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Continuing medical education: Methods of rapid diagnosis

Rapid diagnosis in serology☆

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ABSTRACT

Serological diagnosis of acute phase infections implies the detection of IgM specific response, an effective marker of primary infection, but with less clinical significance in reactivations or reinfections. The aim of this article is to provide an updated view of the rapid diagnosis in serology by detecting the IgM isotype and reviewing its applications and limitations. Point-of-care (PoC) tests are analysed. PoC tests are used in geographical areas where traditional tests are not available, as well as in other circumstances where their use brings the diagnosis directly to the target population. Likewise, their use reduces the response time between taking the sample and the diagnosis, making it easier to make clinical decisions. PoC assays have proven cost-effective, especially in preventing vertical transmission of syphilis and HIV infection.

Diagnóstico rápido en serología

Palabras clave:
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RESUMEN

El diagnóstico serológico de las infecciones en fase aguda implica la detección de la respuesta IgM específica, marcador eficaz de infección primaria, aunque con menos validez en las reactivaciones o reinfecciones. El objetivo de este artículo es proporcionar una visión actualizada del diagnóstico rápido en serología mediante la detección del isotipo IgM y revisar sus aplicaciones y limitaciones. Se analizan especialmente ensayos *Point-of-Care* (PoC) utilizados en áreas geográficas donde las pruebas tradicionales son inaccesibles, y en otras circunstancias donde su empleo acerca el diagnóstico a la población diana, debido a que pueden efectuarse en centros no sanitarios. Asimismo, su empleo disminuye el tiempo transcurrido entre la toma de muestra y el diagnóstico, facilitando al clínico la toma de decisiones. Los ensayos PoC han probado su coste-efectividad, especialmente en la prevención de la transmisión vertical de la sífilis y en la infección por el VIH.

Introduction

Diagnostic microbiology of acute infections involves the detection of microorganisms by direct methods, such as cultures and molecular techniques, or indirect methods, such as serology. In terms of serological methods, the fastest way to obtain an aetiological diagnosis is the detection of specific IgM response. This is an effective marker of primary infection despite its limited usefulness in reactivations and reinfections due to their fugacity and low-intensity manifestation. Nevertheless, demonstrating seroconversion equates to a definitive diagnosis, albeit delayed owing to the time taken to conduct a parallel study of samples obtained during both the acute and convalescent phases.

Serum or plasma clinical samples, usually in liquid phase, tend to be used for serological testing, although blood spots on filter paper are also sometimes used. Other bodily fluids, such as cerebrospinal fluid (for central nervous system infections), saliva and urine may also be used.

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Serological techniques

The classic serological techniques (such as neutralisation, complement fixation and haemagglutination inhibition) detect total antibodies and therefore do not offer a rapid diagnosis. Pre-analytical processes are generally required to eliminate non-specific reactants and the techniques are not automated, making them laborious to carry out. Solid-phase techniques, on the other hand, such as enzyme immunoassay (ELISA), immunofluorescence (IF), immunochemiluminescence (ICL) or immunochromatography (IC) can identify class-specific antibodies, and diagnosis by means of IgM detection is fast. ELISA and ICL assays are available in automatic formats that require expensive equipment.

Rapid serological diagnosis: IgM detection

This is achieved by indirect assays and capture assays. In indirect assays, the specific antibodies in the serum bind to the immobilised antigen in solid phase. The IgM isotype is recognised by anti-IgM antiserum, which conjugates with the assay-specific indicator to produce the reaction. In capture assays, the IgM in the sample binds to an anti-IgM antiserum. If the sample contains specific IgM, it is recognised by adding a conjugated or viral antigen, together with, or followed by, a conjugated antiserum. In both cases, the intensity of the signal generated is directly proportional to the concentration of specific IgM.

Limitations of IgM detection

The validity of IgM as an indicator of recent infection may be compromised by reactivity not directly related to the infection.[1]

a) The presence of rheumatoid factor[2] in individuals with specific IgG. Rheumatoid factor is an IgM autoantibody with anti-IgG specificity. Removing IgG from the sample eliminates this reactivity. Most commercial methods for the detection of IgM that are currently on the market include prior incubation with anti-IgG antiserum, or recommend their use as an option.
b) Certain viruses belonging to a single group share antigenic determinants, expressed as cross-reactivity when measuring IgM.[3] This cross-reactivity is important when dealing with infectious agents that could cause similar symptoms, such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV) in infectious mononucleosis (IM).
c) Multiple reactivity to numerous antigens caused by polyclonal stimulation of memory B cells gives rise to IgM reactivity that is unrelated to the acute infection.[4] It manifests as IgM reactivity to pathogens that had already infected the patient, thereby hindering differential diagnosis.
d) IgM is often present in secondary infections (exogenous reinfection or latent virus reactivation). This poses significant problems in certain situations, such as dengue viral infection or CMV infection during pregnancy. In this case, the primary infection causes congenital infection more often than reactivation, making it extremely important to characterise the infection type in the presence of specific IgM.[5]
e) The persistence of IgM also leads to diagnostic complications, as is the case with *Toxoplasma gondii* infections. Due to the risk it poses to the foetus, its detection in serological testing during pregnancy means having to differentiate the specific IgM response to the acute primary infection.[6]
f) Finally, lack of IgM response is possible in immunosuppressed patients with certain infections, including congenital infection.[7]

In light of the above, assays that are capable of confirming or excluding IgM response, or capable of linking it to primary or secondary infection, are required. These include the establishment of antibody profiles or IgG avidity assays, the latter of which is an effective indicator of primary infection.

Rapid serological techniques

Automated techniques

Most serological tests are available in automated formats, which are especially useful for particularly demanding tests. Their benefits include enhanced performance, accuracy, reduced response time, traceability and less manual work. In short, they are cost-effective. Their drawbacks include the high cost of both equipment and reagents, as well as technical and communication problems with the sample management systems. Table 1 presents a non-exhaustive list of automated assays used by Spanish laboratories, compiled with information from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) (https://www.seimc.org/controldecalidadseimc/).

Point-of-Care assays

Point-of-Care (PoC) assays are performed outside the central laboratory at the patient's bedside, using material and equipment that can be easily transported. Results are either available within minutes or in up to one hour. Specially trained personnel are not required to conduct the assay or to interpret the results. Their uptake is higher in developing countries, taking advantage of their cost-effectiveness to prevent the vertical transmission of syphilis and human immunodeficiency virus (HIV) infection. However, they have also found a role in developed countries as they facilitate the diagnosis of the target population and can be performed outside of healthcare centres.

Immunochromatographic (IC) techniques, also known as lateral flow assays, are currently used to detect IgM and IgG antibodies. The sample passes through a nitrocellulose or nylon membrane where the reaction takes place. The antigens specific to the target antibodies are immobilised on the reaction membrane and bind to any antibodies present. The sample flows to the conjugate pad (antiserum to the target isotype) containing colloidal gold, which changes colour in the presence of antibodies. A control zone is also usually included to ensure that the reaction has taken place successfully. Results are obtained in 15–30 mins.

Infectious mononucleosis (IM)

In young adults and immunocompetent adolescents, the most common clinical manifestation of Epstein–Barr virus (EBV) primary infection is IM. Serological methods such as the heterophile antibody (HA) test and the EBV antibody test are used to establish a microbiological diagnosis. These can differentiate the symptoms associated with EBV from those associated with CMV, HIV or *T. gondii*.

HAs detected in the acute phase of the disease would confirm the diagnosis of IM caused by EBV, as they are not usually present in other conditions and tend to decline rapidly in 2 or 3 months. They are present in 80–90% of patients with IM over the age of 10 years, but the positivity rate is less than 50% for patients below this age, and fail to develop in almost all infants under the age of 3 years.

There are many HA tests available on the market, including variations of the Paul-Bunnell test, latex-particle agglutination tests and IC. Sensitivity ranges from 80% to 95% depending on the age group being tested, while specificity is around 100%.[8] HA false positives are rare but are most likely to occur in patients with autoimmune diseases, leukaemia or certain infections including