First of its Kind Lateral Flow System to Perform "On-Strip" Nucleic Acid Hybridization Detection

Introduction:

Lateral flow assays can do their 'detective' work in multiple ways:

Many conventional lateral flow assays contain affinity reagents that detect a target using the so-called sandwich or competitive systems. In a sandwich format, the presence of target molecules leads to the target being sandwiched between the detector and test line immobilized affinity reagent and formation of a signal. In the competitive format, the presence of target molecules leads to the detector bound affinity reagent no longer binding to the test line bound target and leads to signal reduction.

Emerging nucleic acid based lateral flow methods:

Nucleic acid-based lateral flow assays often use techniques where affinity tags such as biotin, FITC/FAM or DIG are used to detect readouts (signal gain or loss) from upstream enzymatic reactions (e.g. RNase Digestion, DNase Digestion, LAMP, RPA, CRISPR and PCR). Readout systems can be visually detected with versatile lateral flow strip technology using one of the many nucleic acids based Universal Lateral Flow strips offered by Attogene, the world leading resource for Universal Lateral Flow assay kits and services (AU2034-01/02 and AU2034-04/05, AU2035, AU2037, AU2049, AU2052, AU2053). Attogene' s Universal Lateral Flow kits are currently available in two main formats. One of the classes of Universal Lateral Flow products uses detector particles containing streptavidin, and strips containing anti-FAM/FITC and anti-DIG capture probes. The second class of Attogene' s Universal Lateral Flow Strips contain an anti-FITC/FAM detector particles and strips containing anti-DIG and streptavidin capture reagents. Within these two classes of Universal Systems, we also offer several detector compounds that currently include Attogene' s famously robust Gold Quantum Dots, Europium, and Phycoerythrin-based detector particles. This enables a customer to mix and match formats and particles to meet the requirements of the test. Versatility is not only valued by Attogene, but also in everything we do in the lateral flow space.

An emerging lateral flow technique is also in the works that utilize capture probes (affinity reagents) made of nucleic acid sequences immobilized onto the nitrocellulose strips. This nitrocellulose-bound nucleic acid can be used to detect its compliment strand while it passes through and across the immobilized nucleic acid capture probe. This unique technique reminiscent of array-based methodology has enormous potential as it could massively increase the multiplexing capacity of nucleic acid detection reactions derived from isolated DNA or RNA. Now a drumroll please….

Oops, we did it again! Attogene has developed the first of its kind system ("On Strip" Nucleic Acid Hybridization Lateral Flow Starter Kit - AU2061 https://www.attogene.com/shop/on-strip-nucleic-acid-hybridization-lateral-flow-starter-kit/) that enables researchers to perform on strip lateral flow nucleic acid hybridization development techniques. The "On Strip" Nucleic Acid Hybridization Lateral Flow Starter Kit provides all the needed components required to attach a biotinylated nucleic acid to a nitrocellulose membrane securely and run tests with complementary hybridization probes. Useful for optimizing the amount of FAM labeled hybridization probe to obtain a signal in a lateral flow - this "on strip" hybridization technique has a specially formulated and optimized running buffer, biotinylated capture probe and FAM labeled hybridization probes for verification testing. Furthermore, when a system is being designed for on strip hybridization, Attogene has more advanced services to perform direct chemical conjugation of

nucleic acids to carrier proteins to apply lines to nitrocellulose membranes as were don't in Figure 1 and 2. We describe our work with sprayed tagged nucleic acids in the article below.

While this kit is a starting point for research into performing on strip hybridization, the use of proteins chemically conjugated nucleic acid capture probes tends to make for a more stable assay over the longer term and can be developed in a fee for service at Attogene. Just need to contact us at: sales@attogene.com

Results and Discussion:

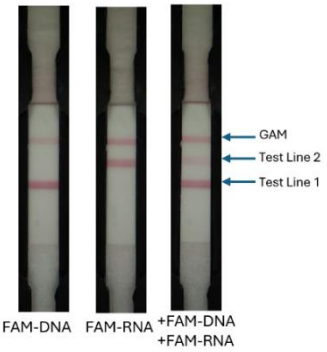

Figure 1. On-strip oligo hybridization which demonstrates specificity. A 74 base single stranded FAM labeled DNA oligonucleotide containing a sequence that is the complement to an oligonucleotide which is immobilized in test line 1 or a FAM labeled RNA complementary to a DNA oligonucleotide immobilized onto test line 2 or both the FAM-RNA and FAM-DNA were added into specialized lateral flow running buffer with 10ul of colloidal gold conjugated to anti-FAM antibody. Dipsticks were applied to wells and incubated for 30 minutes prior to the picture.

One of our key innovations is the ability to stably attach nucleic acid capture probes to the nitrocellulose membrane in the lateral flow format. We have created a system and advanced techniques in finding the optimal oligo-carrier protein conjugate as well as a method of manufacturing and running buffers which provide the optimal utility for on-strip hybridization techniques. The components of this system were integrated into a kit which enables users to perform on strip hybridization. An example of this system is shown in **Figures 1 and 2**.

The on-strip hybridization test shown in **Figure 1** was performed using two different nucleic acids (one of the detection probes is a 74 base FITC/FAM labeled DNA (5'-56-FAM CACGTGGAGCTCG GATCCACGCGCAGTGGGACCAACCCAAGCCGTGGCCTGCCGG GGGGCTAGCGAATTCCGTACG-3') while the second detection probe is an 18 base FAM/FITC labeled RNA sequence (GAUCUGAGCCUGGGAGCU/36-FAM/3'). Capture probes to each of these nucleic acids are specific to their targets which were immobilized to nitrocellulose using a biotin labeled oligo that was mixed with streptavidin. We used this test to generate an on-strip hybridization reaction test with two different capture probes targeting two different types of detection probes. specificity using both RNA and DNA oligonucleotides complementary to specific immobilized capture probes. As is shown in **Figure 1**, we were able to generate specific signals with the specific detection probe on a single strip.

We also designed and tested a complementary three-piece oligonucleotide on-strip hybridization tethering capture system in which one strand is required to tether two of the labeled oligonucleotides together to obtain a signal mimicking an RNA target of GAPDH. Data for this assay is shown in **Figure 2**.
GAPDH RNA Sequence (30 mer): 5'-acaagaggaagaga**g**agagacccucacugcug – 3'
BIO-GAPDH PROBE (15 mer): 5'-/5BiotinTEG/CAGCAGTGAGGGTCT-3'
GAPDH PROBE-FAM (15mer): 5'-CTCTCTTCCT CTTGT/36-FAM/3'

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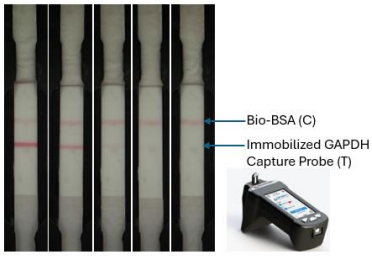
Using the on-strip strip hybridization systems, we applied the biotin oligonucleotide to the nitrocellulose using an optimized streptavidin/oligonucleotide mixture. The Anti-FITC/FAM gold was next mixed with the anti-FAM GAPDH oligo with limiting amounts of the GAPDH RNA. The GAPDH RNA is required for on strip hybridization tethered detection. With the limits of detection of our on-strip hybridization we can typically detect between 1-10 million molecules of target which are well within the range of products in upstream reaction systems (**Figure 2, data not shown**). Having also performed optimized capture probe spraying, lateral flow materials and buffer formulations that enable specific and sensitive on strip strand hybridizations.



	Control	Test	Ratio	%
1pmol GAPDH RNA	164714	667541	4.05	100%
100fmol GAPDH RNA	223775	348384	1.56	39%
10fmol GAPDH RNA	236385	85562	0.36	9%
1fmol GAPDH RNA	220528	40751	0.18	4.5%
0 GAPDH RNA	240056	31269	0.13	3.2%

Figure 2. A GAPDH capture probe was applied to nitrocellulose strips, were incubated with varying concentrations of a complimentary GAPDH synthetic RNA containing a biotin tag and Anti-FITC/FAM gold. This data demonstrates the ability to detect at least 1 fmol of complimentary GAPDH RNA sequence using in-strip hybridization. The readouts of the strips were performed using a DETEKT reader shown to the right, with numerical readouts

Discussion: In conclusion, Attogene has developed a rapid, user friendly lateral flow development kit for performing on strip hybridization. This technique can be used by anyone to develop novel techniques to detect nucleic acids. While this kit is a starting point for research into performing on strip hybridization detection techniques, the use of chemically conjugated nucleic acid capture probes tends to make them more stable and can be developed in fee for service at Attogene. Just contact us at: **sales@attogene.com.** For example, if lines are desired as opposed to spots, Attogene can perform spraying of lines for individualized probes at our facility. This can also be used to increase the number of capture probes on a device and adjust the general architecture of the test. We are here to help.