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Editorial

Infectious disease serology in 2021

A selective history of serology tests

Classically, serology is defined as the study of proteins (such as antibodies) in the blood. The application has been extended to antigens and to other body fluids such as cerebrospinal fluid and aqueous humour. Among the first immunoassays to detect the presence of antibodies are the Widal agglutination test and the complement fixation test (CFT). Agglutination is a serological reaction resulting in the clumping of a cell suspension when a specific antibody is directed against a specific antigen.

The Widal agglutination test was developed in 1896 and named after Georges-Fernand Widal, a French physician. It used a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in serum from patients with fever suspected for *S. typhi* infection [1]. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient against the H (flagellar) and O (somatic) antigens of *S. typhi*. More than 100 years after its invention, this test has been more or less abandoned due the arrival of culture and molecular tests, while the Widal test could not overcome the problems of test variability, lack of reproducibility, and cross-reactivities with other non-salmonella organisms.

The CFT was developed in 1901 by two Belgians, Jules Bordet and his brother-in-law Octave Gengou [2]. The test is based on the ability of complement protein to bind antigen–antibody complexes and to lyse erythrocytes [3]. When an antibody is present in a blood sample, and is incubated with a pathogen-specific antigen, an antigen–antibody complex will form, and this complex will be bound (i.e. fixed) by the complement. Erythrocytes added to the system will be protected from lysis because of this fixation of the complex by the complement.

The CFT has been used to detect antibody responses to various pathogens such as *Mycoplasma pneumoniae*, but many CFT assays have been replaced by less laborious and more sensitive enzyme immunoassays (EIAs) that detect the presence of the antibody by using labelled antibodies. Over the years, EIAs have been transformed from manual to automated techniques. While the CFT is able to detect an antibody at a concentration as low as 1 μg/mL, the lower detection limit of EIAs is approximately 1 ng/mL [4]. Immunoassay technology has been further improved by the chemiluminescence immunoassay (CLIA) that can detect the presence of antibodies at extremely low concentrations (as low as 10^{-21} mol) [4]. Instead of a chromogen substrate, CLIA uses a luminescent chemical as substrate.

Automated EIAs and CLIAs in clinical laboratories allow the turnaround time to be as low as 1 hour. Nevertheless, the patient's sample still needs to be transported to the clinical laboratory, and therefore there is still an increasing demand for rapid immunoassays that can be performed in the proximity of the patient (point-of-care tests), for example in low-resource settings where no laboratory is available. One such assay is the lateral flow assay (LFA). It uses a single chromatographic pad with three areas: an area where the sample is applied, an area where the sample is conjugated with the antigen (or antibody) tagged with a specific label, and the reaction area. In the reaction area, when the target antibody is present in the sample, a secondary antibody (or antigen) specific to the target binds to the labelled conjugated target antibody (or antigen). The LFA is not only rapid but also cheaper than a molecular test. During the current coronavirus disease 2019 (COVID-19) pandemic various antigen LFAs have been tested extensively. While the manufacturers of these tests often report high sensitivity and specificity, in real practice their performance has been suboptimal. A Cochrane review showed a sensitivity of 78.3% (95%CI 71.1–84.1%) during the first week after symptom onset, and a specificity of 99.6% (95%CI 99.0–99.8%) [5]. These LFAs cannot fully replace the PCR test, but they may still be considered in certain settings, such as when quick decisions must be made to determine the patient's COVID-19 status (e.g. to triage patients from the emergency department to COVID-19 wards) when the number of (rapid) PCR tests is limited [5,6]. This situation was experienced by the first author recently in the Curacao Medical Centre in the Dutch Caribbean during the third peak of confirmed COVID-19 cases. Four days before the Easter weekend, 506 new cases were identified on an island with 160 000 inhabitants. This number per capita was the highest in the world that week, and the highest yet measured in Curacao [7]. Due to global competition for COVID-19 tests, the capacity of rapid molecular tests could not be increased rapidly enough. In the setting where the likelihood of COVID-19 test being positive was high, we deployed an LFA test in the emergency department to rapidly triage patients to either COVID-19 or non-COVID-19 wards. We had learned in the week before implementing the LFA test as a screening test that the likelihood of a patient presenting in the emergency department with respiratory insufficiency having a positive COVID-19 test was 63%. Higher prevalence means a higher positive predictive value of a test (and a decrease in false-positive results), and a lower negative predictive value (and an increase in false negatives) [6]. In our two-tiered test algorithm, patients with a positive LFA test needing hospital admission were referred directly to the COVID-19 ward, while patients who tested negative were re-tested using the rapid PCR test. In this high-prevalence setting, the positive predictive value was as high as

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93.1% (95%CI 66.9–98.9) using the LFA test (that we could obtain at that time) with a subpar performance: sensitivity of 65.8% (95%CI 48.7–80.4) and specificity of 91.7% (95%CI 61.5–99.8). Using the approach of screening the patients using the LFA test, we spared 42% of the COVID-19 rapid molecular tests. Surely, this was a unique setting where the likelihood of a COVID-19 test being positive was so high. In a lower prevalence setting, even LFA tests with higher performance would not have this high positive predictive value.

Infectious serology application in clinical practice and public health anno 2021

The technology of infectious disease serology assays has been thus evolving ever since the Widal test and CFT more than 100 years ago, and it has been advancing rapidly in the last decades. The application of infectious disease serological assays is extremely broad since theoretically it can be developed for every pathogen. Choosing topics for a theme issue on serology is therefore challenging, and we have opted to choose reviews giving an overview of the application of serological tests in public health and hospital practice. The narrative reviews will discuss the serology of bacteria (albeit only in a short historical perspective) [8], viruses [9] and fungi [10].

Despite the evolving technology in serological assays, serology is seldom used as the sole diagnostic tool. Only for a few bacterial infections, such as syphilis and disseminated manifestations of Lyme disease, are serological assays still used as the primary test for establishing the diagnosis. In clinical virology, serology is mostly used to determine the stage of infection (acute versus past) by detecting the presence of the IgM antibodies, or by showing significant changes in antibody titres (follow-up serum samples are needed), or by avidity testing (IgG antibody produced early in the infection showing low binding strength between antibodies and virus). Yet, in several clinical situations, serological assays can establish the diagnosis when antibody indexes for specific viral pathogens are calculated. Shamier et al. [9] reviewed the groundwork of Reiber and Felgenhauer that had already been developed half a century ago. They also discussed clinical situations where difference in concentrations of specific antibodies in the cerebrospinal fluid and serum is used for the diagnosis of the central nervous system infection caused by herpes viruses (herpes simplex virus (HSV), cytomegalovirus (CMV) and varicella zoster virus (VZV)), flaviviruses, enteroviruses, measles virus and influenza virus. For this purpose, in certain situations the antibody index performs better than the PCR test. They also discuss the Goldmann–Witmer coefficient, a calculation of antibody index use that is used in diagnosing viral infections of the eye.

Use of antibody tests is limited to the diagnosis of chronic forms of aspergillosis, including allergic bronchopulmonary aspergillosis, while the use of antigen tests has become a reference standard for diagnosing invasive fungal infections. Lass-Flörl et al. discuss the use of serological assays in detecting these acute and chronic fungal infections [10]. The aspergillus antigen (galactomannan) test is also relevant for the COVID-19 pandemic since a positive galactomannan index is often found in respiratory samples of critically ill COVID-19 patients [11]. Positive antigen in sputum or tracheal aspirate may represent colonization and should not be performed in these patients, but when this test is positive in a representative bronchoalveolar lavage, COVID-19-associated pulmonary aspergillosis should be considered.

Considering the emerging infectious diseases, Fischer et al. reviewed the use of serological tests to diagnose emerging infectious diseases such as Zika, Dengue fever and Chikungunya [13].

As a diagnostic test, serological testing can also be used as a screening or surveillance tool. When a large part of the population needs to be screened, testing each individual for screening purposes is laborious, time-consuming, and expensive. An approach to coping with these problems is to combine samples from multiple individuals and test the combination as one group. Group testing (pooling) dates as far back as 1943 when Dorfman proposed this approach to screen World War II soldiers for syphilis using serological assays [12]. In this theme issue, Grobe et al. reviewed this approach by explaining the mathematics of pooling test strategies, and they show several potential practical and clinical applications of this approach using the PCR test for COVID-19. Yet, as explained in their review, when prevalence increases, the pooling strategy is no longer efficient. The grouping or pooling strategy may also be used for surveillance purposes at the hospital level, for example in contact tracing after the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) where a certain number of individuals who came in close contact with the index patient need to be screened.

Concluding remarks

The history of serological assays for infectious diseases and their clinical application covers more than 100 years. This paper accompanies four papers on the practical application of serological assays for use in clinical and public health settings. Since this field is still evolving, it would be compelling to see whether these applications will still be relevant and whether new applications will have found their way into clinical practice a decade from now.

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