avoids also false negative results due to competition with IgG binding with the coated antigen, particularly in high-concentration IgG sera. The percentage of discordance between both ELISA techniques is approx. of 20-30%.

Rekom Biotech has performed the design of a new CMV recombinant polyepitope chimeric antigen (RAG0109), composed by different sensitive and specific antigenic determinants from some CMV proteins. The obtained chimeric protein is soluble, therefore it does not require detergent or chaotropic additives in its storage buffer, being consequently very convenient for coating different platforms or for performing a nanoparticles binding. The purified protein is presented in the following figure 2:

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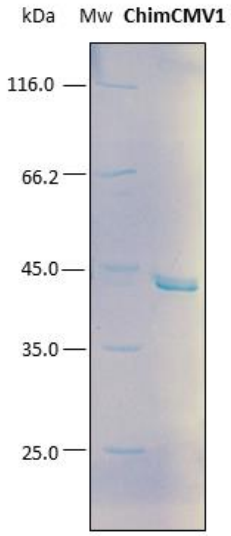

Figure 2. Analysis by SDS-PAGE (12.5%) of 1 µl of recombinant ChimCMV1. Purity is > 95% as determined by gel electrophoresis.

This chimeric recombinant antigen was tested in parallel with other CMV bio-markers such as the Rekom Biotech CMV recombinant proteins pp150 (RAG0091), pp52 (RAG0090) and pp65 (RAG0016). The *in house* indirect IgM ELISA assay was carried out by using a battery of pre-validated sera with the commercial ELISA capture IgM test from Vidas (Figure 3).

In order to detect the performance of our chimeric recombinant antigen in sera discrimination in a IgM and IgG indirect ELISA (figure 4 and 5).

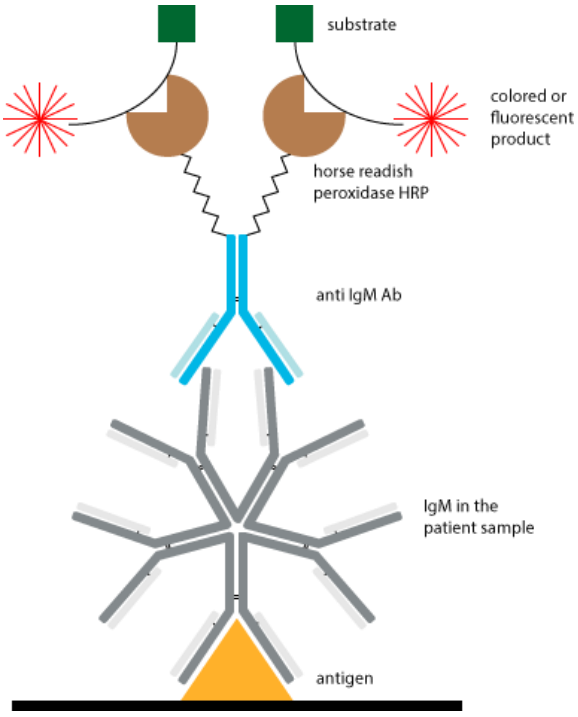

Figure 4. Indirect ELISA assay scheme for IgM detection

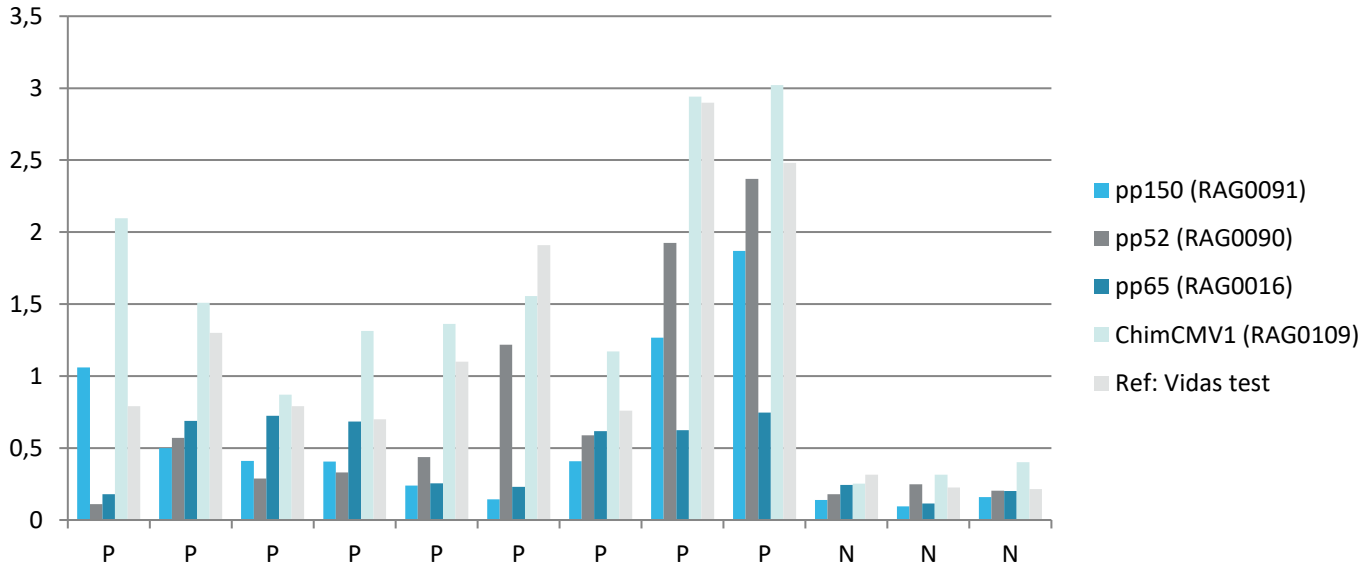

Figure 3. Indirect IgM ELISA assay. Proteins were coating the plates at a final concentration of 1 µg/ml. All these experiments used anti-IgG as sorbent and anti-IgM-HRP. Sera were used in a 1:100 dilution. P means positive pre-validated sera by the Vidas test; N means negative pre-validated sera by the Vidas test.

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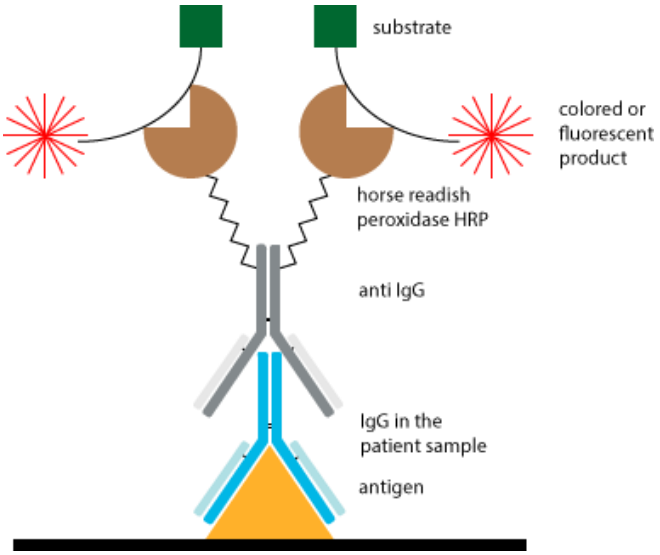

Figure 5. Indirect ELISA assay scheme for IgG detection

The ELISA assays were performed by using pre-validated positive and negative CMV specimen sera, and the target protein was coated at different concentrations, as it is shown in figure 6 and 7.

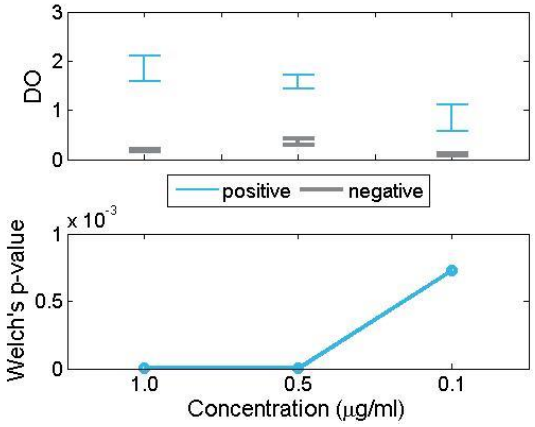

Figure 6. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. Eligible concentrations for the use of the antigen should present statistically significant differences between positive and negative sera. This happens when the intervals at the top do not overlap and, equivalently, when the p-value at the bottom is below 0.05. In the present figure, all p-values are below 0.05 and thus the intervals do not overlap. Therefore, any of the showed concentrations can be used to distinguish between positive and negative sera.

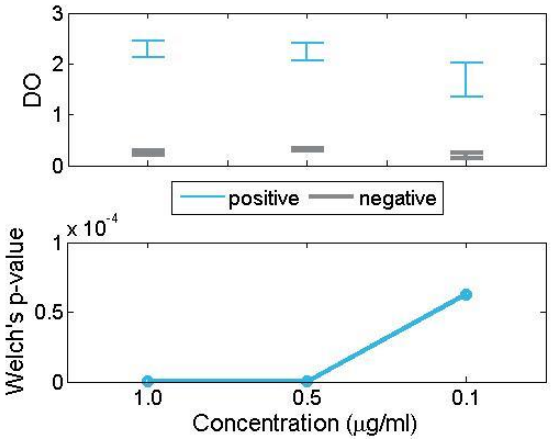

Figure 7. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. Eligible concentrations for the use of the antigen should present statistically significant differences between positive and negative sera. This happens when the intervals at the top do not overlap and, equivalently, when the p-value at the bottom is below 0.05. In the present figure, all p-values are below 0.05 and thus the intervals do not overlap. Therefore, any of the showed concentrations can be used to distinguish between positive and negative sera.

Also, a capture ELISA assay was developed by using the peroxidated recombinant chimeric protein (ChimCMV1-HRP), as it is shown in figure 8.

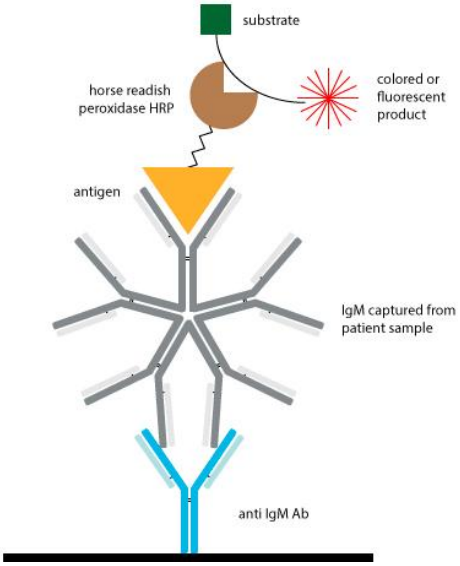

Figure 8. Immune capture ELISA assay scheme

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In a first assay, a capture ELISA assay titration was performed by using different dilutions of the Rekom ChimCMV1-HRP with 1 µg/ml of anti-human IgM coating the plates (figure 9).

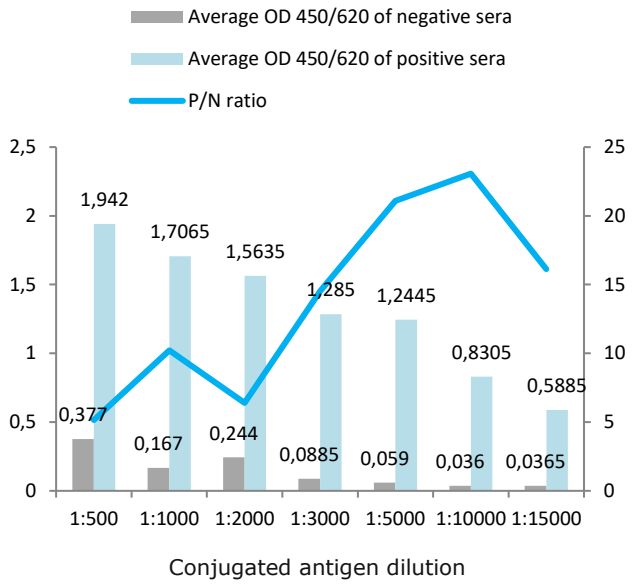

Figure 9. In this plot, the optical density at 450/620 nm obtained in a capture ELISA assay for positive (blue) and negative (gray) CMV **IgM** sera were compared. Seven different conjugated dilutions of the conjugated ChimCMV1 to HRP were tested. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera. Therefore, in the present assay, any of the showed dilutions can be used to distinguish between positive and negative sera (P/N ratio means positive/negative signal ratio).

A second assay was performed: a commercial capture ELISA assay (CMV-IgM-ELA test PKS Medac), was used by replacing its own conjugated antigen by three different dilutions of the Rekom ChimCMV1-HRP (figure 10).

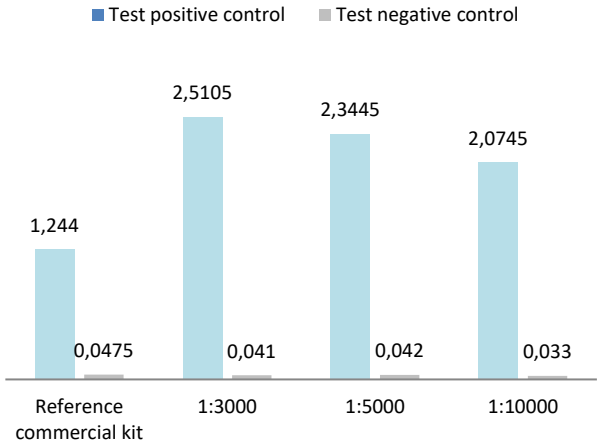

Figure 10. In this plot, the optical density at 450/620 nm obtained in an ELISA capture assay for a positive (blue) and a negative (gray) CMV **IgM** sera were compared. Two different conjugated dilutions and the reference conjugated antigen were tested in the assay. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera. In the present assay, any of the showed dilutions can be used to distinguish between positive and negative sera in a capture ELISA assay.

CONCLUSION

These preliminary experiments suggest that the recombinant polyepitope chimeric antigen designed and produced by Rekom Biotech, RAG0109, recovers some pre-validated IgM positive sera which are not initially detected by two of the best antigens described in bibliography for CMV diagnosis: pp52 and pp150, showing the presence in merely one antigen of some important epitopes for this specific diagnostic.

Furthermore, this recombinant chimera is able to detect IgM Ab in an indirect and a capture ELISA formats. Besides, the IgM detection capacity of the protein has been proven by using a commercial capture assay with very promising preliminary results.

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