ALFA SCIENTIFIC DESIGNS, INC.

White Paper

Driven Flow™ Technology

Accuracy at the Speed of Alfa

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Impact of *Driven Flow*™ Technology

Driven Flow™ Technology represents a breakthrough in point-of-care (POC), rapid diagnostic testing. By greatly enhancing the speed, accuracy, and specificity of the canonical lateral flow assay, the patented *Driven Flow*™ Technology offers a route to true confirmatory and quantitative POC testing for pathogens, drugs and their metabolites, hormones, and other antigens in a wide array of testing environments. Without altering the chemistry of a proven lateral flow assay, *Driven Flow*™ Technology can drastically reduce reaction times, improve the accuracy of the assay, and increase confidence in the specificity of the test. With applications in medical testing, food safety, law enforcement, and biosecurity, *Driven Flow*™ Technology can reduce the time burden on medical professionals, government inspectors, police officers, and border and port control agents. Taken together, the improvements offered by *Driven Flow*™ Technology to existing lateral flow assays signify a drastic technological advancement within a marketplace expected to reach nearly $7 billion by 2020.[1]

DRIVEN FLOW™ TECHNOLOGY IMPACT

- Drastic reduction in reaction times
- Improvement in accuracy and confidence of the test
- Technological advancements expected to reach $7 billion by 2020[1]

Limitations of existing lateral flow assay devices

While lateral flow assays[2] have proven an essential tool for three decades, they remain limited by longer than ideal completion times (5-20 minutes) and typical specificity and sensitivity ranges of 75% to 95%. For instance, a common, qualitative lateral flow assay for detection of Group A Streptococcal antigen (Strep throat), requires a 5 minute development time and returns a specificity range of 91-97% and sensitivity of 78-95% in field testing.[3] As a result, up to a quarter of tests (those returning a false negative result) require a lengthy (2-3 day) confirmatory culture test, during which a patient goes without necessary antibiotic treatment. In this case, decreasing the total analysis time while increasing specificity and sensitivity would greatly enhance the patient experience with POC diagnostics. *Driven Flow*™ Technology was developed to exceed the minimum requirements for specificity and sensitivity in rapid, *in vitro* diagnostic (IVD) testing and to make rapid, cost-effective, simple, and quantitative immunological testing a reality.

What limitations are overcome by *Driven Flow*™ Technology?

INCONSISTENCIES IN DEVICE MATERIALS

Unevenness and variations exist from lot-to-lot, as well as between individual lateral flow devices.[4-5] These inconsistencies are a product of inherent variability in the key materials utilized, such as nitrocellulose (NC) membranes (see **Figure 1**), fiberglass, and cellulose.

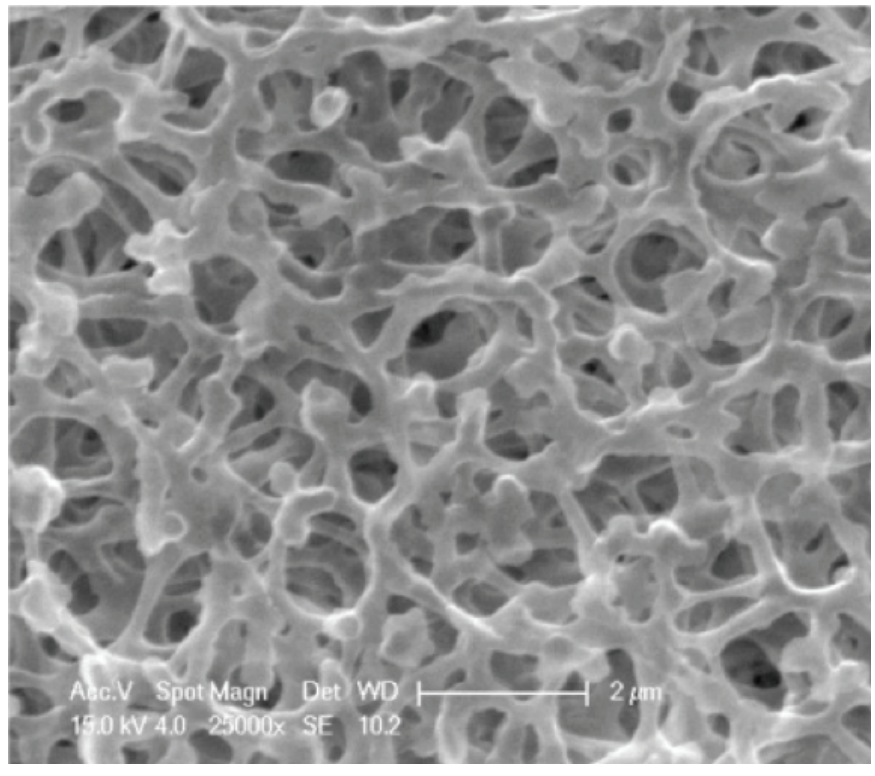

Figure 1: A microscopic view of NC membrane showing uneven pores

CAPILLARY ACTION DRIVING FORCE

Once applied to the test pad, sample is drawn into a lateral flow device, through the reaction zone, and into the detection area by capillary action alone. The process can be slow, which can result in non-specific binding, loss of signal to membrane adhesion, or interference from sample matrix components. All of these issues can decrease the specificity and sensitivity of a given assay.

SAMPLE VISCOSITY

Samples containing large concentrations of protein, such as saliva, are not readily drawn into the reaction zone of a lateral flow device, thus increasing the time required to complete an assay.

The advantages of *Driven Flow*™ Technology

A true one step, one minute, test with high accuracy is attainable. The patented *Driven Flow*™ Technology utilizes a tightly restricted sample flow-path and the additional flow acceleration, termed *Driven Flow*™, provided by mechanically squeezing the liquid sample matrix through the device. Much like the pump used in High Performance Liquid Chromatography (HPLC), this squeezing action produces the powerful driving force to push the flow past the reaction area evenly, at high speed while generating maximum, thorough reaction of the binding complexes. The extra motivational forces found in *Driven Flow*™ Technology devices can reduce the reaction time to less than one (1) minute. Forward and downward pressure also provides a strong self-washing function during the flow process, resulting in minimization of non-specific binding in the reaction area. Only the specifically bound analyte-conjugate complex remains on the Test line following the self-wash process. Therefore, *Driven Flow*™ devices:

- have a self-wash function that helps eliminate non-specific, adhesive binding.
- accelerate the flow of sample matrix and components, thereby defeating the obstruction issues of uneven, porous materials
- hasten the completion of the test with high accuracy, making confirmatory rapid testing feasible.
- defeat the sticky feature of protein-rich specimens, making it an exceptional option for testing human saliva.

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Comparison of Lateral Flow to *Driven Flow*™ Assays

In a comparative study between lateral flow and *Driven Flow*™ assays, cassettes, saliva multi-drug devices, and cups were compared per the schematic in **Figure 2.** All experiments were performed in a controlled laboratory setting and used equivalent test strips in the *Driven Flow*™ and lateral flow assays. Results were analyzed for time of conjugate release and accuracy.

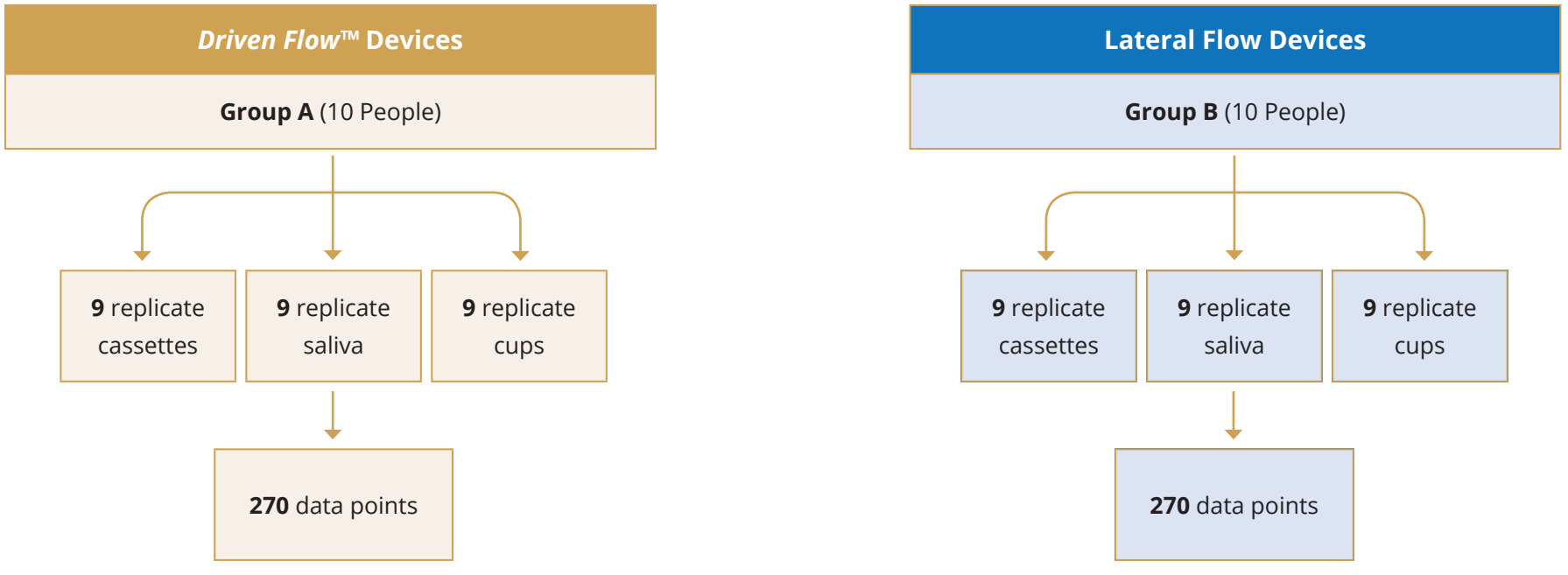

Figure 2: Experimental schematic for comparison of lateral flow and *Driven Flow*™ technologies.

As shown in **Table 1**, *Driven Flow*™ devices are able to return results more than 3 times faster than their lateral flow counterparts.

Device Format		Cassette	Saliva	Cup
Time (min)	Lateral Flow	> 4	> 10	> 4
	Driven Flow™	< 1	< 3	< 2

Table 1: Time, in minutes, required to complete conjugate release

Device Format		Average % Accuracy		
		Cassette	Saliva	Cup
Negative	Lateral Flow	95-99	85-95	95-99
	Driven Flow™	99-100	99-100	99-100
75% Cutoff	Lateral Flow	90-96	65-80	90-96
	Driven Flow™	95-99	90-96	95-99
125% Cutoff	Lateral Flow	90-95	65-80	90-96
	Driven Flow™	95-99	90-96	95-99

Per the data presented in **Table 2**, both lateral flow and *Driven Flow*™ devices properly identified all negative samples. However, *Driven Flow*™ technology outperformed lateral flow devices at the 75% and 125% cutoffs (tests spiked at known analyte concentrations), never falling below 97% accuracy in cassette, saliva, and cup formats.

Table 2: Average % accuracy of samples that are negative for the queried analyte(s), or spiked at the 75% or 125% cutoff.

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Conclusion and Future Applications

Driven Flow™ Technology is an ideal, convenient methodology for on-site use that satisfies the need for fast and highly accurate results. Depending on the application, the testing time may be reduced to less than 1 minute.

In areas where faster and more accurate screening test results are mandatory, *Driven Flow*™ Technology allows the user to obtain test results at the right time, at the required accuracy, in the right place.

Given the increased accuracy and speed of analysis inherent in *Driven Flow*™ devices, a number of gaps in the marketplace will inevitably be bridged using this innovative, patented technology. With applications in law enforcement, biosecurity, and food safety, *Driven Flow*™ Technology is poised not only to capture a large portion of the existing lateral flow testing market, but also to create new growth opportunities outside of the healthcare system.

Looking forward, lateral flow POC testing must become compatible with electronic recording devices, such as strip readers and smart phones. With less ambiguity inherent in results from *Driven Flow*™ devices, the technology is particularly amenable to this type of automated documentation. In addition, by adorning reagents with fluorescent labels, or other means of signal amplification, the enhanced specificity and sensitivity afforded by *Driven Flow*™ devices can perform effectively in high-challenge testing.

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