

# Updates in Immunoassays: Bacteriology

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## LEARNING OBJECTIVES:

1. List various immunoassay methodologies available for bacteriological assay use.
2. Review the principle of latex agglutination assays for bacteriology.
3. Compare and contrast syphilis screening and confirmatory tests.
4. Describe the CDC guidelines for syphilis testing.
5. Discuss FDA-cleared assays for tuberculosis testing.
6. Explain the principle of lateral flow immunoassays.

**ABBREVIATIONS:** BCG - bacille Calmette-Guerin; CDC - Centers for Disease Control and Prevention; CFP-10 - culture filtrate protein; CLIA - chemiluminescent immunoassays; DFA - direct fluorescent antibody; EIA - enzyme immunoassay; ELFA - enzyme-linked fluorescent assay; ELISA - enzyme-linked immunosorbent assay; ESAT-6 - early secreted antigen of 6 kDa; FDA - Food and Drug Administration; FTA-ABS - fluorescent treponemal antibody absorbed test; IFA - indirect fluorescent antibody; IgG - immunoglobulin G; IgM - immunoglobulin M; INF-g - interferon-gamma; NTM - nontuberculous mycobacteria; QFT-G - QuantiFERON®-TB Gold Test; QFT-GIT - QuantiFERON®-TB Gold In-Tube test; RPR - rapid plasma reagin; SPR - solid phase receptacle; TB - tuberculosis; TP-PA - *Treponema pallidum* particle agglutination; T-Spot - T-SPOT.TB test; VDRL - Venereal Disease Research Laboratory.

**INDEX TERMS:** Immunoassay, lateral flow immunoassay, immunochromatographic assay, infectious agent; syphilis; rapid plasma reagin (RPR); enzyme-linked fluorescent assays; fluorescent antibodies; latex agglutination assays, QuantiFERON tuberculosis testing.

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The use of immunological assays in the area of infectious disease has allowed for rapid and accurate identification of various bacteria, especially when identifying fastidious organisms such as *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Legionella pneumophila*.<sup>1</sup> Rapid latex agglutination assays, fluorescent antibody assays whether direct (DFA) or indirect (IFA), enzyme-linked immunosorbent assays (ELISA), enzyme immunoassays (EIA), lateral flow immunoassays also known as immunochromatographic assays and enzyme-linked fluorescent assays (ELFA) are available for *in vitro* diagnostic use to identify, screen for, or confirm the presence of infectious agents. The advent of the previously identified above rapid immunoassays has not only shortened turn-around times when compared to routine culture, but enabled the medical laboratory professional to report accurate results to the clinician in a timely manner ensuring administration of the appropriate antimicrobial therapy.

For many years, the serology department in most hospitals and medical centers was housed in the microbiology laboratory. Since several immunoassays are automated and require specific instrumentation to read and interpret results, most serological assays are now performed in the clinical chemistry department. Simple agglutination procedures however such as rapid plasma reagin (RPR) screening for syphilis along with group A strep, *Salmonella*, *Shigella*, and *E. coli* 0157 serogrouping are still performed in the microbiology and serology laboratories. This article will give an overview of some of the common and up-to-date

serological methods and assays used in bacteriology whether performed in the microbiology, serology, clinical chemistry or the special chemistry department.

**Rapid Latex Agglutination Assays**

A visible precipitate forms when equal concentrations of soluble antigen and soluble antibody bind forming an insoluble complex.<sup>1,2,3,4</sup> This procedure may require 48–72 hours to complete, which is not acceptable when a rapid diagnosis is needed.<sup>2</sup> By using insoluble particles bound with either antigen or antibody the reaction time is shortened, producing a more rapid, positive reaction that is easier to visualize.<sup>1,2,3,4</sup>

In latex agglutination, a latex particle (bead) is coated with either known antigen or antibody and allowed to react with the patient sample. After performing the procedure, visible clumping is observed if the reaction is positive. Most assays produce a qualitative result, although serially diluting the sample and obtaining an end-point will yield a quantitative result. It is important to note that false positive results (cross reacting antigens) and false negative results due to prozoning (antibody excess) or postzoning (antigen excess) can occur.<sup>2</sup> Conversely, there are several advantages of using latex agglutination assays, which include rapid results, cost effectiveness, and ease of use. Various latex agglutination assays are available for diagnostic use in the detection of infectious agents. Table 1 lists several assays currently available.<sup>5</sup>

**Flocculation Assays**

Flocculation assays are a variant of agglutination assays in which soluble antigen and soluble antibody react and form a fine precipitate that is visible either microscopically or macroscopically.<sup>1,3,4,6</sup> The precipitin product is visible since the assay takes place in a very small space, i.e. a plastic coated test card as seen in the RPR test or on a glass slide used in the Venereal Disease Research Laboratory test (VDRL). The RPR and VDRL are two routine assays used to screen for *Treponema pallidum*, the causative agent of syphilis.

Most laboratories use the RPR test on patient serum. Results are read macroscopically as the primary screening test for syphilis. Since autoimmune diseases, hepatitis, infectious mononucleosis, and additional treponemal species can cause a false positive result, presumptive positives must be confirmed with a

treponemal test specific for *Treponema pallidum* antibodies.<sup>1,2</sup> Confirmatory tests include the fluorescent treponemal antibody absorbed test (FTA-ABS) or the *Treponema pallidum* particle agglutination (TP-PA). The FTA-ABS uses an indirect fluorescent antibody (IFA) methodology while the TP-PA uses gelatin particles coated with treponemal antigens.<sup>2,4</sup> If positive, both assays confirm a diagnosis of syphilis.

**Table 1.** Rapid Latex Agglutination Kits.<sup>5</sup>

Manufacturer	Assay
Aleré™	WAMPOLE® Staph Latex Test
Arlington Scientific, Inc.	ASO Latex Test Staphslide Latex Test
Becton-Dickinson, Inc.	BBL™ Pneumoslide™ Test for <i>Streptococcus pneumoniae</i> BBL™ Staphyloslide™ Latex Test for <i>Staphylococcus aureus</i> BBL™ Streptocard™ Enzyme Latex Test BD Directigen™ Group B Strep Test Kit BD Directigen™ <i>H. influenzae</i> type b Test Kit BD Directigen™ Meningitis Combo Test BD Directigen™ <i>N. meningitidis</i> Groups A, C, Y and W135 Kit BD Directigen™ <i>N. meningitidis</i> Group B/E. coli K1 Test Kit BD Directigen™ <i>S. pneumoniae</i> Test Kit
Cardinal Health	SP® Staph Latex Test Kit
LifeSign®	Staph Latex
Pro-Lab Diagnostics	Prolex™ Streptococcal Grouping Latex Kit
Remel	PathoDx® Strep A Hospital Latex Test Kit PathoDx® Strep D Kit PathoDx® Strep Grouping PathoDx® Strep Universal Grouping Kit Rapid Identification Method™ E. coli Latex Test RPR Card Test Seradyn Seratest™ ASO Latex Test Staphaurex® Plus Test Kit Staphaurex® Test Kit Streptex® Wellcogen® Bacterial Meningitis Antigen Latex Test Kit Wellcolex® Colour Salmonella Test Wellcolex® <i>E. coli</i> 0157
Stanbio Laboratory	RaPET® ASO Test kits

**Abbreviations:** ASO: Anti-streptolysin O, RaPET: Rapid particle enhanced technology, RPR: Rapid plasma reagin

The current algorithm used in most laboratories employs initial screening with a non-treponemal test (e.g. RPR) followed by an antibody-specific treponemal test (e.g. FTA-ABS). With the development of automated enzyme immunoassays (EIA) and chemiluminescent immunoassays (CLIA), and considering their reported high sensitivity and specificity,<sup>7</sup> some laboratories are using a reverse syphilis screening algorithm where the initial screening is done with either an EIA or CLIA and then confirmed with a non-treponemal test such as RPR. If the EIA or CLIA is positive and the RPR is positive, it is confirmatory for syphilis. If the EIA is positive and the RPR is negative, it is followed up with a FTA-ABS. If the FTA-ABS is positive it could be an indication that the patient has either late syphilis or a prior history.<sup>4,8</sup> If the FTA-ABS is negative, the final result is negative. Although the Centers for Disease and Control and Prevention (CDC) still recommend the traditional procedure for syphilis testing, if reverse syphilis testing is done, the recommendation is as follows: for a positive EIA/CLIA test, the sample should automatically be tested with a qualitative nontreponemal test (e.g. RPR or VDRL). If the RPR is negative and the EIA is positive, the specimen should be tested automatically with a confirmatory treponemal test.<sup>8</sup> While the FTA-ABS is the most commonly used confirmatory test for syphilis, the test has lower specificity and apparent lower sensitivity compared to other treponemal-specific tests.<sup>9</sup> Based on recent studies, data suggest the TP-PA assay yields better sensitivity and specificity and is the recommended test for confirming cases of syphilis over the FTA-ABS.<sup>8,10</sup>

### Fluorescent Antibody Assays

Fluorescent labels provide rapid, specific and sensitive results due to the use of specific monoclonal or polyclonal antibodies attached to a fluorochrome.<sup>1,2,3,4,6</sup> Immunofluorescent assays are of two types: direct (DFA) or indirect (IFA). Direct assays are used mainly to detect infectious agents present in tissues and body fluids whereas indirect assays detect antibodies found in both serum or body fluids.<sup>3</sup>

In the DFA, a glass slide is fixed with the tissue or body fluid suspected of containing the antigen. A conjugated fluorescent antibody is then placed “directly” on the tissue and, after the appropriate incubation and wash step, the slide is placed under the fluorescent

microscope and examined for fluorescence. In the indirect assay, the patient’s own antibody is allowed to react with the known antigen fixed to the glass side. After the wash phase, a fluorescent-labeled antibody is added to the reaction. If the antigen/antibody complex was initially formed, the labeled antibody will attach to the patient’s antibody. After a second wash to remove any unbound antibody, the slide is placed under a fluorescent microscope where fluorescence is observed.<sup>1,2,3,4,6</sup>

Fluorescent assays are commonly used to identify a number of bacterial agents such as *Bordetella pertussis*, *Legionella pneumophila*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Borrelia burgdorferi*, and *Rickettsia rickettsii*.<sup>2,4</sup> The advantages include sensitive, specific, and rapid results that are easy to perform; however, disadvantages include time-consuming preparation and the purchase of an expensive fluorescent microscope.<sup>3</sup>

### ELISA and EIA

ELISA and EIA technology provide rapid, sensitive and specific results through the use of monoclonal antibodies bound to an enzyme.<sup>1,2,3,4,6</sup> The principle of these methodologies along with a discussion of the various semi-automated and automated analyzers available are discussed in an accompanying article of this focus series entitled *Updates in Immunoassays: Virology*.

ELISA and EIA technology is widely used in the clinical laboratory for the detection of infectious agents. In bacteriology, assays are available that detect IgM and IgG levels to the following infectious agents: *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Clostridium difficile*, *Legionella pneumophila*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Brucella species*, *Bordetella pertussis*, and *Mycobacterium tuberculosis*.<sup>2,11</sup>

### Tuberculosis Testing

According to the World Health Organization, one person becomes infected with tuberculosis (TB) every second.<sup>12</sup> The first immunological test developed to determine if a person was infected with tuberculosis was the TB skin test also referred to as the Mantoux Tuberculin skin test.<sup>3,13</sup> Unfortunately, this test cannot distinguish between individuals infected with TB to those that have been administered the bacille Calmette-

Guerin (BCG) vaccine. In 2005, the Food and Drug Administration (FDA) cleared a new blood test called QuantiFERON<sup>®</sup>-TB Gold (QFT-G, Cellestis Limited, Carnegie, Victoria, Australia).<sup>14</sup> In 2007 and 2008, the FDA cleared two additional tests: QuantiFERON<sup>®</sup>-TB Gold In-Tube test (QFT-GIT, Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT.TB test (T-Spot, Oxford Immunotec Limited, Abingdon, United Kingdom), respectively.<sup>15</sup> The QFT-GIT and T-Spot tests use ELISA and enzyme linked immunospot technology, respectively.<sup>3,14,15</sup> Both assays measure T-cell interferon gamma (IFN- $\gamma$ ) responses to two or three *Mycobacteria tuberculosis* specific antigens ESAT-6, CFP-10 and TB 7.7.<sup>3,14,15</sup> *M. tuberculosis*, *M. kansasii*, *M. marinum*, and *M. szulgai* are the only species that secrete these proteins; therefore, patients who have received the BCG vaccine or are infected with another nontuberculous mycobacteria (NTM) would render a negative result. However, since *M. kansasii*, *M. marinum*, and *M. szulgai* also release IFN- $\gamma$ , false-positive results may occur.<sup>16</sup> Turn-around times for the T-SPOT test and QFT-GIT are two days and one day, respectively.<sup>3</sup> Due to the simplicity of the ELISA test and the ability to report out results in one working day, several laboratories have opted to utilize the QFT-GIT test using either semi-automated or automated EIA instrumentation.

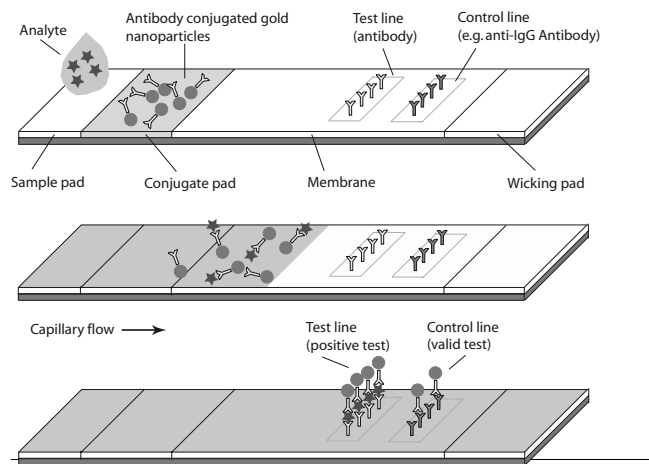
Neither test can distinguish between latent and active infection. In 2010 the CDC published “*Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection—United States, 2010.*”<sup>15</sup> Refer to this document for additional information including estimates of sensitivity and specificity, limitations of testing as well as guidelines for testing in adults and children.<sup>15</sup>

**Lateral Flow Assays**

Lateral flow assays or immunochromatographic assays are strip tests that are simple to use and can produce accurate results in under 30 minutes.<sup>3</sup> The test strip or chromatographic membrane can detect both antigen and antibody and contains three areas: the sample area contains the antibody that is adsorbed onto the membrane and where the sample is added; the conjugate or reagent pad which can use various conjugate types such as colloidal gold, dye, or latex beads to produce the signal; and the reaction membrane or capture line contains a second antibody that binds to

the antigen/antibody complex producing a positive color reaction.<sup>2,3,17</sup>

Once the sample is added, if the antigen is present, it will bind to the first antibody labeled with gold, dye, or latex on the test strip. The complex continues to flow laterally by capillary action through the membrane and will attach to the second antibody at the capture line.<sup>2,3,17,18</sup> Enough of the complex will form resulting in a color reaction at the test line. See Figure 1.<sup>18</sup> An internal control is incorporated into the test strip to ensure the results are valid.<sup>2</sup>



**Figure 1.** Principle of the Lateral Flow Immunoassay. Image Reprinted with Permission by CytoDiagnostics Inc.<sup>18</sup> www.cytodiagnostics.com.

The test strip can detect both antigen and antibody and contains three areas: the sample area (pad) which contains the antibody that is adsorbed onto the membrane and where the sample is added; the conjugate pad which in this case uses gold nanoparticles to produce the signal; and the reaction membrane or capture line which contains a second antibody that binds to the antigen/antibody complex. The analyte is added and if the antigen is present, it will bind to the first antibody labeled with gold nanoparticles on the test strip. The complex continues to flow laterally by capillary action through the membrane and will attach to the second antibody at the capture line. A control is incorporated into the test strip which validates the test. A positive reaction results in a color reaction at the test line.<sup>18</sup>

This technology provides accurate, sensitive results that are cost effective and easy to perform. Many companies market lateral flow technology in the detection of infectious disease. Table 2 lists several lateral flow assays currently available.<sup>19</sup>

**Enzyme-Linked Fluorescent Assays**

ELFAs combine both ELISA testing with a fluorescent final detection process.<sup>20</sup> The Vidas by bioMérieux is one of the most commonly used instruments that employ this methodology.

**Table 2.** Lateral Flow Assays.<sup>19</sup>

Manufacturer	Assay
Alere™	BinaxNOW® Streptococcus pneumoniae, legionella, strep A Clearview® chlamydia, H. pylori, strep A
AT First Diagnostic LLC	inSTIcheck™ syphilis, gonorrhea, chlamydia
Atlas Link Biotech Co., Ltd	FirstVue™ H. pylori, strep NOVAtest® chlamydia, gonorrhea, H. pylori, M. pneumoniae, strep A, tuberculosis
Becton-Dickinson Diagnostic Systems	BD Chek™ strep A
Beckman Coulter Inc.	ICON® strep A, H. pylori
Chembio Diagnostics, Inc.	TB STAT-PAK® II
Meridian Bioscience, Inc.	ImmunoCard STAT!® HpSA® (Helicobacter pylori stool antigens) ImmunoCard® C. difficile antigen
Quidel Corporation	QuickVue® H. pylori, strep A, chlamydia
Remel	Oxoid RAPID H. pylori StAR™ Oxoid Xpect™ legionella

The Vidas and Minividas are automated immunoassay detection systems that offer cost effective options such as single and batch testing abilities, user-friendly instrumentation, computer, printer, and keyboard, and ready to use reagents with minimal cross over and contamination.<sup>16</sup> Each kit contains a solid phase receptacle (SPR) which is coated with antigen or antibody and a test strip which contains all reagents needed for the assay. The SPR acts as a pipette and transfers reagents throughout the reaction to the various wells incorporated on the test strip. The last well measures the intensity of the fluorescent determining whether the reaction is positive or negative.<sup>20,21</sup> Most results are final within 30 minutes and are sensitive and specific due to the specific enzyme and fluorescent tags.<sup>20,21</sup>

The Vidas and Minividas offer a variety of tests including tumor and cardiac markers, coagulation, and hormone assays. In the area of infectious disease, many assays are available for the detection of viruses and

parasites. In bacteriology, immunoassays are available for *Clostridium difficile* toxin A and B detection, Lyme IgM and IgG, *Chlamydia*, *H. pylori*, *E. coli* 0157, *Listeria*, *Campylobacter*, *Staph* enterotoxin, and *Salmonella*.<sup>22</sup>

**Summary**

There are many immunoassays available that provide rapid, accurate and sensitive results. The intent of this article was to provide a brief overview of some of the products and methodologies available for clinical use and to discuss some of the principles behind the methodology and instrumentation. In the area of infectious disease, the use of immunoassays ensures rapid turnaround times that will result in the administration of prompt, accurate treatment for the patient. Ultimately, this will improve overall patient outcomes while possibly decreasing the costs associated with increased hospital stay. In conclusion, immunoassays are essentially easy to perform, cost-effective, produce highly sensitive and specific results, and allow the medical laboratory professional the ability to report accurate results in a timely manner.

The author does not endorse any company or product and has no financial gain or otherwise interest in the products presented.

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