



EXPERT'S CORNER: A PERSONAL APPROACH

Febrile reactions in the 21st century



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Febrile reactions are a group of serological agglutination assays which originated in the beginning of the last century. They are still used in developing countries because of their speed and technical ease of application, as well as their low cost. For almost 100 years, these studies were used for the diagnosis of diseases caused by the salmonella bacteria, such as typhoid and paratyphoid fever (Widal reaction) and brucellosis (Huddleston reaction), as well as for the detection of some diseases caused by rickettsias (Weil–Felix reaction in *Proteus OX19* infections). The presence of fever in the clinical picture of all these infectious ailments was the origin of the "febrile reactions" denomination and the "febrile agglutinins panel" concept for patients with fever of unknown origin; both terms are semantically and clinically inappropriate.

Nowadays febrile reactions are within the group of assays considered obsolete. They were undoubtedly of great use in the past; however, for more than 30 years, they have been substituted by other methodologies, and are now a part of the history of medicine. When these assays were initially implemented, sanitary conditions in our country were poor. Consequently, these infectious diseases had a high prevalence. Today, even though eradication of these ailments has not been accomplished, it has been significantly lowered. However, there are still endemic zones, mainly for the salmonella and brucella bacteria. In biostatistics, the lowering of the prevalence of a disease

in a given population invariably generates a proportional decrease in the predictive diagnostic values for a lab assay. This concept is evidently applicable for febrile reactions as well.

This is the reason for which these studies have significantly decreased in their diagnostic sensitivity, specificity and predictive values indicators.

Additionally, there are two other more important factors which limit the diagnostic usefulness of febrile reactions as laboratory assays. The first one refers to the error inherent in the agglutination analytical method, the second one to the fact that, in the majority of cases, the physician makes an inadequate interpretation of the results of said studies.

Analytical method

The commercial reactive consists of a suspension of dead bacteria which are reacted with serial dilutions from the patient's serum. Because this is an agglutination assay, "human error" is present, since no analytical measurement instruments are used and the reading of results depends on the visual appreciation of the observer. The last dilution presenting agglutination from the patient's serum is the result of the antibodies titer of the report. Technically, a subtle difference between the last two tubes of the agglutination reaction may not be detected between two different observers, thus this variation may cause the possibility of two discrepant results of the same sample, whose titers may vary (i.e. between 1:160 and 1:320).

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Major considerations regarding febrile reactions are:

1. The standardization of commercial antigen is very deficient. This explains the discrepant results with the same serum sent to different clinical laboratories; even when using different batches of the same commercial brand antigen, there may be different results in antibody titling.
Lack of appropriate commercial antigen standardization also generates a lack of proper internal quality control in clinical laboratories, which do not have trustworthy accuracy and reproducibility parameters for these assays. According to the above, within the external quality controls of serum sample exchange between different laboratories, there is an evident lack of concordance in the results.
2. Another technical problem that may occur, specifically in some patients with brucellosis, is false negatives, caused by the zone phenomenon. This eventuality is not exclusive to febrile reactions, since it may occur in every type of agglutination or precipitation reaction. This is a result of the lack of equivalencies between the amounts of commercial antigen and the antibody present in the patient's serum. This is a prozone phenomenon, or antibody excess. In order to identify this possible eventuality, serial dilutions of the patient's serum in the presence of the same amount of commercial bacterial antigen will have to be performed.

Considerations of the clinical interpretation of febrile reactions

1. The main problem of febrile reactions is that when interpreting results, it has not been possible to have a uniformed and standardized criteria of which antibody titer is to be considered a significant cut-off point which definitely establishes the diagnostic criteria of the disease. This has led some doctors to prescribe an antimicrobial therapy without justification, for the mere reason of presenting reactivity in any of the assays. The latter occurs even in asymptomatic patients.
2. An antibody titer by itself cannot be associated with a definite diagnosis. In the endemic areas of the disease, a significant interpretation of the antibody titer results is difficult.
3. Febrile reactions may show positive results in a healthy population, and antibodies' titration values may also present significant variations with age. This can be explained by many different concepts, including analytical errors, immunological memory in patients who had the disease, anamnestic immune responses, vaccinated individuals, or by the presence of unspecified crossed reactions with types of different bacteria which have been described in Widal reactions with enterobacteria, in Huddleson with tularemia, cholera and certain types of yersinia enterocolitica, as well as the Weil Felix reaction in patients with previous urinary infections, or who present leptospirosis or Lyme disease.
4. There is also the possibility of false negative reactions in patients who are using antibiotics or who are under

steroid treatment or other types of immunosuppressants. On the other hand, in patients with brucellosis, Huddleson's reaction may result in a negative due to the presence of the prozone effect. This is just a technical problem caused by the inhibition of the agglutination reaction due to an excess of antibodies in the patient's serum. It is clear that like in any other laboratory assay, all results must be interpreted according to the patient's symptomatology and taking into account the epidemiological factors of these diseases.

5. Antibody detection in febrile reactions occurs at a late stage in the evolution of the disease. This occurs between the second and fourth week of the clinical picture onset. It is not possible to identify the type of immunoglobulin in the results; it may be IgM, IgG or IgA. Once formed, the antibodies may persist after the healing of the disease for months, and even years.

What can be rescued from febrile reactions?

We are now able to say that out of the febrile reactions panel, the Huddleson traditional assay, despite some inconveniences mentioned above, is the most useful. Brucellosis is a worldwide zoonotic disease and its main contamination source is the consumption of non-pasteurized dairy products. *Brucella melitensis* is the main agent linked to the infection of human beings. It is a major healthcare issue in the Mediterranean Basin, Asia (specifically the Middle East) and Africa. Its incidence is unknown. However, between 1 and 200 cases per 100,000 habitants have been reported. In Latin America, and in our country, there are well-delimited endemic zones. Culture and isolation of the responsible agent in blood and bone marrow are slow (from 7 to 21 days), and it can sometimes takes up to 35 days. Despite these difficulties, bone marrow culture specifically remains the gold standard. Within the clinical laboratory, *Brucella* is highly infectious and requires a biosafety level 3 facility. Thus, serology in the early stages of the disease is of great importance in diagnosis. The traditional agglutination assay developed by Huddleson has been modified, standardizing the commercial antigen. Nowadays, a strain of the same biotype 1 bacteria is used (strain 99s or strain 119-3), acidified and dyed with rose bengal to make the antigen more visible. It is the same plaque agglutination assay, yet consistently more specific in the detection of antibodies against *Brucella* spp., while it does not discriminate the type of immunoglobulin and it is considered a good screening assay. It is currently used in blood banks in our country in the prevention of brucellosis transmission through blood transfusion.

Since 1970, and with the advent of new technologies like immunoassays, these assays were substituted by the specific detection of IgG or IgM immunoglobulins against their respective infectious agents. New techniques, such as the immunoenzymatic assay or the ELISA (*Enzyme-Linked ImmunoSorbent Assay*) and the chemiluminescence, have proven to have greater diagnostic sensitivity and specificity, improving analytic reproducibility and accuracy as well. Thus, nowadays it is possible to identify, in an earlier manner and more specifically, the type of antibody produced by the organism in the

immune response generated by bacteria and rickettsias, which participate as etiological agents of the diseases which in the past were diagnosed through febrile reactions.

Molecular biology plays, without a doubt, a major role in the diagnosis of these groups of infectious ailments, as well as in many others. The challenge is the high cost of the new

technology, which is currently impeding its use on a more routine basis.

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