RESEARCHERS HAVE CONVERTED SOPHISTICATED TECHNOLOGY INTO A RAPID SIMPLE TESTING METHOD

While the basis for rapid tests were initiated over 50 years ago, continual improvements have refined the technology and simplified the tests utility.

This incredible amount of work has made it possible to take time intensive testing needing:

1. Expensive equipment
2. Highly trained personnel
3. Research laboratory environment

Into a test that can be performed:

1. Rapidly
2. Simply
3. By unexperienced users
4. Under worldly conditions and environments
5. Any location

Because of this, it is not surprising that the range of portable flow-based rapid assays for clinical, veterinary, agricultural, bio-defense and environmental applications continues to rapidly grow. Rapid tests of mention here include assays such as lateral, horizontal, vertical solution flow devices that can be used to detect virtually any compound on this planet.

A typical rapid test consists of the following components:

1. Sample pad - an adsorbent pad onto which the test sample is applied.
2. Colored Particles – these particles contain binders specific to the target analyte bound to colored particles (Gold nanoparticles). Attogene offers high quality, robust gold nanoparticles reagents for development of rapid assays.
3. Reaction membrane – typically a nitrocellulose or cellulose acetate membrane onto which anti-target analyte binders are immobilized in a line that crosses the membrane to act as a capture zone or test line (a control zone will also be present, containing binders specific for the item associated to the colored particle).
4. Wick or waste reservoir – a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it.

The components of the strip are usually fixed to an inert backing material and may be presented in a simple dipstick format or within a plastic casing with a sample port and reaction window showing the capture and control zones.

Attogene manufactures a full product line of gold nanoparticles (colloidal gold) for use in a variety of lateral flow assays. Our diverse product line of different type of nanoparticles offers you products with a narrow size distribution (CV of less than 12%), exceptional adsorption and conjugation properties and with greater than 95% spherical particles. In addition, our batch to batch variability is extremely low (+/- 2nm), which assures that you our customer will always end up with a product within the specified size range that you ordered.

The high shape uniformity of our colloidal gold will minimize the variability within your assay by allowing control over the available surface area while conjugating proteins to our gold nanoparticles. It will also ensure a more uniform flow rate across your membrane for improved reproducibility and overall results. A critical step in the development of a lateral flow assay is gold conjugate optimization.

OPTIMIZING GOLD CONJUGATION

One of the first steps in developing a lateral flow assay entails optimizing conditions for conjugating the antibody to the gold nanospheres. This can be done in 96 well plates to enable the evaluation of many different conditions for conjugation optimization including buffer, pH and antibody concentration as outlined below.

Conjugation pH evaluation – finding the optimal buffer and pH:

1. Add 100 ul of 1 OD colloidal gold to each well of a 96 well plate
2. Add 10 ul of 22mM buffer at pH from 5.5 to 10 in 0.5 pH unit increments. NOTE: (suitable buffers to evaluate are MES from pH 5.5 to 6.5, HEPES from 7.0 to 7.5, and Borate from 8.0 to 10)
3. Add 10 µl of antibody diluted in the same buffer and pH as used for the gold at 100 ug/ml with immediate gentle mixing upon addition. This will yield final concentration of 10 ug/ml.
4. Incubate for 15 minutes at room temperature
5. Add 25 µl of 1.5M NaCl
6. Analyze aggregation by visual analysis as witnessed by a shift from red to gray purple
7. The optimal conditions are the buffers and pH's that prevents aggregation
   Note A. The pH of 7.5 is found to be acceptable for many monoclonal antibodies. HEPES buffer is very convenient to use since it dissolves to give a pH of 7.5 without the need to titrate the solution Further. Antibodies dialyzed against or buffer exchanged on a column to 10mM HEPES tend to work well.

Conjugation Optimization – finding the optimal antibody concentration in optimal buffer:

1. Add 100 ul of 1 OD colloidal gold to each well of a 96 well plate
2. Add 10 ul of 22mM of optimal buffer
3. Add 10 µl of antibody diluted in optimal buffer at 100 ug/ml, 50 ug/ml, 37.5 ug/ml, 25 ug/ml, 18.75 ug/ml, and 12.5 ug/ml with immediate gentle mixing upon addition. This will yield final concentrations of 10 ug/ml, 7.5 ug/ml, 5.0 ug, 3.75 ug, 2.5 ug, 1.875 ug, and 1.25 µg of antibody respectively.
4. Incubate for 15 minutes at room temperature
5. Add 25 µl of 1.5M NaCl
6. Analyze aggregation by visual analysis as witnessed by a shift from red to gray purple color.
7. The minimum amount of protein required to stabilize the gold solution is the optimal condition

Note B. An important point to consider is that the minimum amount of protein required to stabilize the gold solution does not represent saturation values. Excess antibody may be used to attain maximum binding however, the excess ligand must be removed, otherwise it would compete with the gold-conjugated ligand during an assay.

Removal of excess ligand may be required and is can be done using:

1. Centrifugation: a low-speed centrifugation an be used to remove large particles before high-speed centrifugation is applied. To minimize the amount of solution to be centrifuged after conjugation, concentrated native unconjugated colloidal gold can be used as the starting material.
2. Tangential flow membranes. When using this method, it is preferable to first pre-wet the membranes with a solution containing bovine serum albumin to prevent excessive loss of the conjugate.

DOT SPOT ANALYSIS CAN BE USED TO OPTIMIZE ASSAY

1. **Apply antibody with concentration of 0.1 mg per ml or greater onto membrane in spots with a pipette capable of dispensing 1 µl.**
2. **Dry membrane for a minimum of 1hr at 37oC.**
3. **Mock lateral flow test strips may be made using a vinyl backing, the spotted nitrocellulose, and an absorbent pad.**
4. **To perform this test, 50–100 µl of test antigen or buffer control is mixed with 10–50 µl of the colloidal gold antibody preparation of another antibody aimed at finding a potential sandwich pair it is generally advantageous to have surfactant and some proteins in the buffer solution (e.g., 0.1%Tween 20 and 0.1–1% bovine serum albumin).**
5. **The strips are placed directly into the antigen gold solution and allowed to develop for 5–10 minutes.**
6. **Review the spots**

Note A. All antibodies in this evaluation should be tested as both the capture antibodies (membrane antibodies) and as detector particles.