A rapid magnetic particle-based enzyme immunoassay for human cytomegalovirus glycoprotein B quantification

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Keywords: Magnetic beads; immunoassay; mpEIA; Human cytomegalovirus; Glycoprotein B

Abstract

Human cytomegalovirus (HCMV) is a herpes virus that can cause severe infections. Still, the available methods for its diagnostic have the main disadvantage of requiring long time to be performed. In this work, a simple magnetic particle-based enzyme immunoassay (mpEIA) for the quantification of glycoprotein B of Human cytomegalovirus (gB-HCMV) in urine samples is proposed. The immunosensor scheme is based on the analyte protein gB-HCMV sandwiched between a primary monoclonal antibody, (MBs-PrG-mAb1), and a secondary anti-gB-HCMV antibody labelled with *Horseradish peroxidase* (Ab2-HRP) to allow spectrophotometric detection.

The mpEIA analytical performance was tested in urine samples, showing a linear dependence between gB-HCMV concentration and the absorbance signal at 450 nm in a range of concentrations from 90 to 700 pg mL^{-1}. The calculated detection limits for gB-HCMV were 90±2 pg mL^{-1} and the RSD was about 6.7% in urine samples. The immunosensor showed good selectivity against other viruses from Herpesviridae family, namely varicella zoster and Epstein Barr viruses. The recoveries of spiked human urine samples at 0.30-0.50 ng mL^{-1} concentration levels of gB-HCMV ranged between 91 to 105%. The proposed mpEIA method was validated following the guidelines of the European Medicines Agency (EMEA-2014), and allows rapid, successful and easy quantification of gB-HCMV in urine samples.

1. Introduction

Human cytomegalovirus (HCMV) is one of the eight herpesviruses that commonly infect humans, being its transmission facilitated by mucous contact [1,2]**.** It is recognized as the most common congenital viral infection in human and an important cause of morbidity and mortality in immunocompromised hosts [3]. Primary infection by HCMV results in the establishment of a lifelong latent infection, and its reactivation may occur in situations of immune system dysfunction or low maturity, resulting in severe clinical symptoms [1,2, 4, 5]. Still, the diagnosis of HCMV remains controversial because of the difficulty in separating patients who are asymptomatic (but carry HCMV) from patients who have

the symptomatic disease [1]. The standard for diagnosis of HCMV infection are serological tests based on IgM and IgG detection [6]. IgG detection is only valuable for establishing past infection, or confirming seroconversion, while IgM has poor sensitivity and specificity to detect a recent infection. These tests usually require a minimum of two days [3]. On the other hand, the traditional method for direct free HCMV detection is viral isolation, that detect viral antigens in tissue, urine or saliva samples. Nevertheless, time analysis requirements for this method (≈ 20 days) makes it disadvantageous to be routinely used in clinical diagnosis as point of care [5,78].

As an alternative, the use of capture antibodies against the envelope glycoproteins of HCMV open the possibility of faster immunochemical methods of analysis. Glycoprotein B of HCMV (gB-HCMV) is a viral glycoprotein that plays a crucial role in virus entry into the cell and emerges during the early stages of a HCMV infection [8]. In addition, gG-HCMV is the dominant antigen in the envelope of HCMV, being possible its determination in body fluids like urine, where viral loads are higher [9]. This is quite advantageous since the collection of these biological samples is simple and ~~is~~ performed by noninvasive procedures. In consequence, the development of new methods for the accurate detection of gB-HCMV in body fluids is of great interest, unlike the up mentioned analytical method based on the determination of antibodies generated by the HCMV virus [5].

Enzyme-linked immunosorbent assays (ELISA) have also great presence in clinical diagnostic tests [10]. However, conventional ELISAs have some limitations, such as high costs (e.g. high quantities of antibodies immobilized on the wells of the microtiter wells), slow kinetics, and nonspecific adsorptions of conjugates and/or matrix interferers on the microplate wells [10]. Much of these limitations have been greatly improved with the recent use of surface-functionalized magnetic micro-beads (MBs), in the so-called magnetic particle-based enzyme immunoassays (mpEIA) [10]. The use of MBs improves the affinity interaction thanks to a faster assay kinetics of the dispersed beads. The surface area is higher than a flat solid phase of the wells (in a classical microtiter plate), and the washing and separation efficiencies are enhanced by using an external well-controlled magnetic field, allowing the analysis of complex samples without any pre-enrichment or purification steps [10, 11].

Thus, in this work, we propose a spectrophotometric mpEIA method for the rapid and simple gB-HCMV determination in urine samples (see the immunoassay scheme of Fig. 1). Magnetic particles functionalized with protein G (MBs-prG), allows the antibody (mAb1) oriented immobilization, resulting in a more effective recognition of gB-HCMV [12]. The spectrophotometric detection was performed through a secondary antibody labelled with *Horseradish peroxidase* (Ab2-HRP)[5,8,10]. Special attention was given to the non-specific adsorptions (NSA) of gB-HCMV and Ab2-HRP, as they have a very negative influence in quantitative immunoassays [13]. After optimization of the experimental conditions, the validation of the mpEIA method was carried out according to the "Guideline on bioanalytical method validation", from the European Medicines Agency (EMEA) (2014) [14]. The developed immunosensor was shown to be a fast and effective method for gB-HCMV quantification in human urine samples for the useful determination of HCMV infections.

2. Material and methods

2.1. Apparatus

Spectrophotometric measurements were made with a Bio-Rad model 680 Microplate Absorbance Spectrophotometer (Bio-Rad, Hercules, CA, USA). Standard 96-well polystyrene microplates (ref. 82.1581) were supplied from Sarstedt (Nümbrecht, Germany). A magnetic 96-well separator (ThermoFisher Scientific, Waltham, MA, USA) was used to separate the MBs from the supernatants to make easier the removal of solvent from the standard ELISA plates. Incubation process was carried out using Grant Bio POS-300 Orbital Shaking Platform (Cambridge, UK). A magnetic separation stand for eppendorf vials (Z5342, 12 positions, 1.5 mL volume) was purchased from Promega (Madison, WI, USA).

2.2. Reagents

Human cytomegalovirus glycoprotein B (gB-HCMV) and Anti-Cytomegalovirus glycoprotein B primary and secondary antibodies (mAb1 and Ab2-HRP) were purchased

from Abcam (ab43040, ab69245, ab6499, respectively) (Cambridge, UK). Bovine serum albumin (BSA), peroxidase substrate 3,3',5,5'-tetramethylbenzidine liquid substrate system for ELISA (TMB), Tween-20 and monosodium phosphate were obtained from Sigma-Aldrich (Madrid, Spain). Disodium phosphate and sulfuric acid were acquired from Panreac (Barcelona, Spain). Sodium chloride was purchased from Fisher Scientific (Bishop's Stortford, UK) and dynabeads® protein G immunoprecipitation kit and Pierce Protein-Free were products from Thermofisher Scientific. Urine samples were obtained from healthy volunteers after informed consent. All the reagents used were of analytical grade and Milli Q water Millipore (Burlington, MA, USA) was used for preparing all solutions.

2.3. Primary antibody immobilization on functionalized magnetic beads

Dynabeads® were re-suspended in the vial (vortexed >30 sec or tilted and rotated for 5 min). Then, 50 µL (1.5 mg) of MBs-PrG Dynabeads® were transfer to a tube**,** washed three times with 500 µL of PBS buffer in order to remove NaN_3 preservative and the surfactant. Each washing step consisted on the re-suspension of the beads in PBS buffer, followed by supernatant clearance under a magnetic field that retain the beads and separate them from solution.

After MBs-PrG cleaning, the desired amount of primary antibody (mAb1), diluted in PBS, was added to them and left to incubate for 10 min at room temperature. The antibody modified MBs (MB-PrG-mAb1) were washed three times with PBS buffer, reconstituted in PBS (1.0 mL), and stored at 4 ºC when not in use. According to the manufacturer, it is not recommended to freeze (−20 °C) these kind of MBs.

2.4. Immunoassay determination of gB-HCMV

All the immunoassay incubations were made on standard polystyrene flat-bottom microtest plates coupled with a magnetic support with 96x individual magnets to hold back the modified MBs on each of the microplate wells. In order to avoid nonspecific adsorption, the wells of the microplate were, firstly, blocked overnight by using the commercial solution Pierce Protein-Free. Following emptying of the wells, 25 µL of MBs-

mAb1 (containing 5 µg of MBs) were added to each well and the remaining sites of the beads surface blocked with BSA 4% during 60 minutes at room temperature. After this, 25 µL of gB-HCMV standard (or the clinical sample) were added to obtain a final concentration over the range from 0 to 800 pg mL^{-1} gB-HCMV. Incubations were carried out for 60 minutes at room temperature with gentle orbital agitation. Finally, 25 µL of the Ab2-HRP was added to each well and left to incubate for 60 minutes at room temperature with gentle orbital agitation.

Between each of the incubation steps, the wells were washed three times with 300 µL of PBS and the solvent removed under an external magnetic field for retaining the modified MBs. For the mpEIA spectrophotometric measurements of Ab2-HRP, 50 µL of the peroxidase substrate TMB reagent was added to each well, left to react for 10 min at room temperature, stopped with 0.5 M H_2SO_4 and the absorbance measured at 450 nm with an ELISA reader.

3. Results and Discussion

As it is well known, immunoassays are greatly dependent on experimental conditions, being imperative their detailed optimization for achieving the best analytical properties. A sandwich assay scheme was used for the construction of our immunosensor, as it improves selectivity against a competitive immunoassay scheme, once two selected antibodies are used for the specific recognition of different parts of the same gB-HCMV protein. After incubation with the primary antibody, a HRP-labeled secondary antibody (Ab2-HRP) was used to generate the analytical signal for gB-HCMV detection. The scheme of this mpEIA immunosensor assay is shown in Fig. 1.

Special attention was paid to avoid NSA of the gB-HCMV protein and the Ab2-HRP conjugated antibody, both on the wall of the microtiter wells and on the modified surface of MBs-PrG-mAb1, since this would falsify the analytical signals, not due to the extension of the incubation reactions. It was also considered the possibility of unwanted interactions between primary and secondary antibodies [15].

After optimizations, the mpEIA analytical performance in quantifying gB-HCMV was tested. In order to evaluate the feasibility of the proposed assay for real samples

analysis, the mpEIA was used for the determination of gB-HCMV in human urine samples, and cross-reactivity evaluated with Epstein-Barr virus (EBV) and Varicela-zoster (VZV) antigens, both viruses from the *Herpesviridae* family.

3.1 Optimization of the experimental conditions

It is well known that NSA can occur on the surface of the 96 well polystyrene ELISA plate through physical adsorption by electrostatic and/or van der Waals forces and/or by hydrophobic interactions [16]. In order to avoid NSA of the biological reagents on the wells, three blocking agents were tested, namely the SuperBlock and Pierce Protein-Free commercial solutions and a 4% bovine serum albumin (BSA) solution prepared in 0.1 PBS (pH=7.4). After blocking the wells overnight with these solutions, incubations of the secondary Ab2-HRP antibody were carried out (25 µL for 60 min.), and the absorbances measured after reaction with TMB, as explained in section 2.4. The results are shown in Fig. 2. As it can be seen, in presence of SuperBlock no difference is observed compared to when no blockage is performed, demonstrating secondary antibody nonspecific adsorption to the well's walls and the inefficiency of this blocking agent. The lowest absorbance values were obtained with 4% BSA and Pierce Protein-Free solutions. Despite the similarity between their absorbance mean values (0.175 and 0.180 respectively), Pierce Protein-Free solution was chosen as the optimal blocking agent, once the relative standard deviations obtained in replicated tests were of 17.3% for Pierce Protein-Free and 27.4% for BSA blocking solutions.

Although hydrophobic interactions play an essential role in epitope-antibody binding, these forces may also promote nonspecific interactions between primary and secondary antibodies. Low levels of a detergent, such as Tween-20, are known to reduce surface tension and as consequence this type of interaction [17]. Accordingly, in order to further lower the background signal, a PBS + 0.02% Tween-20 solution was used during the secondary antibody incubation step. No changes of the absorbances were observed using tween-20 solutions, showing that hydrophobic interactions do not significantly contribute to the global NSA.

With the use of MBs modified with capture antibody in the immunosensor scheme, a high background signal was observed after incubations with Ab2-HRP (without gB-HCMV), indicating nonspecific adsorptions of the conjugated Ab2-HRP antibody on the unblocked MBs surface. Thus, the MBs blockage was considered to avoid this problem [18]. Two blocking agents (BSA and Pierce Protein-Free) were evaluated. The lower absorbance signals due to NSA of Ab2-HRP were obtained with 4% and 5% of BSA solutions, as it is shown in Fig. 3. However, a decrease in gB-HCMV detection absorbances were found with more than 5% of BSA solutions, suggesting that BSA blocks some antigen binding sites of the mAb1 antibody, reducing the ability to detect gB-HCMV. The best concentration for this purpose was 4% BSA, once it shows lower blank signals and it did not interfere with the gB-HCMV antigen detection. Experimental conditions strongly influence the sensitivity of the sandwich immunoassays. In particular, it is important that the number of available binding sites of the primary (mAb1) and secondary (Ab2-HRP) antibodies be in excess of the stoichiometric amount of the gB-HCMV analyte. Under these conditions, adequate absorbance values are produced (Absorbance ≤ 1.0) according to Fig. 4 a linear range of concentrations from 70 pg mL^{-1} to 800 pg mL^{-1} was obtained.

3.2 Immunosensor analytical performance

The analytical performance of the immunoassay was evaluated following the guidelines of the European Medicines Agency on bioanalytical method validation [14]. Accordingly, the main characteristics that are essential to ensure the acceptability of a bioanalytic method are: calibration curve performance, lower limit of quantification, matrix effect, precision, selectivity and accuracy.

Calibration standards were prepared measured in PBS buffer and urine matrices for comparison purposes. Urine was chosen to evaluate the immunosensor performance, once high virus load is found in excreted urine from HCMV infected individuals [9]. In presence of PBS buffer, absorbance at 450 nm increased linearly with analyte concentration in the narrow range from 70 pg mL^{-1} to 800 pg mL^{-1}, showing the calibration curve a good linear correlation coefficient (Table 1). Higher concentrations produced saturation of the binding sites of primary antibody. Although this range of

concentrations quantification is small, it provides clinically useful and sensitive information for the control of HCMV, once the appropriate dilution of the samples have been carried out.

To test the biological matrix effect on the magnetic particles based immunoassay, gB-HCMV standards were prepared in a 2-fold diluted urine sample from healthy patients in PBS buffer pH 7.4. Replicated calibration graphs were carried out in PBS 0.1 M. buffer and in diluted urine samples. The linear range in presence of urine (between 90 pg mL^{-1} to 700 pg mL^{-1} of gB-HCMV) was slightly smaller than that observed in PBS buffer, possibly due to the presence of other matrix components in the urine samples, that might block some antigen binding sites of the primary mAb1, leading to an earlier saturation. Nevertheless, there was not significant differences between the slopes of both calibrating graphs in the two matrices (95% level of confidence). In both calibration curves, three replicates for each gB-HCMV concentration were performed and the RSD exhibits an average value of 13.7% when PBS matrix is used and 6.25% in urine samples. These RSD values are acceptable for this kind of immunosensors and the very low concentrations of gB-HCMV measured, at the pg mL^{-1} order [19].

The lowest detection (LOD) and quantification (LOQ) limits are one of the most important properties in applying immunosensors to clinical samples and to obtain the least number of false negatives. The first stage in LOD calculation was the determination of a linear relation between gB-HCMV concentration and absorbance signal at 450 nm (Table 1). Anomalous points were avoided using least median squares regression (LMS) [20,21]. Then, in order to check the LOD values, results from the calibration curves were evaluated by using the DETARCHI software [22], that calculates detection limits with an evaluation of the probability of false positive (α) and negative (β), according to ISO11843-2, 2000 [23]. A limit of detection of 70±10 pg mL^{-1} for gB-HCMV in buffer and 90±20 pg mL^{-1} for gB-HCMV in urine samples were obtained for $\alpha=\beta=0.05$. These two values are very similar, reinforcing the non-existence of interferences from the matrix in the immunosensor response.

To our knowledge, very few immunosensors for the direct determination of gB-HCMV have been reported. A piezoelectric affinity sensor has been proposed, which is based on the immobilization of anti gB-HCMV antibodies on gold electrodes [24]. The

sensitivity of this immunosensor is, however, very low (LOD about 1 μg mL^{-1} of gB-HCMV), owing to the intrinsic low sensitivity of this analytical technique.

We have also previously reported an electrochemical immunosensor for gB-HCMV detection, by using capture anti-gB-HCMV antibodies absorbed on screen-printed carbon electrodes, and secondary anti-gB-HCMV antibodies labelled with gold nanoparticles [5]. gB-HCMV detection was carried out through electrochemical stripping analysis of silver nanoparticles deposited quantitatively on the immunosensor, catalyzed by the nanogold labels [5]. However, the reproducibility of this method (RSDs of about 12%) was not very good owing to the random immobilization of the primary antibody on the working electrode, which resulted in small efficiency of antigen detection, low signals compared to the large amount of this antibody used, and the nonspecific deposition of silver on the sensor surface in this kind of voltammetric detection [25].

In this way, the results obtained with the proposed mpEIA compares favorably to those obtained in other reports of gB-HCMV detection shown in Table 2, in terms of analytical performance. Of all the methods for the detection of gB, the proposed method is the one that presents lowers limits of detection with shorter sample preparation times (the only need to prepare the urine samples are dilutions with 0.10 M. PBS buffer).

The proposed immunochemical method must discriminate the gB-HCMV analyte of interest from other related viruses in the studied biological samples. The presented approach might have the possibility of false positive results, caused by cross-reactions with some virus of Herpesviridae family, once they are morphologically and structurally related [1,28]. To evaluate the selectivity of the antibody, one virus from each sub-family of Herpesviridae family was selected as possible interfere, namely the Epstein-Barr (EBV) and Varicela-zoster (VZV) viruses. In order to evaluate cross reactivities, concentrations of 300, 400 and 500 pg mL^{-1} of the above virus antigens were assayed, and the gB-HCMV concentration was measured following the proposed method. The obtained absorbances were compared with a control assay, which consisted in a blank test, i.e. PBS buffer was added instead of the analyte to the immunassay. A false positive was considered when the assay with these viruses produced higher spectrophotometric signals compared to the control assay. According to the results, given in Fig. 5, all the

assays with EBV and VZV antigens showed lower signals than the control, meaning that the secondary Ab-HRP did not bind during the assays. In contrast, all the tests with gB-HCMV showed higher signals, demonstrating the primary and secondary antibody specificities to gB-HCMV with the sandwich immunoassay scheme.

In order to evaluate the analytical applicability of the proposed mpEIA method, urine samples, collected from a healthy human, were spiked with 300, 400 and 500 pg mL^{-1} of gB-HCMV and analyzed immediately. The absorbances of the spiked samples, following the mpEIA procedure (section 2.4), were measured and were interpolated in the calibration curve obtained with urine. The results are shown in Table 3. The measurements of each sample were replicated (n=5), and the results of the Table are the mean±SD.

The precision of the mpEIA assay was estimated based on repeat measurements of standard diluted urine samples with spiked concentrations of gB-HCMV (Table 3). The relative standard deviations have acceptable values, lesser than about 8.1% in all cases (n=5), in accordance with the usually obtained for these types of immunosensors based on magnetic particles compared with classical ELISAs [10]. The relative errors at these very low levels of gB-HCMV concentrations are also smaller than about 4.7% in all cases, demonstrating the successful applicability of the method in urine biological matrices.

It should also be noted the high sensitivity of the developed mpEIA method, being able to detect gB-HCMV concentrations of few pg mL^{-1}, and a limit of detection of 90 pg mL^{-1} of gB-HCMV, much smaller than others reported for the same analyte (Table 2). For example, as it was reported in our previous work with a disposable electrochemical sandwich immunosensor, the limit of detection was 3.2±0.2 ng mL^{-1} for the same glycoprotein [5].

The overall analysis time of samples with the proposed immunosensor is shorter when compared to the usually applied methods of HCMV virus isolation, DNA amplification, or antibody screening [1]. ≈24 h using techniques such as antibody detection and shell-vial and ≈6 h for polymerase chain reaction. The mpEIA can analyze a sample within ≈3 h, a little more compared with our previous work (≈1h) owing to the incubations time needed in this sandwich assay, but with a lower limit of detection and better sensitivity, accuracy, and reproducibility (Table 2).

Despite, the most common target analyte used for HCMV quantification being virus DNA, is important to emphasize that our method targets the structural protein, gB-HCMV, present on the HCMV virus envelope. HCMV is released in urine, during active infection, and our method will allow the infection identification for a minimal concentration of 90±20 pg mL^{-1} gB-HCMV in this specific body fluid. We expect that the viral load will be directly proportional to gB-HCMV concentration. Ongoing work is being developed to establish the correlation between gB-HCMV concentration and viral load, which will be of great interest to monitor patient response to therapy.

4. Conclusions

A sensitive mpEIA is proposed for the rapid (about 3 h) determination of gB-HMCV protein in urine samples, with the objective of early screening and quick diagnostics of HCMV infections. Magnetic beads functionalized with PrG allow the oriented immobilization of the primary mAb1 antibody by its Fc part, which has more affinity and efficiency to bind to the antigen protein. Incubations in a dispersed mode, due to the magnetic beads, improve kinetics and also the washing and separating steps of the immunoassay compared with a classical ELISA in which the detecting antibodies are immobilized on the solid surface of the wells of a microtiter plate.

The sandwich immunoassay scheme improves selectivity over other human herpes viruses, given that gB-HCMV is recognized through two epitopes by both capture and secondary antibodies. The secondary antibody labeled with HRP provides a convenient way to simply obtain the spectrophotometric signals.

The developed method was partially validated following the recommendations of the EMEA, and allows the determination of gB-HCMV in urine samples at a relevant range of concentrations from 90 to 700 ng mL^{-1} of gB-HCMV.

The calculated detection limit in urine samples was 90±20 pg mL^{-1}. Reproducibility exhibits an average RSD value of 6.25 %. Spiked of human urine samples at pg mL^{-1} levels provided relative errors lower than approximately 4.7%, demonstrating a very good accuracy. The presence of other similar herpes viruses (varicella zoster and Epstein Barr) does not interfere.

ELISA readers are frequently available in biochemical laboratories, but additionally, this

same mpEIA method can be easily adapted to electrochemical transduction on screen-printed electrodes. Multiplexed electrochemical transduction of mpEIA provides simplicity, sensitivity and portability of the instrument, as we have previously demonstrated with competitive assay schemes in the determination of drugs of abuse and mycotoxins in foods [29].

Acknowledgments

This work is supported by FEDER funds through the POCI - COMPETE 2020 - Operational Program Competitiveness and Internationalization in Axis I - Strengthening research, technological development and innovation (Project No. POCI-01-0145-FEDRER-007491), Portuguese Funds by FCT - Foundation for Science and Technology (Project UID/Multi /00709/2013) and Spanish Ministry of Science and Innovation (MICINN), Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (FEDER) (TEC20013-40561-P and MUSSEL RTC-2015-4077-2).

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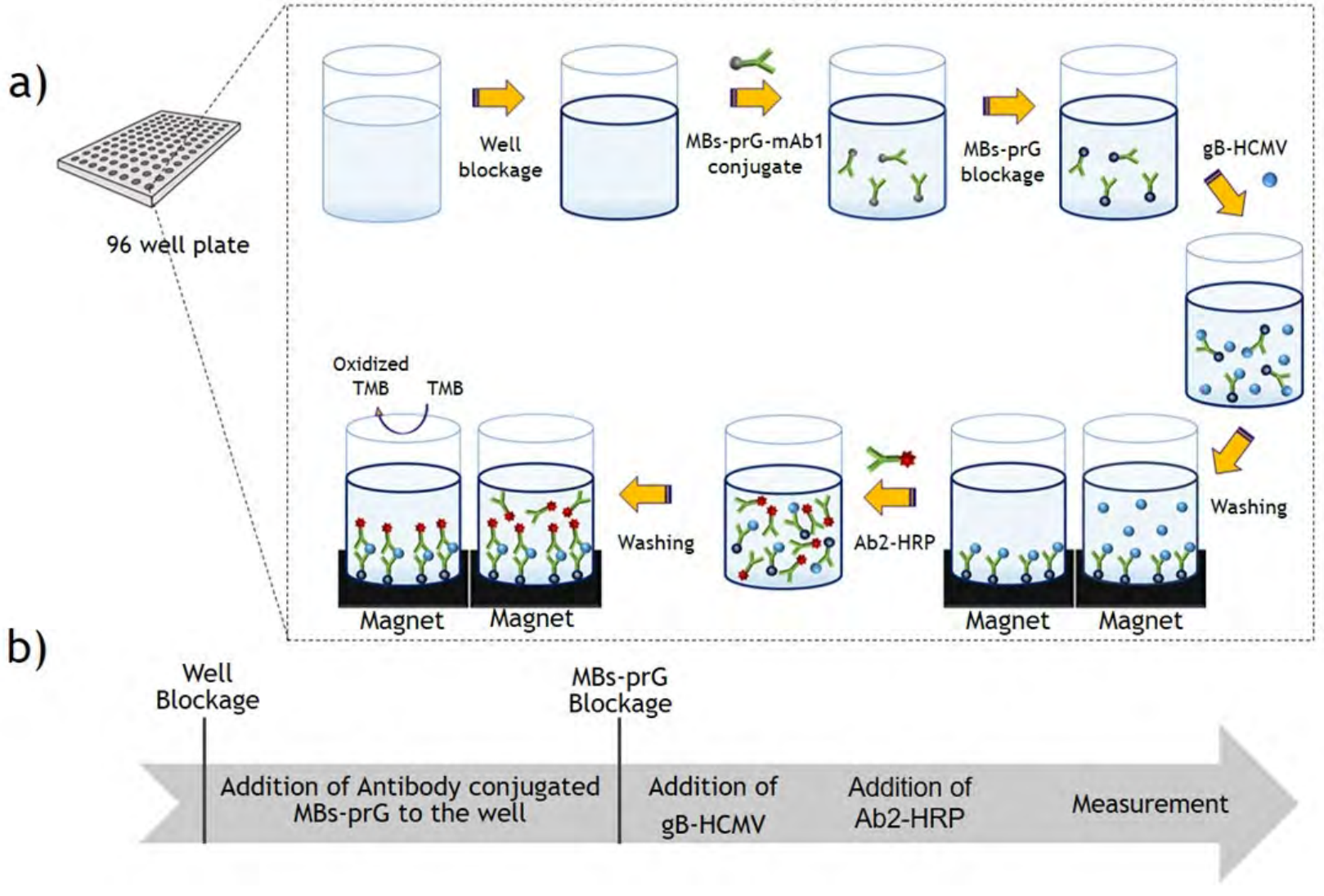
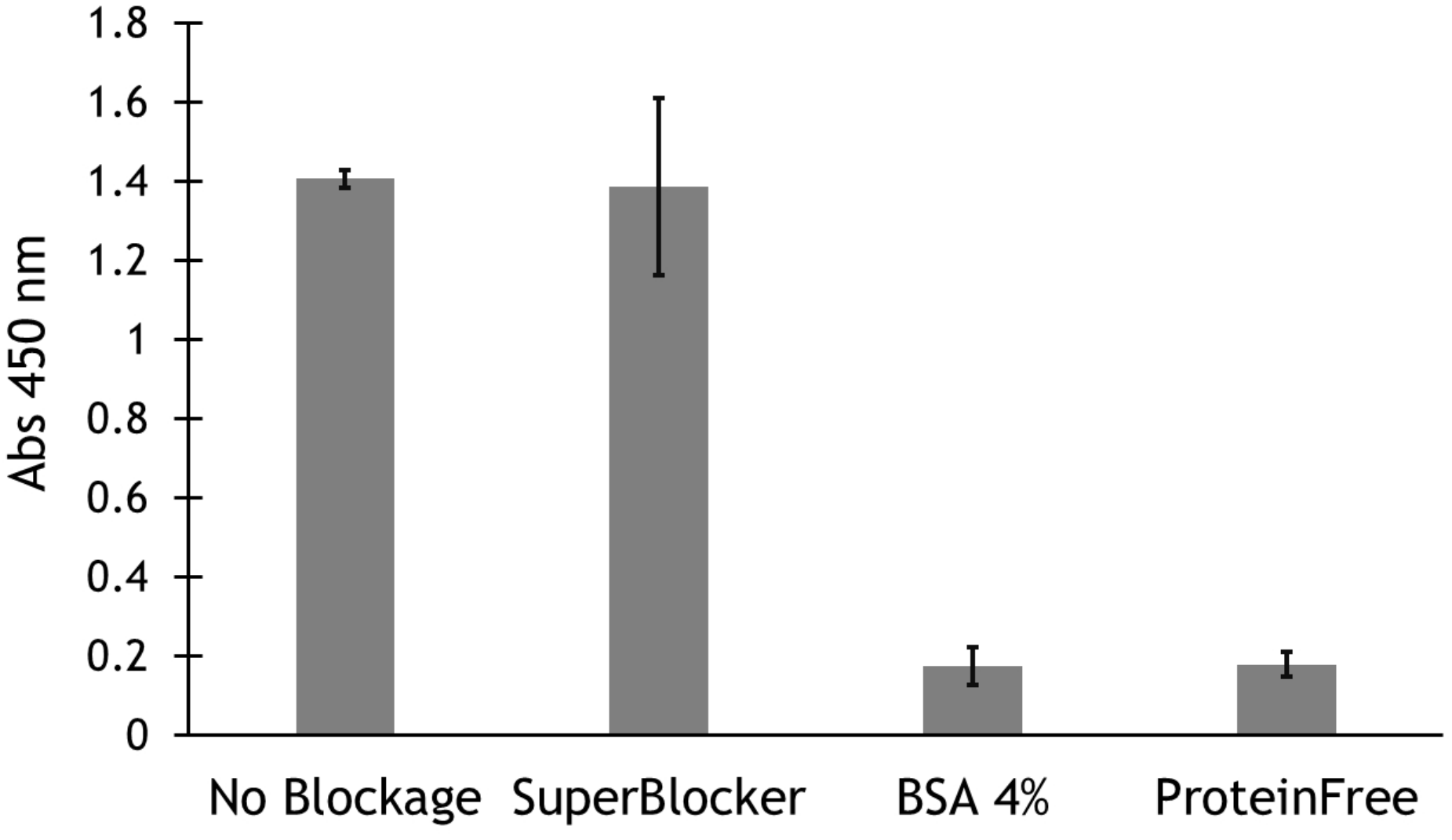
Figure 2. Absorbances measured after the well blockage with SuperBlock, Pierce ProteinFree and BSA blocking agent solutions, after incubations with Ab2-HRP for 60 min. The absorbance values are the mean±SD of n=5 replicates.

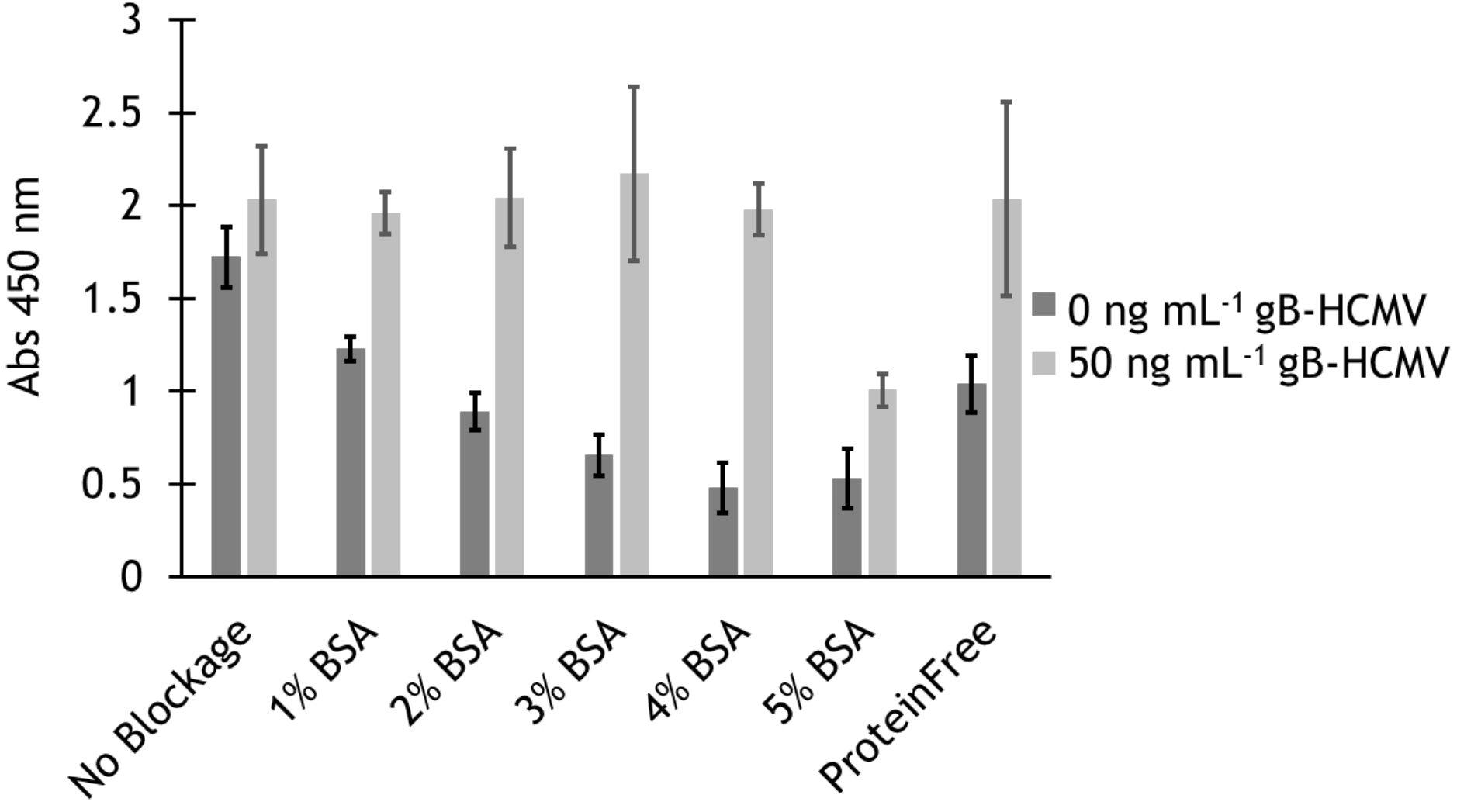
Figure 3. Influence of the MBs-PrG-mAb1 blockage on background absorbances from NSA of Ab2-HRP and on absorbances from gB-HCMV detection. An excess of concentration of 50 ng mL^{-1} gB-HCMV was used. Results are the mean±SD of *n=5 replicates*.

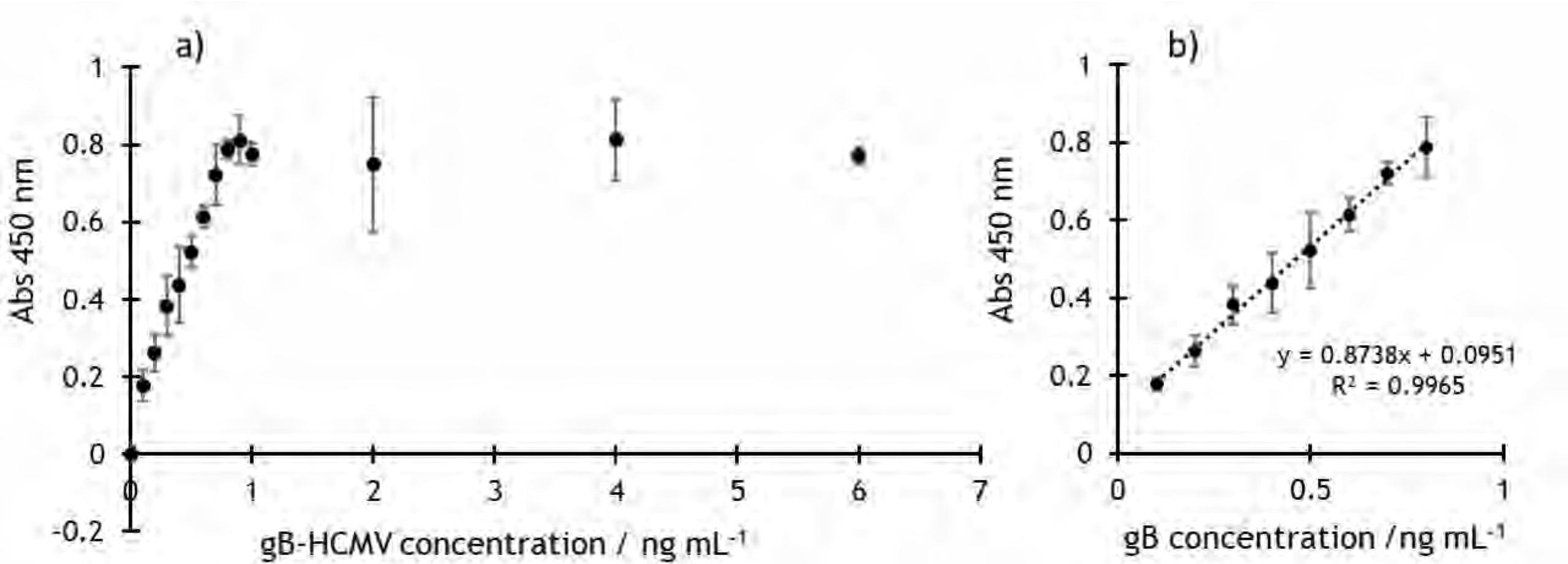
Figure 4. Absorbance signals of the mpEIA assay method for gB-HCMV concentrations from 0.00 to 6.00 ng mL^{-1} in 0.1 M. buffer matrix (a) and calibration curve from 0.07 to 0,80 ng mL^{-1} (b). Experimental conditions: 5 µg of saturated with antibody MBs added to the wells (8 µg of mAb1 per mg of MBs-PrG). Results are the mean±SD of n=3 *replicates*.

Figure 5. Study of the cross-reactivity of EBV and VZV viruses on gB-HCMV determination, and comparison with control solutions without gB-HCMV. Results are the mean±SD of n=3 *replicates*.


a)
96 well plate
Well blockage
MBs-prG-mAb1 conjugate
MBs-prG blockage
gB-HCMV
Washing
Ab2-HRP
Washing
Oxidized TMB
TMB
Magnet
Magnet
Magnet
Magnet
b)
Well Blockage
MBs-prG Blockage
Addition of Antibody conjugated MBs-prG to the well
Addition of gB-HCMV
Addition of Ab2-HRP
Measurement


Abs 450 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
No Blockage
SuperBlocker
BSA 4%
ProteinFree


Abs 450 nm
3
2.5
2
1.5
1
0.5
0
No Blockage
1% BSA
2% BSA
3% BSA
4% BSA
5% BSA
ProteinFree
0 ng mL-1 gB-HCMV
50 ng mL-1 gB-HCMV


a)
Abs 450 nm
1
0.8
0.6
0.4
0.2
0
-0.2
0
1
2
3
4
5
6
7
gB-HCMV concentration / ng mL-1
b)
Abs 450 nm
1
0.8
0.6
0.4
0.2
0
0
0.5
1
y = 0.8738x + 0.0951
R² = 0.9965
gB concentration /ng mL-1

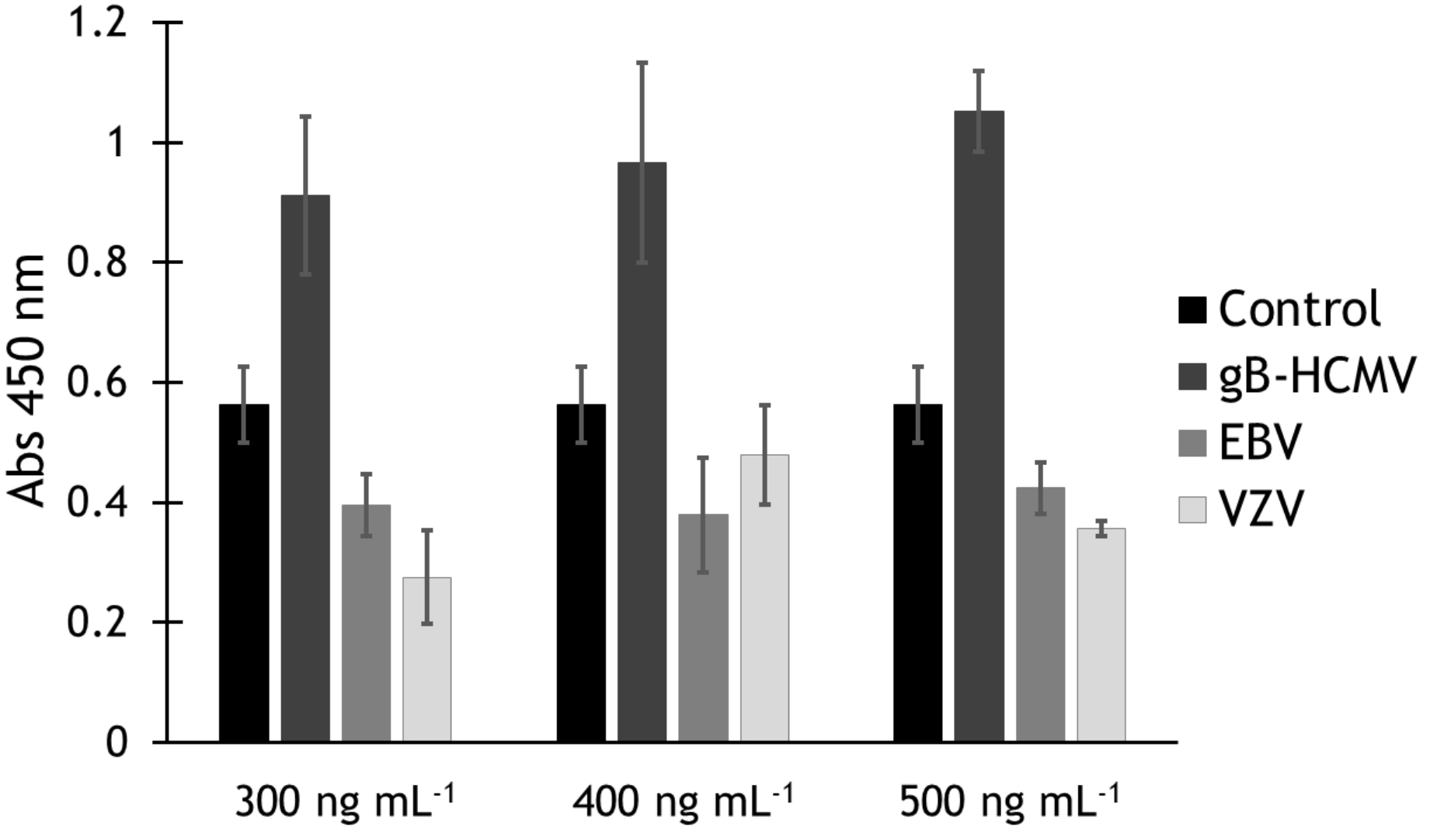

1.2
1
0.8
0.6
0.4
0.2
0
Abs 450 nm
Control
gB-HCMV
EBV
VZV
300 ng mL-1
400 ng mL-1
500 ng mL-1

Table 1.

	Slope	R^2	Linear concentration range, ng mL^{-1}	Detection limit, ng mL^{-1}
PBS	0.97 ± 0.02	0.996	0.10 –0.80	0.07 ± 0.01
Urine	1.17 ± 0.04	0.993	0.10 – 0.70	0.09 ±0.02

Table 2.

Method	Biological matrix	Analyte	Concentration range	Limit of detection	Overall analysis time	Reference
ELISA	Blood	IgG	0.4 – 0.6 IU mL^{-1}	---	---	[7]
Biosensor Based on Imaging Ellipsometry	Blood	IgG	0.1-1.0 IU mL^{-1}	0.01 IU mL^{-1}	---	[33]
Quartz crystal microbalance immunosensor	---	gB-HCMV	2.5 -5 µg mL^{-1}	1 µg mL^{-1}	~20h	[13]
Electrochemical immunosensor	Urine	gB-HCMV	5-15 ng mL^{-1}	3.2 ± 0.2 ng mL-1	~1h	[4]
Electrochemical Immunoassay	Saliva	pp65	0.1 – 80 ng mL^{-1}	30 pg mL-1	>24h	[34]
mpEIA	Urine	gB-HCMV	90 - 700 pg mL^{-1}	90 ± 20 pg mL^{-1}	~3 h	This work

Table 3.

Spiked gB-HCMV concentration, ng mL^{-1}	Calculated gB-HCMV concentration, ng mL^{-1}	% Recovery	RSD %
0.30	0.28±0.01	93.3%	4.7%
0.40	0.41±0.03	102.5%	6.5%
0.50	0.53±0.01	106.0&	4.5%