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A SIMPLE, RAPID, AND HIGHLY SENSITIVE MAGNETIC BEADS ELISA FOR DETECTION OF SARS COV-2 ANTIBODIES (IgG) IN HUMAN PLASMA SAMPLES AS A POINT OF CARE ASSAY

The pandemic outbreak of coronavirus (SARS CoV-2) has been going on over the last 3 years. The people are vaccinated with different vaccines targeting the S protein. ***Aim.*** *Therefore, it is essential to have an assay that can detect different parts of the virus as a serological assay and can be performed as a point of care test. Hence, in this work, we decided to develop such an assay with the help of magnetic beads.* ***Methods****. The magnetic beads ELISA (MB ELISA) was developed in a microtube. The viral ligand- specific magnetic beads were used to detect the nucleoprotein (NP)-specific IgG antibodies in human plasma samples. The results were read with the naked eye as well as with professional ELISA readers.* ***Results****. 7 µL magnetic beads were suitable to detect the presence of NP-specific antibodies. The assay needs only a magnetic rack and a pipettor to be performed. The results were available within 30 min. The positive results were observed as yellow color visually but also read in ELISA reader as OD values. The sensitivity of this assay was 1:10^8 dilutions. The cross-reaction panel was negative with different pathogens and negative human plasma.* ***Conclusions****. This work may be the first report in literature about the development of a magnetic beads ELISA as a point of the care assay, which is reproducible, highly sensitive, robust, and easy to perform. It was used to detect the presence of NP-specific IgG antibodies in the plasma samples successfully. This assay can be used as a professional assay, where the results can be measured with an ELISA reader. This assay may be suitable in small clinics also under field conditions. It can be used to detect the SARS CoV-2 infection in vaccinated persons (S protein-based vaccines) along with non-vaccinated population in latent and active phase.*

Keywords: *coronavirus, serology, SARS CoV-2, magnetic beads.*

Magnetic beads have been used in biomedical and clinical research for different purposes including the isolation of stem cells and mononuclear cells like T and B cells over many years [1, 2]. The recent pandemic outbreak of SARS CoV-2 (Wuhan strain and its mutations) has been going on over the last 3 years since the beginning of 2020. To detect this virus and its

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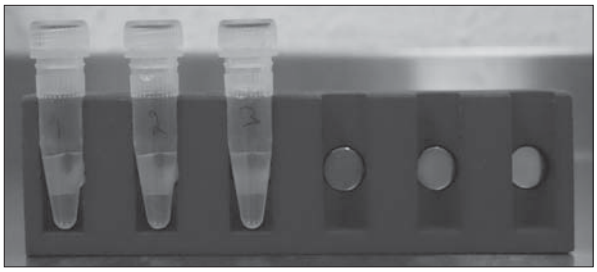

Fig. 1. Magnetic rack with microtubes showing a washing step

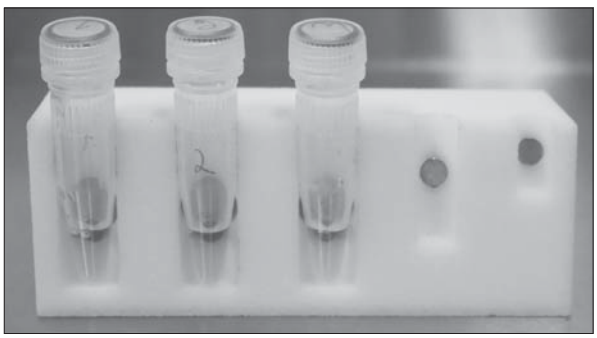

Fig. 2. The yellow color indicates positive samples, and the transparent solution — negative control

antibodies, there are different methods, e.g., the real-time PCR tests and rapid tests [3]. To detect the presence of SARS CoV-2 specific antibodies in the blood and its fractions such as plasma and serum, serological tests, e.g., ELISA based on plates and conventional ELISA need many hours to provide the results. Therefore, the scientific community is looking for alternatives to perform ELISA in a shorter time [4—8].

Magnetic beads are discussed in the literature as an alternative as M magnetic beads tests can give the results shortly, moreover, can be read with the naked eye as well as with an ELISA reader. Such tests can act as a point of care as well [8]. In the literature, there are a few reports on MB ELISA tests [9—12].

Most of the vaccinations against SARS-CoV-2 in Europe and the USA are based on S-protein, hence vaccinated persons should have antibodies against S-protein only. In case of natural infection, such persons are going to have antibodies against the other parts of the virus, e.g., Nucleoprotein (NP). Therefore, we decided to develop an MB ELISA assay to detect NP- specific antibodies in human plasma samples.

Materials and methods. 100 μL Blocking puffer (Genekam, Germany) was pipetted in 1.5 mL microtubes (Starstedt, Germany), and to this, 7 μL or 10 μL of SARS CoV-2 ligand-specific magnetic beads (Genekam) were added. The fluid was kept for 5 min at room temperature. After that, the fluid was removed, while keeping the tubes for 2 min in a 6-tube magnetic rack (Genekam). The plasma or serum samples were diluted with diluting buffer 1:2 or 1:4, or more, depending on the application. 100 μL of the diluted serum or plasma samples was added to magnetic beads and kept for 3 min at room temperature.

The samples were washed with 1.5 mL washing buffer (Genekam) 3 times (Fig. 1). 100 μL of conjugate anti-human IgG was added to each well and kept at room temperature for 5 min. The magnetic beads were washed 4 times with 1.5 washing buffer. 100 μL of the substrate was used in each well and kept for 7 min at room temperature in the dark. The reactions were stopped with a sulfuric acid solution (Applichem, Germany). This will turn the color yellow in positive samples but in negative samples and control, there will be no color, i.e., the solution remains transparent. The results can be read with the naked eye (Fig. 2). To quantify the results, 100 μL of solution from each tube was put on a 96 well plate (Sarstedt, Germany).

The plate was read with an ELISA reader (BioBase, China) at 450 nm wavelength. The test was completed within 30—45 min depending on the number of samples. The whole procedure is a modification of the conventional ELISA on the plates of the author's research work.

The test was conducted with positive samples and positive or negative controls. In the negative control, there were ligand-specific magnetic beads, and only diluting buffer was added. All experiments were conducted under sterile lam-

inar flow. The positive controls were obtained from NHS, UK. The test was performed on unknown plasma samples.

The sensitivity was calculated with a10-fold serial dilution of 1:2 diluted positive samples. The cross-reaction panel included known positive samples for influenza, measles, Epstein Barr virus, etc.

Results. The MB ELISA detects specifically positive samples for NP-specific antibodies in plasma samples. The negative control was negative. There were around 4 unknown samples, positive for the presence of NP- specific antibodies. These samples were repeated 3 times, and they were also positive. The OD values measured with the ELISA reader varied between 1.155 and 2.856 (Table 1). The results of 10 experiments can be seen with the naked eye (Fig. 2), which means that this assay can be used as a point of care under the field conditions. The cross-reactions panel with related pathogens showed negative results (Table 2). The control samples from NHS, UK, were positive. ElISA's readings were between 0.580 to 1.900 for different positive samples. Two different volumes of plasma 25 and 50 μL were used successfully.

The sensitivity limit of the assay was 10^8 (Table 3).

It was found that 7 μL of ligand-specific magnetic beads is sufficient to conduct the assay. |In the beginning, 10 μL of beads was used, but it was found that 7 μL is the optimal concentration to conduct the assay. The negative controls were negative with 7 μL magnetic beads against 10 μL magnetic beads, where there is some problem of cross-talking.

The time needed to perform this assay was 30 min. It was found that it can be cut to 15 min, but the washing steps need a lot of time, which increases the time of conducting the assay. Genekam magnetic racks are made in such a way that the microtube fits the rack tightly so that the fluid can be thrown away just by tilting the rack. The rest of the fluid can be removed with a pipette.

Discussion. In this research work, we have developed an MB ELISA for detecting the presence of NP-specific antibodies (IgG) of SARS CoV-2. It can be used as a point of care assay under the field conditions as this assay can be performed at room temperature and needs only a magnetic rack, pipettors, and pipette tips. This assay is highly sensitive: it can detect up to 10^8 dilutions, which is more than the detection limit of the conventional ELISA. The reason may be that the magnetic beads present the antigen in the 3D dimension against the conventional ELISA, where

Table 1. **The OD values of different plasma samples with magnetic beads**

Experiment	Plasma (OD values)				
	1	2	3	4	5
Plasma sample	2.116	2.852	2.840	1.155	2.386
Negative control	0.082	0.091	0.078	0.069	0.09

Table 2. **Cross-reaction panel**

Pathogen	Result
Influenza A	negative
Mumps	negative
EBV	negative
Human Plasma	negative
Measles	negative

Table 3. **Sensitivity range with different dilutions**

Dilutions	Result
1 : 0	positive
1 : 10	positive
1 : 100	positive
1 : 1000	positive
1 : 10000	positive
1 : 100000	positive
1 : 1000000	positive
1: 10000000	positive
1 : 10000000	positive
1: 100000000	negative
1: 1000000000	negative

the antigens bind to the receptors on the plastic surface in the 2D dimension. Another advantage is that the MB ELISA lasts within 30 min, while the conventional ELISA takes 3 to 24 h. Our MB ELISA needs only 7 μL of antigen beads, which is very little, thus it can give the results with as little as 25 μL plasma from the patient.

Using this basic method, we have developed a number of MB-ELISA tests against other common pathogens, which are being validated**,** and the work will be published later. Therefore, only a small number of samples are shown in this paper. Moreover, this test has some other important applications as well, which will be published later. The time needed to develop this whole assay was more than 5 years of research in our laboratory. We have designed a new type of magnetic rack, where the tubes fit tightly in order to throw the supernatant while tilting the rack against the magnetic racks available on the market, where tubes shake and consume many pipette tips to remove the supernatant and many times suck away the magnetic beads, leading to failure.

Huergo et al. have developed another MB ELISA for the detection of SARS CoV-2-specific antibodies using a 96-well plate system, but that test cannot be used under field conditions as it needs expensive instruments, e.g., a special magnetic rack and an ELISA reader, in addition to the 96-well plate system, hence it cannot be used as a point of care assay. It is for high-level laboratories, where the user has labs with a full set up of instruments for serological assays, while our MB ELISA can be used worldwide as a point of care test, where the results can be read with both the naked eye and an ELISA reader, if available. The chemicals are in a position to be transported easily [13].

This assay is available as a commercial test so it can be used under field conditions for small clinics and mobile laboratories with limited sources and worldwide. The chemicals can be shipped at both 4 °C and room temperature. This assay can be performed at room temperature. Its cost is going to be around Euro 1 per test.

As this assay detects NP-specific antibodies, it can be used to detect the presence of these antibodies in vaccinated and infected persons with both active and latent infection.

Conclusions. It may be the first report in the literature about the magnetic ELISA test for the detection of SARS CoV-2 antibodies in plasma samples with a high sensitivity as a point of care test along with the possibility to quantify the concentration of antibodies with an ELISA reader. Point of care assay needs only simple instruments, namely a magnetic rack, pipettors, and pipette tips. It can be conducted anywhere worldwide.

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Conflict of Interests. There is no conflict of interests.

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ПРОСТИЙ, ШВИДКИЙ ТА ВИСОКОЧУТЛИВИЙ МАГНІТНИЙ ІФА-ТЕСТ
ДЛЯ ВИЯВЛЕННЯ АНТИТІЛ (IgG) ДО ВІРУСУ SARS CoV-2 У ЗРАЗКАХ ПЛАЗМИ КРОВІ ЛЮДИНИ
ЯК ЕКСПРЕС-ТЕСТ ДЛЯ НАДАННЯ МЕДИЧНОЇ ДОПОМОГИ

Пандемія коронавірусу SARS CoV-2 триває вже 3 роки. Люди вакцинуються різними вакцинами, націленими на білок S. **Мета.** Розробити тест на основі магнітних кульок, який може виявляти різні частини вірусу як серологічний аналіз і може бути виконаний як швидкий тест на місці надання медичної допомоги. **Методи.** ІФА-тест з магнітними кульками був розроблений з використанням мікропробірок. Специфічні до вірусного ліганду магнітні кульки використовувалися для виявлення специфічних до нуклеопротеїну (NP) антитіл класу IgG у зразках плазми крові людини. Результати зчитували як неозброєним оком, так і за допомогою лабораторних ІФА-сканерів. **Результати.** 7 мкл магнітних кульок здатні виявляти наявність NP-специфічних антитіл. Для проведення аналізу потрібен лише магнітний штатив та піпетка. Результати доступні через 30 хвилин. Позитивні результати спостерігали візуально як по наявності жовтого кольору рідини, так і по показниках оптичної густини (ОГ) ІФА-рідера. Чутливість цього аналізу становила $1:10^8$ розведень. Панель перехресних реакцій була негативною щодо інших різних патогенів, а також щодо людської плазми. **Висновки.** Ця робота є першим в літературі повідомленням про розробку ІФА з використанням магнітних кульок. Розроблений метод є відтворюваним, високочутливим, надійним і простим у виконанні. Він був використаний для успішного виявлення специфічних IgG антитіл до NP у зразках плазми крові. Цей аналіз може бути використаний як професійний, де результати отримують за допомогою ІФА-рідера, і бути придатним для тестів у невеликих клініках, а також у польових умовах для виявлення інфекції SARS CoV-2 у вакцинованих осіб (вакцини на основі S-протеїну), а також у невакцинованого населення у латентній та активній фазах.

Ключові слова: *коронавірус, експрес-тест, серологія, SARS CoV-2, магнітні кульки.*