

MFIA™ Anti-Ig Control Improves Accuracy of Serology Results Interpretation

The Multiplexed Fluorometric ImmunoAssay™ (MFIA™) became our primary serologic test method in January of 2006, replacing the singleplex enzyme-linked immunosorbent assay (ELISA). The adoption of a multiplexed assay format has made it feasible for us to incorporate into MFIA™ panels additional control tests for more accurate and reliable interpretation of assay results.

Irrespective of the diagnostic assay technique, controls are essential for assuring that results are meaningful. Controls fall into two general categories. System-suitability controls show whether overall assay performance meets established acceptance criteria. For example, tests of standard immune and nonimmune control sera are used to verify that the analytical sensitivity and specificity of an assay are adequate. The entire assay run may be considered invalid when one or more system-suitability control results are unsatisfactory. The second category comprises controls that assess various sample properties to assure that the sample is suitable for a particular assay. A sample-suitability control commonly employed in ELISA, MFIA™, and other serologic assays is referred to as a tissue control (TC) because the reactant attached to the solid-phase (i.e., ELISA plate well or MFIA™ bead) is most often an extract of uninfected tissue culture. The TC reaction measures nonspecific binding of serum immunoglobulin to the solid-phase. It is subtracted from the microbial antigen reaction to determine the specific serological response. When the TC reaction intensity exceeds an acceptable level, however, a positive response is interpreted as nonspecific because the sample is unsuitable for the assay. High levels of nonspecific antibody binding occur most often with sera from animals that are old, that have autoimmune disease, or that have been parenterally inoculated with biologics.

Because the MFIA™ is multiplexed, the TC is performed along with all other assays in a single test well, whereas a separate test well is required for the ELISA TC. This difference illustrates important benefits of the MFIA™. First, the significance of control results for a sample is maximized because control tests and microbial antibody assays are performed together in the same well, under the exact same conditions. Furthermore, control tests can be added to MFIA™ panels without a commensurate increase in the required sample volume or number of test wells per sample. We have taken advantage of these benefits by incorporating into our MFIA™ panels extra control bead sets. These include an Ig set, coated with immunoglobulin from the animal species being tested, and an Anti-Ig (or Anti-IgG) set, coated with anti-immunoglobulin, for example, goat IgG anti-mouse IgG.

The Ig test is a unique system-suitability control in that it evaluates assay conditions, reagents, and reader performance for each test well. The results for the Ig control test and those for the standard control sera are compared to established acceptance criteria to determine whether they are satisfactory. Since assay results are approved and reported to you only if system-suitability control results are satisfactory, Ig control outcomes are not included in results reports.

TC and Ig controls have been part of mouse and rat MFIA™ panels since they were initially offered. The Anti-Ig control, on the other hand, was not a standard of these panels until several months ago. Similar to the TC, the Anti-Ig control evaluates sample suitability; unlike the TC, however, it is not antibody-assay specific. Rather, the Anti-Ig control shows whether the concentration, species, and type of immunoglobulin in a serum sample are in general suitable for the MFIA™ panel. We therefore have decided to report Anti-Ig results separately. If the Anti-Ig control score for a sample is below an acceptable level, it is reported as F for failed; a P is reported to indicate that the sample passed. Before reporting an F, the sample is retested to rule out pipetting mistakes and other laboratory errors as the cause of the failure.

When the Anti-Ig control result for a sample is reported as having failed, negative and borderline MFIA™ results for that sample may be reported as inconclusive (I). Conversely, positive findings are still considered valid. A sample can fail because it is:

- Too dilute or degraded because of improper preparation or storage;

- Labeled with the wrong species (for example, a mouse sample labeled as rat) or dilution (e.g., labeled as neat even though it had been diluted five-fold);
- Derived from an immunocompromised host or a species different from the one(s) for which the MFIA™ panel was developed.

Depending on the probable cause(s) for the Anti-Ig failure, we might retest the sample by MFIA™ or by another serologic method that does not utilize a specific anti-species immunoglobulin, such as protein-G immunofluorescence assay (IFA). We may further suggest that you submit additional serum samples from immunocompetent sentinels of the appropriate species as well as other animal and environmental specimens for infectious agent polymerase chain reaction (PCR) testing.

For additional information on our MFIA™ serology services, please contact Technical Services (1-800-338-9680 or comments@crl.com).