Go with the Flow

Adapting Lateral Flow Assays for Nucleic Acid Detection

How to incorporate high sensitivity nanoparticles to rapidly detect amplified DNA and RNA

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Most people are familiar with lateral flow assays (LFAs), especially pregnancy or COVID-19 rapid tests, that detect a protein's presence or absence. LFA-based tests are fast and easy, enabling point-of-care and at-home testing. However, the low sensitivity and narrow testing capacity of LFA-based assays have curbed their widespread adoption for other applications.[1] To overcome these limitations, scientists optimized assay designs and adopted new types of nanoparticles for reporter probe conjugates. Advances in these technologies have supported the development of nucleic acid LFAs NALFAs.[2]

Lateral Flow Fundamentals

LFAs contain three key components to detect an analyte (protein, RNA, or DNA)

- A sample and wick pad to direct fluid flow through the assay
- A conjugate pad with reporter probes to visualize analytes
- A nitrocellulose membrane with capture molecules, immobilized on strips to form one or more test and control lines

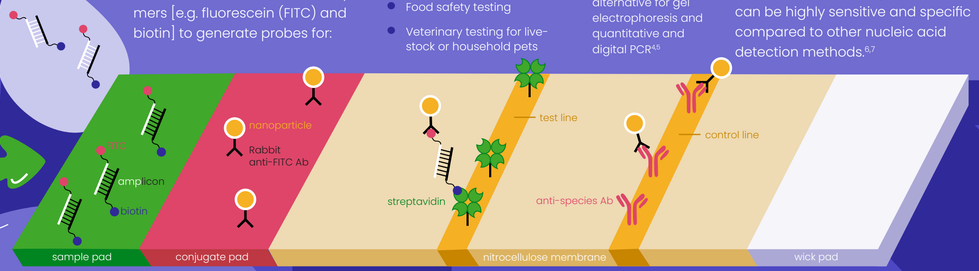
These components must overlap by at least 1 millimeter for uninterrupted sample flow.[3]

Expanding LFA Applicability with Nucleic Acids

Scientists pre-amplify a sample's nucleic acids with dual labeled primers (e.g. fluorescein (FITC) and biotin] to generate probes for:

- Infectious microbe or disease biomarker detection
- Food safety testing
- Veterinary testing for livestock or household pets
- Rapid amplicon quantification as an alternative for gel electrophoresis and quantitative and digital PCR[4,5]

NALFAs can be quantitative, offer versatile detection capabilities, and can be highly sensitive and specific compared to other nucleic acid detection methods.[6,7]



Common Nanoparticle Details

To detect labeled amplicons, researchers generate reporter probes by conjugating tag-specific antibodies to strongly-colored or fluorescent nanoparticles and embed them in the conjugate pad.

Particle sizes between 20 nm and 500 nm provide the best signal while still being small enough to easily flow through the assay.[3]

Common nanoparticles include[8]

	Probe Color	Probe Size	Limit of Detection	Antibody Loading
Gold Nanospheres	Red	40 nm	Moderate	Low
Dyed Latex Beads	Varies	300 nm	Low	High
Gold Nanoshells	Green / Blue	150 nm	Low	Low
Europium Nanoparticles	Fluorescent	300 nm	Moderate	High

Key Considerations for Efficient Assays

Because reporter probes impact assay sensitivity, specificity, cost, and performance, scientists must carefully weigh all factors, including assay development time and instrument requirements for signal read out, when designing probes.

- Visual assays offer the greatest ease of use but are qualitative or semi-quantitative.
- Optical or fluorescent NALFAs are reproducible and quantitative but increase assay complexity[4]

Increasing Assay Sensitivity with Nanoshells

Even after preamplification, samples often contain few labeled nucleic acids. Therefore, scientists must use sensitive probes to improve assay performance. nanoComposix 150 nm gold nanoshells are ultra-bright reporter particles that offer unique optical properties, have improved visual signal, and require fewer antibodies per test compared to latex-based LFAs.[8]

Learn more about our lateral flow assay development services: fortislife.com/lateral-flow

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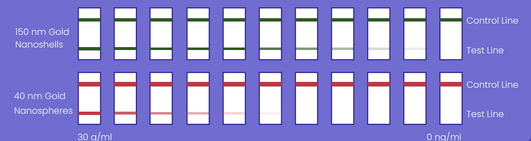

A comparison of troponin protein concentrations ranging from 30 ng/mL (left) to 0 ng/mL (right), detected with 150 nm gold Nanoshells (top panel) or 40 nm gold Nanospheres (bottom panel).[8]

Ensuring Assay Functionality

An essential part of every NALFA assay is its control line. To visualize this line, scientists typically select a control antibody that will recognize the test antibody (the blue anti-FITC antibody in the central image) and immobilize it to the nitrocellulose membrane (red antibody in image).

Alternatively, researchers develop control probes that recognize a second amplicon generated from the same sample. 40nm gold nanospheres are excellent nanoparticles for this kind of control probes because they are robust and cost-effective.[3,8]

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