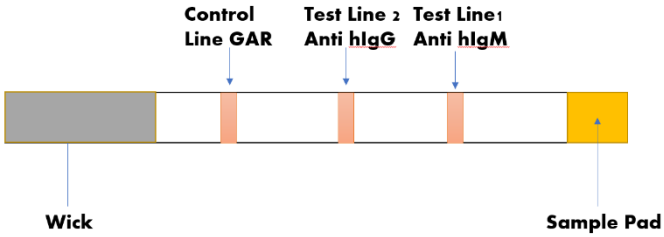
Universal Fluorescent Human IgG/IgM Detection Assay: A Highly Versatile Human Serology Testing Tool

Introduction:

While necessity is the 'mother of invention', versatility in assay development is not far behind. Attogene's Human IgG/IgM Universal Lateral Flow Assay Kits (colloidal gold and fluorescent readouts) are ready-to-use, universal test strips. They are based on lateral flow technology that employs either colloidal gold or quantum dot streptavidin conjugate particles embedded into the sample pad to conveniently capture biotinylated antigens. The strips are designed to aid in the development of qualitative or quantitative rapid test systems for detection of anti-human IgG and IgM antibodies that react to the any antigen that can be biotinylated (i.e. viral antigen, autoimmune antigen, allergen). Importantly, the assays are easily customizable, providing every laboratory with the possibility to adapt the assay to their specific needs.



Antibody tests are a method of choice to determine if a person has been exposed to a pathogen. They are also incredibly valuable in the detection of autoantibodies (against intrinsic antigens) that can be found in human autoimmune disorders. In our Universal IgG/IgM Lateral Flow Test, any user supplied biotinylated antigen can be mixed with a biotinylated rabbit IgG (binds to anti rabbit control line) and sample (human sera) in a specially designed assay running buffer in a well of the supplied 96-well plate. Next, a lateral flow strip is then added to the well, and the reaction is complete in 10-15 minutes. We show data below that depicts the use of our Universal Lateral Flow Assay Test kit.

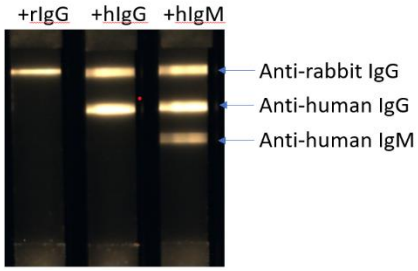


Figure 1. Biotinylated rabbit (r) IgG, human (h) IgG and human (h) IgM were sequentially mixed onto lateral flow running buffer. Next, strips that contained goat anti rabbit IgG (GAR), mouse anti human IgG or mouse anti human IgM capture lines were added into the well and run for 15 minutes. Results indicate a specific association of the capture lines with the desired biotinylated detector antibody using the universal lateral Human IgG/IgM lateral flow assay.

Results: As shown in Figure 1, the Universal Fluorescent IgG/IgM Lateral flow serology test can be used to specifically detect human IgG and IgM antibodies. For example, the Universal Lateral Flow Buffer was mixed with biotinylated rabbit IgG (rIgG) in lane 1, Biotinylated human IgG (hIgG) and rIgG in lane 2, rIgG + hIgG + biotinylated human IgM (hIgM) in lane 3. Capture lines are illuminated only when the designated detector antibody is included in the running buffer. This demonstrates the high specificity of the universal lateral flow test and its ability to detect human antibodies. We have performed many optimization tests including (1) varying the concentration of antibody while keeping the amount of streptavidin quantum dots the same and (2)

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Figure 2. Biotinylated rabbit IgG and SSB (La) antigen were sequentially incubated with lateral flow running buffer either with or without the inclusion of a human anti SSB antibody cat #K07104. Next, strips that contained goat anti rabbit IgG, mouse anti human IgG or mouse anti human IgM capture lines were added into the well and run for 15 minutes.

varying the amounts of quantum dots and keeping the biotinylated antibody constant. Thus, we've left nothing to chance in ensuring that the sensitivity and specificity of our assay is top notch.

Next, we put our lateral flow assay to a real-world application scenario using the human La autoantigen (SSB). The human La autoantigen (SSB) is a common target for autoantibodies identified in patients with autoimmune disorders such lupus erythematosus or Sjögren's syndrome. To illustrate the power and utility of our universal fluorescent lateral flow test kit, we generated purified recombinant human La protein and biotinylated it using Attogene's protein biotinylation kit (Cat number C02028). The biotinylated protein was added into lateral flow running buffer either with or without added control anti-human La (SSB) supplied by Meridian Biosciences (Cat number K07104). We next added strips from our Fluorescent Universal IgG/IgM lateral flow kit (AU2044-01), incubated for 15 minutes, and detected signal using a fluorescent lateral flow reader. The presence of the clear and prominent anti-human IgG line in **Figure 2** indicates the reactivity of this antibody with the biotinylated quantum dot bound La antigen.

Finally, we validated this real-world readiness using human sera. Specifically, we use pooled normal sera and sera from a patient suffering from lupus erythematosus. In this experiment, biotinylated human La protein was added into the lateral flow running buffer either with no serum (Lane 1), 1ul of normal human serum (Lane 2) or 1ul of serum from a patent with lupus erythematosus (Lane 3). As described above, an Attogene Universal Lateral Flow strip was added into the well, incubated for 15 minutes, and read using a fluorescent lateral flow reader. Figure 3 clearly shows that sera from a patient with lupus erythematosus has appreciable IgG antibody that reacts with the biotinylated human La autoantigen. Importantly, the lack of a signal in the normal sera lane demonstrates the specificity of our assay. Thus our assay appears to be more than ready for real world scenarios.

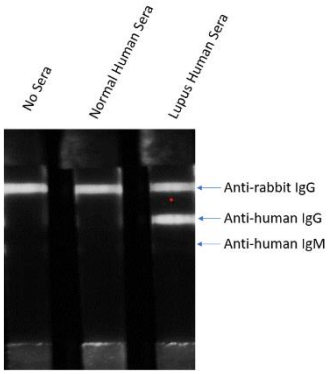

Figure 3. Biotinylated rabbit IgG and La protein was mixed with lateral flow running buffer with no serum (Lane 1), normal human serum (Lane 2) or serum from a patient with lupus erythematosus.

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Discussion: We have developed and used a Fluorescent Universal Lateral Flow Assay for detection of anti-human antibodies targeting a common autoimmune target - the La antigen- for serology testing utility. This product also enables the user a highly cost-effective and rapid way to develop additional lateral flow assays for any target antigen reactivity to human IgG and IgM in serum samples. It is very important to note that the optimization Attogene has already done for this assay will save the User an enormous amount of time and resources. In particular, the relative stoichiometry between the biotinylated antigen, biotinylated rabbit IgG, and the streptavidin gold is absolutely critical for assay optimization. The appropriate concentration of biotinylated antigen to use with strips is dependent upon its purity - and a standard curve can readily be used to determine the best ratio (generally between 1ng-100ng per test in our experience). To provide added confidence in our assay, a positive control line (biotin-rabbit IgG will bind to the goat anti rabbit (GAR) line on the test) is present to ensure the assay is running appropriately. So, if you're serious about serology testing, this product may be a gamechanger for you.