Infectious serology, how to test large series without pooling samples

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Abstract:
Pooling samples for serological testing was used first during the second world war. It was described later as a cost-effective technique permitting large screening of populations, especially for new infectious diseases. However, the dilution effect is responsible for decreasing sensitivity, limiting its use in practice, especially blood banking. In this paper, we describe a modification of the classic enzyme-linked immunosorbent assay (ELISA) procedure, which permits the test of indefinite samples using just one well. Specimens are tested pure one by one without any dilution, so sensitivity remains unchanged. This new procedure is time-consuming but can be considered as a revolution in qualitative ELISA testing.

Keywords: ELISA, pooling sera, infectious serology, Algeria

Background
ELISA testing was developed in the 1960s as a safety method to replace radioimmunologic assays. In the 80s, ELISA was generalized in all laboratories with the discovery of the human immunodeficiency virus. However, in the first times, the cost was high and couldn't be supported in low-income countries. Pooling sera for testing was considered a solution to reduce the cost of screening large populations; six to ten sera were mixed and tested. The negative result excluded the infection in all samples, while the positive reaction requires analyzing them one by one [1, 2]. Unfortunately, mixing sera shows to reduce the test's sensitivity, especially when diluting some low positive samples. In this case, the reactivity will be lower than the cutoff of detection [3, 4]. Thereby, Novack found a diminution of the sensitivity of detecting antigen S of B hepatitis from 99% to 93% when pooling and an extension of the serological windows for five days [5]. In another study, Novack also found a reduction of the sensitivity by 3% to detect antibodies of C hepatitis when using a pool of 6 samples [6]. In addition to the effect of dilution, there is a risk of neutralization of antigens such as antigen S of B hepatitis when mixed with sera containing antibodies against antigen S [5]. For these reasons, pooling was prohibited in blood banking, even in developing countries [7]. We presented a modification of the procedure of qualitative ELISA permitting testing theoretically an infinite number of samples using just one well.

Presentation of the hypothesis
In ELISA testing, wells are coated with an antibody (or antigen for sandwich ELISA), forming a solid phase [8]. In the first step, the sample is added, and the eventual antigen (or antibody) present in sera will set to the solid phase. After washing, this reaction will be completed by the addition of a conjugate containing an antibody (or antigen) associated with a specific enzyme such as alkaline phosphatase (EC 3.1.3.1) or glucose oxidase (EC 1.1.3.4). This enzyme catalysis a colorimetric reaction, which means a positive reaction.

The proposed idea consists of the incubation in one well of samples one after one before washing and adding conjugate. In the first step, a sample is incubated, and in the second step, the first sample is eliminated from the well, and a second sera is added for incubation. These operations can be repeated many times (eliminating sera and incubation of a new one in the same well). If one of the multiple samples tested contains the specific antibody (or antigen), it will be irreversibly fixed in the solid phase even if all other samples are negatives. The procedure is completed without modifications with washing, the addition of conjugate followed by colorimetric revelation.
A positive reaction means that at least one of the samples is reactive and impose analyzing them one by one or by smaller groups.

**Evaluation of the procedure**

To be validated, this method must present the same sensitivity as classical ELISA testing. For evaluating this procedure, positive control, or a known positive sample (confirmed containing antibodies or antigens of an infectious disease) should be tested first and followed by a series of known negatives sera in the same well as described above. In the end, the reaction must be positive.

The sensitivity of this new procedure can also be evaluated and compared to classical methods. For this, the positive sample should be diluted half to half (1/2, 1/4, 1/8, 1/16, 1/32…). Each dilution is tested in duplicate with classical ELISA and with the proposed procedure. The last dilutions giving a positive reaction with the two methods are compared and must be the same.

**Consequences of the idea**

The main disadvantage of pooling sera is the dilution effect as described above. The proposed procedure permits to test of pure samples, so the sensitivity remains unchanged and avoids antigens' neutralization. Successive incubations of samples are time-consuming, but the financial benefit is significant.

This hypothesis's benefit is also important, for example, during the first times of a pandemic, especially with new emerging infectious diseases. In these situations, the need for screening tests exceeds their production rate. This has been observed in the 80s with the apparition of the acquired immunodeficiency disease syndrome and, more recently, with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [9]. The other benefit is economic; this hypothesis's application permits a drastic reduction in screening populations' cost, particularly in low-income countries.

**Conclusion**

Incubating samples one by one in ELISA testing, as described in this paper, seems to be a real alternative to pooling sera without any disadvantage.

**Abbreviation**

ELISA: Enzyme-Linked Immunosorbent Assay; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

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**Availability of data and materials**

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**Authors' contributions**

Mohammed Nazim Bennoum (MNB) is the principal investigator of this manuscript (Viewpoint). MNB proposed the idea, and AD, and MC have contributed to the writing, reviewing, editing, and approving the manuscript in its final form. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

We conducted the research following the Declaration of Helsinki. However, Viewpoint Articles need no ethics committee approval.

**Consent for publication**

Not applicable

**Competing interest**

The author declares that he has no competing interests.

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