ALFA SCIENTIFIC DESIGNS, INC.

White Paper

DRIVEN FLOW™: A Breakthrough in iFOB Testing

ALFA's Patented Technology Improves Accuracy and Speed

DRIVEN FLOW™ TECHNOLOGY

Instant-view plus™

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Introduction and Background

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 a multitude of analytes. In combination with ALFA's benchmark immunochemical fecal occult blood (iFOB) test,[1] *Driven Flow*™ Technology can drastically reduce reaction times, improve accuracy, reduce the sample volume requirements, and increase confidence in the specificity of this crucial test that prescreens for early detection of colorectal cancer (CRC) or precancerous polyps.

Early Detection of Colorectal Cancer

CRC is the third most common cancer diagnosed in the United States. Affecting men and women equally, CRC is the second leading cause of cancer death, killing more people annually than either breast or prostate cancer.[3], [12] Reports have shown that an early diagnosis of CRC results in about a 90 percent cure rate, making early detection through screening an essential tool for CRC eradication. A large-scale study of 46,000 people by the Centers for Disease Control and Prevention proved that using fecal occult blood (FOB) tests could save up to 33,000 lives nationwide every year by helping to detect CRC in its early stages, when treatment outcomes are favorable. The findings prompted the U.S. Senate to declare March "National Colorectal Cancer Awareness Month". The National Cancer Institute, the National Institutes of Health, the American Cancer Society, and many medical laboratories also recommend the FOB test as an effective screening tool for CRC.

THE BENEFITS OF EARLY DETECTION

There are over **1 million colorectal cancer survivors** due to early detection.

Colon cancer diagnosis rates have dropped by **30%** due to more Americans engaging in regular testing.

Colorectal Cancer Symptoms

Pre-cancerous polyps and early-stage CRC do not always cause symptoms initially. Therefore, an individual could have polyps or CRC and not know it, making a simple, non-invasive screen so important to early detection. Overwhelming scientific evidence shows that the FOB test is the best and most reliable form of colon cancer screening. Based on the rationale that the earliest sign of pre-cancerous polyps or CRC is blood in the stool, and this blood is most often occult (not visible to the human eye),[9-10] screening for FOB and subsequent removal of pre-cancerous polyps could saves tens of thousands of lives annually.[3-4, 7, 11]

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Colorectal Cancer Screening Methods

Whether alone or in combination, several different screening tests are used to detect pre-cancerous polyps or CRC. The U.S. Preventive Services Task Force (USPSTF) recommends CRC screening for men and women aged 50–75 using FOB testing, sigmoidoscopy, or colonoscopy. The American Cancer Society, the USPSTF, and Centers for Disease Control recommend high sensitivity FOB tests for CRC screening mainly due to the advantages of the FOB test over other methods, which include:

- Cleansing of the colon is not necessary
- Samples can be collected at home
- Low cost compared to other CRC screening tests
- Does not cause bleeding or tearing/perforation of the colon's lining
- High sensitivity FOB detects CRC at relatively high rates
- Access to colonoscopy and other invasive tests may be limited or nonexistent for many patients
- FOB testing is non-invasive

Most importantly, a Japanese study demonstrated that using immunochemical FOB tests reduced mortality by 60%.[2-6]

Knowing is Critical: A Patient's Comments about FOB tests

"Both my father and grandfather died of colon cancer—that's what motivates me to get screened. To have the ability to check things before they get too far along is reassuring. My father did not get screened. My fear of colonoscopies was nothing compared to my fear of dying from colorectal cancer! I started getting screened with FOB tests and I've had them regularly. It is easy, simple and accurate. I am no longer afraid."

Denise, Ohatchee, Alabama
http://www.cdc.gov/cancer/colorectal/basic_info/stories.htm

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]

Impact of *Driven Flow*™ Technology

While essential to rapid point-of-care testing, traditional lateral flow assays[13] for FOB are limited by longer than ideal completion times (5-20 minutes) and accuracy around the cutoff ranges of 80%-90%.[14] By reducing both the time required to complete a test, and improving the sensitivity and specificity of results, *Driven Flow*™[17] Technology offers a robust alternative to standard iFOB testing. But how does *Driven Flow*™ defeat the difficulties inherent to these tests? An examination of lateral flow's limitations shows how in the next section.

Driven Flow™ Overcomes the Limitations of Lateral Flow

Inconsistencies in device materials

Unevenness and variations exist from lot-to-lot, as well as between individual lateral flow devices.[15-16] These inconsistencies are a product of inherent variability in the key materials utilized, such as nitrocellulose (NC) membranes (see **Figure 1**), fiberglass, and cellulose.

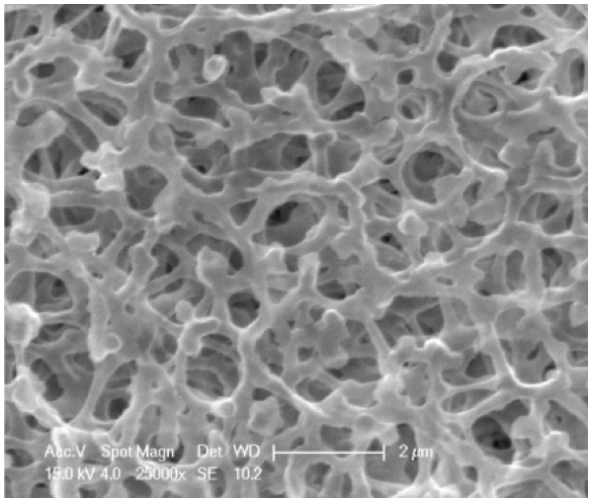

Figure 1: A microscopic view of NC membrane showing uneven pores

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.

Uncertainty near the detection cutoff

Of particular concern with traditional lateral flow assays is performance at, or near, the limit of detection. For a traditional lateral flow assay, the concentration range from 75-125% of the test cutoff (analyte concentration at the detection limit) is typically marred by an expected accuracy as low as 70-85%. Thus, up to 30% of tests near the limits of the device are likely to return false positive or false negative results.

Driven Flow™ technology abrogates these deficiencies by accelerating analyte and carrier flow through the reaction matrix. This serves to minimize the effects of inconsistent materials and speeds the delivery of the positively reacted complex past matrix components which can alter signal strength. This leads to an assay that performs much more reliably at or near the limit of detection. A more detailed portrait of this capability is discussed below.

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 along with an 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 a 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. By restricting the flow-path, sample volume requirements are also reduced by up to 75%. Forward and downward pressure 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.
- hasten the completion of the test with high accuracy, making confirmatory rapid testing feasible.
- accelerate the flow of sample matrix and components, thereby defeating the obstruction issues of uneven, porous materials.

Comparison of *Driven Flow*™ to Lateral Flow iFOB Assays

Driven Flow™ assays offer three main advantages over traditional lateral flow tests: (1) a significantly reduced reaction time for analysis, (2) a much lower sample volume requirement, and, most importantly, (3) greatly increased accuracy, particularly within 75-125% of the test cutoff (analyte detection limit), as shown in **Table 1**.

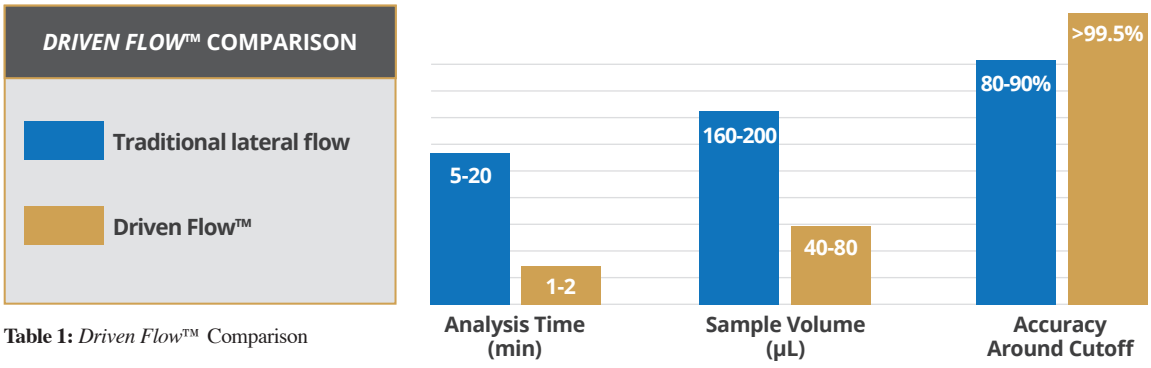

Table 1: *Driven Flow*™ Comparison

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A Semi-quantitative Collection Tube Enhances Accuracy

To further enhance the accuracy and reliability of testing, controlling the amount of stool specimen introduced into the sample enclosure is essential. The scientists at Alfa Scientific Designs, Inc. have developed and patented[18] a revolutionary semi-quantitative collection tube designed to combat false positive results by limiting the fecal sample size. This semi-quantitative collection tube[1] relies on an internal metering device and double closure to ensure both sample quality and sample integrity, thus reducing expenditures on unnecessary and invasive medical examinations.

The Instant-view-PLUS™ iFOB Test with *Driven Flow*™ Technology – the better choice for CRC screening

The Instant-view-PLUS™ Fecal Occult Blood rapid test is an immunochemical device made in the US by Alfa Scientific Designs, Inc., the pioneer of iFOB tests. Recognized in the industry for developing and manufacturing products with high accuracy and speed, ease-of-use, and cost-effectiveness, ALFA's *Driven Flow*™ iFOB test is the new generation for CRC prescreening. With ALFA's revolutionary technology, analysis times are reduced by 5-10 fold and assay accuracy is boosted to >99%.

Conclusion

The Instant-view-PLUS™ iFOB Test with *Driven Flow*™Technology is the better choice for CRC screening because early detection is essential to boost CRC survival rates. ALFA's *Driven Flow*™ Instant-view-PLUS™ iFOB device provides a revolutionary rapid test for CRC prescreening with high accuracy in as quick as one minute.

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References

1. ALFA's Patented Semi-Quantitative Collection Tube Increases Accuracy of iFOB Test, ALFA Scientific Designs, White Paper.
2. Colorectal Cancer. The American Cancer Society. Available at: http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-key-statistics. Accessed June 3, 2011.
3. Rozen P, Knaani J, Samuel Z. Comparative screening with a sensitive guaiac and specific immunochemical occult blood test in an endoscopic study. Cancer 2000; 89:46-52.
4. Young GP, St John DJB. Faecal occult blood tests: choice, usage and clinical applications. Clin Biochem Rev 1992;13:161-167.
5. Burch JA, Soares-Weiser K, St John DJ, et al. Diagnostic accuracy of faecal occult blood tests used in screening for colorectal cancer: A systematic review. Journal of Medical Screening. 2007;14(3):132–137.
6. Young GP, St John DJB. Selecting an occult blood test for use as a screening tool for large bowel cancer. Rozen P, Reich CB, Winawer SJ (eds); Large Bowel Cancer: Policy, Prevention, Research and Treatment. In Front Gastrointest Res 18; pp 135-156. Basel, Karger 1991.
7. Rozen P, Knaani J, Samuel Z. Performance characteristics and comparison of two immunochemical and two guaiac fecal occult blood screening tests for colorectal neoplasia. Dig Dis Sci 1997; 42(10):2064-2071.
8. Macrae FA, St. John DJB. Relationship between patterns of bleeding and Hemoccult sensitivity in patients with colorectal cancers or adenomas. Gastroenterol 1982; 82: 891-898.
9. Rosenfield RE, Kochwa S, Kaczera Z, Maimon J. Nonuniform distribution of occult blood in feces. Am J Clin Path 1979;71:204-209.
10. Herzog P, Holtermüller KH, Preiss J, Fischer J, et al. Fecal blood loss in patients with colonic polyps: A comparison of measurements with 51chromium-labeled erythrocytes and with the Haemoccult test. Gastroenterol 1982; 83(5):957-962.
11. The American College of Radiology Colon Cancer Committee, Screening and Surveillance for the Early Detection of Colorectal Cancer and Adenomatous Polyps, CA Cancer J Clin 2008;58:130–160
12. FDA Website http://www.fda.gov/MedicalDevices/ ProductsandMedicalProcedures/InVitroDiagnostics/HomeUseTests/ucm125834.htm
13. May, et. al., U.S. patent number 5,656,503
14. Jeanette M. Daly, Camden P. Bay, and Barcey T. Levy, Evaluation of Fecal Immunochemical Tests for CRC Screening, J Prim Care Community Health, 4(4), 245-250.
15. Conclusion advantages/disadvantages of lateral flow systems, by michielberge on April 14, 2013 . Literature: Raphael C. Wong, Harley Y. Tse, Lateral Flow Immunoassay, Springer, ISBN: 978-1-58829-908-6
16. Geertruidia A. Posthuma-Trumpie, Jakob Korf, and Aart van Amerongen, Lateral flow (immun0)assay: its strengths, weaknesses, opportunites and threats. A literature survey. Anal Bioanal Chem, 2009, 393: 569-582, DOI 10.1007/ s00216-008-2287-2.
17. Wang, et. al., U.S. patent number 9,377,457 B1
18. Zhou, et. al., U.S. patent numbers SD477,669S; 6,780,160 B2; 6,922,370 B2; 7,049,693 B2.