Abstract

A 2014 paper in the American Journal of Tropical Medicine concluded “Use of IgM antibodies should be reconsidered as a basis for diagnosis and public health reporting of RMSF and other spotted fever group rickettsiae in the United States.” We suggest this warning be confined only to assays using whole-cell Rickettsia antigens, the IgM assay on which this conclusion was based, and to extend this to include typhus group rickettsiae. Such warnings should not be taken to mean that properly designed IgM assays should not be utilized in confirming acute rickettsial infections. The assumption has too often been that IFA or MIF are the only assays available, although both western immunodiffusion and ELISA assays have been shown to be both accurate and sensitive. Comparative results of MIF and ELISA performed in our laboratory demonstrate that the removal of LPS from the immunodominant protein antigens (native rOmp A and/ or rOmp B) produces spotted fever and typhus group IgM ELISA assays that are both sensitive and specific. As removal of LPS from whole cell antigens is not realistic due to the crystalline nature of the lipopolysaccharide, whole cell assays should not be utilized for IgM antibody assays due to unacceptably high false-positive rates. Attempts were made to adsorb anti-LPS reactivity in serum samples using LPS-coated microbeads (SFG or TG-specific) as a pre-treatment step, but the decrease in false-positive titers by MIF was less than a single two-fold dilution. Similar results were found using Weil-Felix antigens for adsorption.

Reagents

For MIF testing, our Fuller Laboratories 2-antigen MF slides (R20-12) were utilized according to a assay kit protocol. These slides contain separate reagents of Rickettsia rickettsii B. lycophila in each well as a background matrix. In short, the sera were pre-treated with high Titer goat anti-human IgG (chain-specific) to precipitate IgG-class antibody and, after allowing 20-30 minutes for this reaction, further diluted in PBS to 1:4. Treated serum dilutions were incubated in slide wells for 30 minutes, washed with PBS and treated with rabbit anti-human IgM (chain-specifc) for 30 minutes.

For ELISA testing, our Fuller Laboratories protocols for spotted fever (RMM-96K) and typhus group (OTM-96K) were used as described in the kit insets. In short, serum specimens were pre-treated with goat anti-human IgG (chain-specific) to precipitate IgG-class antibody and, after allowing 20-30 minutes for this reaction, further diluted in PBS to 1:10 in a plastic containing TMB 20 and bovine serum albumin. These pre-treatment sera were incubated in test wells at ambient temperature (370C) for 60 minutes, then washed 3 times with PBS-Tween 20 wash buffer. Anti-human IgG (chain-specific) was added for another 60 minutes at ambient temperature. Following a 5 wash step using PBS-Tween 20, the wells were incubated with a TMB substrate solution. The color developed was measured at 450 nm and absorbance values compared with Cutoff Calibrator values. Values higher than the Cutoff Calibrator is a 100% positive result.

Discussion

The ELISA assays used in this comparison have been commercially available since 2009 and we have supplied reagents for numerous clinical validations around the world. Our original Internal validations compared results with a qualitative Western Immunodot to the Cutoff Calibrator. The coating antigens for these assays are native Outer Membrane Proteins (OMP), purified while specifically removing any trace of the lipopolysaccharide (LPS) antigens found in the rickettsial cell wall. The spotted fever assay (RMM-96K) utilizes the Omp A + Omp B heterosubunit from Rickettsia rickettsii, although the range of reactivity across the major pathogenic clade is rather broad due to the antibody class. The assay for typhus group utilizes the purified Omp B from Rickettsia tsutsugamushi, which also selects reactivity to 6, prowazekii. Cross reactivity of IgM class antibody to enterobacterial LPS is often mentioned in discussing false-positive results in Rickettsia assays and it is most often mentioned in relegating all IgM assays to the “clinically unreliable” category. This information regarding Rickettsia IgM assays is not common knowledge among clinicians or clinical laboratory personnel, who are generally unaware of the types of assays that have been developed to generate the high sensitivity and specificity required.

A series of remedial assays were attempted to remove anti-LPS reactivity from clinical sera, including pre-incubations with liquid phase LPS, solid-phase LPS on micro-beads and Weil-Felix antigens. The lack of meaningful decreases in titer plot to an excessive expense involved. The preferred method in this case is to simply remove the LPS antigen from the test assay and use only the immunodominant protein antigens.