

UC Riverside

UCR Honors Capstones 2016-2017

Title

A Method for Depositing Microspheres for Use in Lateral Flow Assays

Permalink

<https://escholarship.org/uc/item/5kn9s636>

Author

Roper, Jenna Marie

Publication Date

2017-12-08

A METHOD FOR DEPOSITING MICROSPHERES
FOR USE IN LATERAL FLOW ASSAYS

By

Jenna Marie Roper

A capstone project submitted for
Graduation with University Honors

May 11, 2017

University Honors
University of California, Riverside

APPROVED

Dr. Hideaki Tsutsui
Department of Mechanical Engineering

Dr. Richard Cardullo, Howard H Hays Jr. Chair and Faculty Director, University Honors
Interim Vice Provost, Undergraduate Education

ABSTRACT

Lateral flow tests are simple devices used to detect of a wide variety of analytes. These devices are portable, stable, low-cost, and user-friendly, making them ideal for point-of care testing. The purpose of this project is to develop a lateral flow device, with a simple and rapid read-out, for quantitative detection of DNA. This device utilizes oligomer-conjugated microspheres that aggregate in solutions containing the targeted DNA. When deposited onto a porous substrate, such as filter paper, the wicking distance of the microspheres is dependent on the size and presence of the aggregates. In lateral flow devices, pre-depositing reagents simplifies user input, making the device more user-friendly. This report details a method of pre-depositing microspheres onto the device so that microspheres dry onto the paper and are able to be rehydrated and wick through the paper. Microspheres are negatively charged, hydrophobic particles. While stable in solution, the drying process forces microspheres close together; when the microspheres touch they irreversibly aggregate. The goal is to block microspheres from aggregating by adding water-soluble inert polymers, proteins, and surfactants to the deposited microsphere solution. The solution must be stable in dry form, yet dissolve easily upon re-hydration to allow the microspheres to wick through paper. We found that depositing microspheres in a buffer containing bovine serum albumin (BSA), sucrose, Tween-20, and PVP-40 allowed the microspheres to wick through Whatman® Fusion 5 upon addition of solution.

ACKNOWLEDGEMENTS

I would like to thank my graduate student mentor, Brent Kalish, and all members of the Tsutsui Lab. I would also like to thank the MARCU* Program at UCR for their support.

TABLE OF CONTENTS

Abstract.....	ii
Acknowledgements	iii
Table of Contents	iv
List of Figures.....	vi
1. Background	1
1.1 Low Cost Diagnostics	1
1.2 Lateral flow devices.....	1
1.2.1 Sample pad.....	2
1.2.2 Conjugate pad.....	2
1.2.3 Membrane	2
1.2.4 Absorbent Pad	2
1.3 Nucleic Acid Testing.....	3
1.4 Instrument-Free Quantitative DNA Detection	3
1.4.1 Pre-depositing microspheres	4
2. Materials and Methods.....	5
2.1 Reagents and membranes	5
2.2 Microsphere Preparation.....	5
2.3 Preparation of the test strip.....	5
2.4 Assay procedure.....	5
3. Results and Discussion.....	6
3.1 Material of Test Strip.....	6
3.2 Buffer Composition	6
3.3 Effect of Material on Microsphere Re-Hydration	7

3.4 Effect of Microsphere Size on Wicking Distance.....	9
3.5 Effect of Surfactant and Blocker Concentrations on Microsphere Re-Hydration.....	10
4. Conclusion and Future Works	11
5. References.....	13

LIST OF FIGURES

Figure 1 Lateral Flow Assay Schematic 1

Table 1 Conjugate Release Buffers 6

Figure 2 Nitrocellulose..... 8

Figure 3 Filter Paper. 8

Figure 4 Fusion 5 8

Table 2 Conjugate Release Buffers, increased Tween-20, sucrose, or BSA 9

Figure 5 Effect of Buffer Concentration 9

Figure 6 Effect of Tween-20, sucrose, and BSA 10

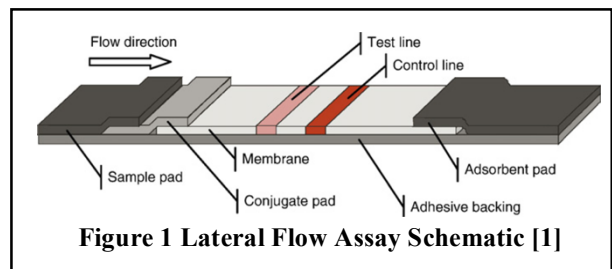
1. BACKGROUND

1.1 Low Cost Diagnostics

Many diagnostic technologies that are extensively used in developed nations are not useful in developing countries. Developing countries often lack the necessary equipment, trained personnel, or even basic infrastructure, such as electricity and running water that are necessary for these high-tech diagnostics. The World Health Organization (WHO) has set forth guidelines for developing diagnostics for use in resource-limited settings; a successful diagnostic must be ASSURED. It must be affordable to those who need it, sensitive and specific, user-friendly, rapid and robust, equipment-free, and delivered to end users. Paper is an ideal platform for point-of-care diagnostics in resource-limited settings because it is lightweight, ubiquitous, low-cost, and easy to manufacture.

1.2 Lateral flow devices

Lateral flow assays (LFAs) are a class of paper-based devices that can be used to detect the presence or absence of a wide-variety of analytes. Lateral flow



assays are an ideal diagnostic technology for developing countries because they are rapid, low-cost, user-friendly, and do not require any external machinery. In a LFA, liquid wicks through a porous substrate via capillary action, combating the need for a pump or other external driving force. LFAs are used for a many applications. LFAs can detect proteins, pathogens, metabolites, or nucleic acids in biomedical [2] [3], agricultural [4], environmental [5], and food safety [6] applications.

In a lateral flow device, a sample containing a target analytes moves along a strip, passing zones that contain molecules that will react with the target. LFAs typically consist of a sample pad, conjugate-release pad, membrane and absorbent pad all affixed to a sturdy backing [7].

1.2.1 Sample pad

The sample application pad is typically made of cellulose or glass fiber. It functions to collect the sample and transport it in a continuous manner to the rest of the strip. The sample pad may pre-treat the sample, such as filtering out certain components or adjusting the sample pH.

1.2.2 Conjugate pad

Typical materials for the conjugate pad are cellulose, glass fiber, and Fusion 5. The conjugate release pad functions as storage for labelled analytes or the colored recognition element. The conjugates must dry without aggregating or being damaged and remain stable for long-term storage, then easily release when a sample solution is applied.

1.2.3 Membrane

The membrane is typically made of nitrocellulose. It contains a test line and a control line where the target, typically bound to a colored particle, binds. It is important that the membrane have strong binding, to capture probes, and low non-specific adsorption, to ensure maximum assay performance.

1.2.4 Absorbent Pad

The absorbent pad, typically cellulose, is the final component of a lateral flow device. It functions to allow a greater sample volume to be used and still wick through the device without any external driving forces or machinery.

1.3 Nucleic Acid Testing

Nucleic acid testing has critical functions in areas of food safety, environmental monitoring, personalized medicine, health, and agriculture [8]. There are many technologies used extensively in the developed countries for detection of nucleic acid sequences including polymerase chain reaction (PCR) and quantitative PCR (qPCR); these methods rely on costly and sometimes inaccessible equipment and reagents, trained technicians, and require infrastructure that is largely lacking in developing countries. There is an emerging class of lateral flow devices that detect nucleic acids in point-of-care applications where a laboratory and trained personnel are not readily available [9]. For example, Mao *et al.* developed a nucleic acid biosensor using gold nanoparticles (Au-NP). In the presence of the target strand, the Au-NP probes are immobilized on the test zone, creating a red band that enables qualitative visual detection. However, in order to quantify the amount of DNA, a strip reader must be used to image the red band and calculate the intensity of the color [10]. Thus far, nucleic acid detection in lateral flow assays is limited to either qualitative yes/no detection or quantitative detection that requires a camera, smart phone, strip reader, or other external piece of equipment for analysis

1.4 Instrument-Free Quantitative DNA Detection

Our lab is developing a paper-based method for determining the concentration of a certain target DNA in a sample. Our method utilizes a three-component system in which two different strands of ssDNA, which are complimentary to either the 5' or 3' end of a target DNA sequence, are conjugated to the surface of two populations of polystyrene latex microspheres. When the conjugated microspheres are in solution with the target strand, the single stranded DNA hybridizes causing the microspheres to form aggregates. A higher

concentration of target DNA causes larger aggregates to form. When a solution containing these aggregates is deposited onto a porous substrate, such as filter paper, the wicking distance is dependent on the size of aggregates. Small particles wick farther than larger particles; therefore, a solution containing a high concentration of the target DNA will travel a shorter distance than a solution containing a lower concentration. This allows for a quantitative assay with a read-out visible to the naked eye.

1.4.1 Pre-depositing microspheres

The goal of this paper is to determine a method of pre-depositing microspheres onto a test strip such that the microspheres dry without aggregating and then re-hydrate and wick through the test strip upon addition of a sample solution. This would minimize user input, which is critical for successful point-of-care testing.

Microspheres are negatively charged and hydrophobic. While stable in solution, they are forced close together during the drying process. If they come in contact with each other while drying, they irreversibly aggregate. In this paper, we aim to determine a material for the test strip, appropriately sized microsphere, and conjugate release buffer that allows the microspheres to be dried onto the test strip but still wick through the strip upon re-hydration. Materials commonly utilized in lateral flow assays: nitrocellulose, filter paper, and Fusion 5 were tested. The material must not permanently bind to microspheres and exhibit the inverse wicking relationship used in our detection mechanism. Additionally, conjugate release buffers containing different concentrations of surfactant, blocking protein, water-soluble inert polymers, and stabilizers. The buffer must prevent microspheres from irreversibly aggregation when drying; it must be stable when dry but quickly dissolve upon addition of a sample.

2. MATERIALS AND METHODS

2.1 Reagents and membranes

A 10% solid solution of 0.15 μm , 0.30 μm , 0.33 μm , 0.66 μm , 0.68 μm and 1 μm colored, carboxylated, polystyrene latex microspheres purchased from Magsphere, Pasadena, CA. 10x phosphate buffered saline, bovine serum albumin (BSA), Tween-20, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP40), and sucrose purchased from Sigma-Aldrich, St. Louis, MO. Backed nitrocellulose, Hi-Flow plus cellulose ester membranes (HF135), purchased from Millipore, Darmstadt, Germany. Whatman[®] qualitative filter paper, Grade 1 and Whatman[®] Fusion5 were purchased from GE Healthcare Life Sciences, Pittsburgh, PA.

2.2 Microsphere Preparation

Microspheres were diluted 10-fold to a 1% solid solution in 1mM PBS. Microspheres were centrifuged at 7,000 rpm for 15 minutes. The supernatant was discarded and replaced with fresh PBS. After three wash cycles, the microsphere were re-suspended to a 10% solid solution. The microspheres were added to various conjugate release buffers to make a 1% solid solution.

2.3 Preparation of the test strip

Filter paper was backed with clear packing tape and then cut into 5x60 mm strips. The strips were then affixed to cardstock with double-sided tape. The nitrocellulose and Fusion 5 were cut into 50x600 mm strips and affixed to cardstock using double-sided tape.

2.4 Assay procedure

Using a micropipette, 5 μL 1%-solid microspheres in various buffers was deposited onto the test strips. The microspheres were allowed to dry at least 24 hours at room

temperature. In order to test the microsphere’s ability to re-hydrate, 50 μL of the same conjugate release buffer used deposit microspheres was added to the test strips. Microspheres in 1 mM PBS was used as a control.

3. RESULTS AND DISCUSSION

3.1 Material of Test Strip

Nitrocellulose, filter paper, and Fusion 5 were chosen because they are commonly used in lateral flow devices. Nitrocellulose almost exclusively functions as the membrane where the test strip and control strip are bound in classic LFAs. Filter paper is made of cellulose, which is sometimes used as the sample application pad and/or conjugate release pad. Fusion 5 is an “all-in-one” material that can serve as each component of a lateral flow device, though it is often utilized as simply a conjugate release pad. Six different sized microspheres from 0.15 to 1 μm were tested, this range is was chosen due to the pore sizes of the three materials tested. Fusion 5 has a pore size of approximately 2.5 μm . Grade 1 filter paper has a particle retention size 11 μm , Nitrocellulose has a pore size of around 0.45 μm . The microspheres chosen are small enough to travel through each substrate.

3.2 Buffer Composition

Three different conjugate release buffers were tested (**Table 1**). Each buffer is a 1 mM PBS buffer with 1% (w/v) PVA and 0.5% (w/v) PVP-40, both water-soluble inert polymers. The buffers have a high, medium, and low concentration of

Buffer 1 low concentration	Buffer 2 medium concentration	Buffer 3 high concentration
1mM PBS	1mM PBS	1mM PBS
0.5% PVP40	0.5% PVP40	0.5% PVP40
1% PVA	1% PVA	1% PVA
0.1% Tween-20	0.5% Tween-20	1% Tween-20
1% BSA	10% BSA	20% BSA
15% Sucrose	20% Sucrose	25% Sucrose

Tween-20, BSA, and sucrose. The concentration of Tween-20 in conjugate release buffer for microspheres in Fusion 5 is often between 0.1% (w/v) and 1% (w/v), the concentration of sucrose from 15%-25% (w/v) and the concentration of BSA is between 1% and 25% (w/v) [11]. Tween-20 aids in the release of microspheres upon re-hydration, BSA acts as a blocking protein to prevent non-specific binding and aggregation, and the sucrose functions as a blocker, protecting the microspheres during the drying process. Sucrose also aids in the release of microspheres, since sugar dissolves easily upon addition of solution.

3.3 Effect of Material on Microsphere Re-Hydration

Nitrocellulose, filter paper, and Fusion 5 were tested by depositing 1% microsphere solutions in the different buffers onto a test strip and comparing the microspheres in the conjugate release buffers to microspheres deposited in PBS. Microspheres in PBS irreversibly aggregated when dried and did not wick through the test strip upon addition of buffer regardless of the material of the test strip, as expected (**Fig 2-4a**).

In nitrocellulose (**Fig 2**) and filter paper, (**Fig 3**), none of the microspheres exhibited conjugate release. The three buffers showed no change in behavior from the PBS control. In Fusion 5 each combination appears to move slightly upon re-hydration (**Fig 4**). The microspheres in each conjugate release buffer are slightly lighter and have a larger diameter than the PBS control indicating the dried microspheres moved slightly upon re-hydration. However, the blue 0.68 μm microspheres in the medium-strength buffer, buffer 2, shows a much higher conjugate release than the other sized microspheres; nearly all the microspheres rehydrate and wick through the test strip. The weak buffer, buffer 1, does not block aggregation (**Fig 4b**), while the most concentrated

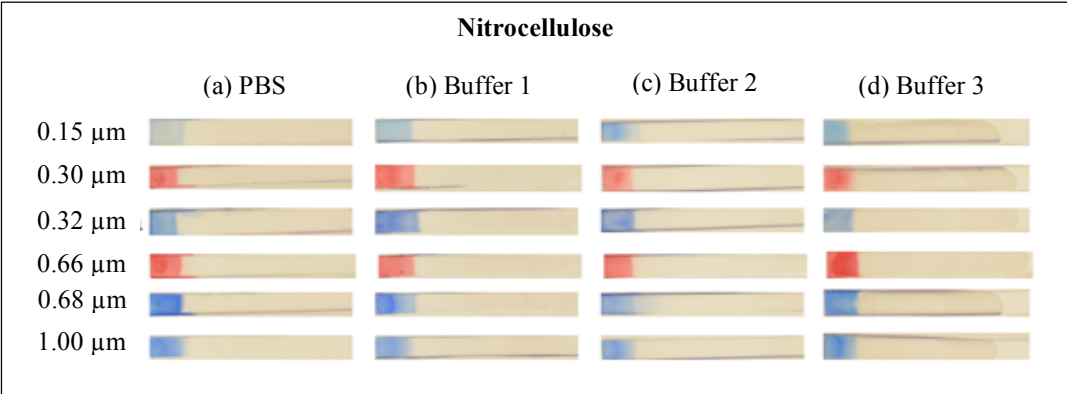


Figure 2 Nitrocellulose Various sized microspheres deposited in PBS (a), and buffer with a low (b), medium (c), and high (d) concentration of sucrose, BSA, and Tween-20.

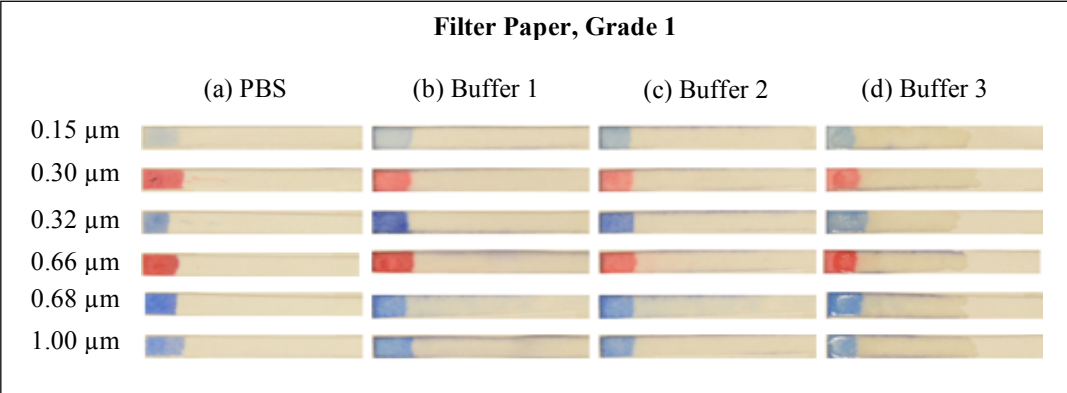


Figure 3 Filter Paper Various sized microspheres deposited in PBS (a), and buffer with a low (b), medium (c), and high (d) concentration of sucrose, BSA, and Tween-20.

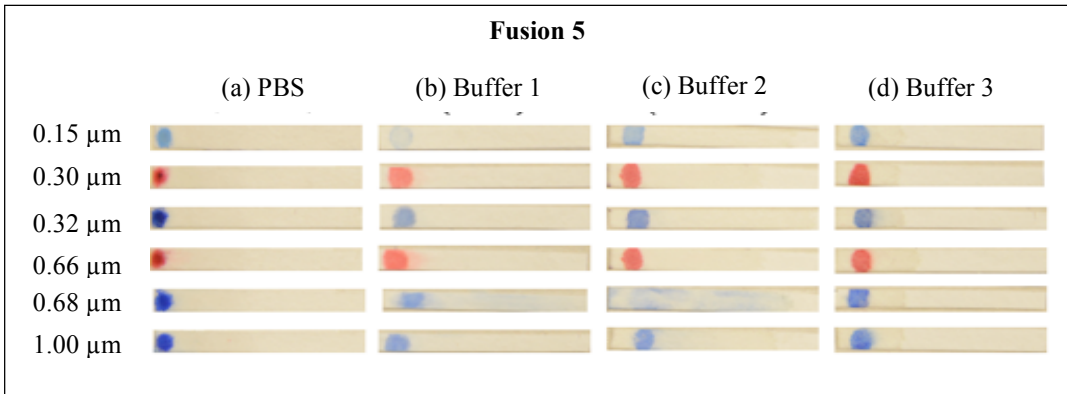


Figure 4 Fusion 5 Various sized microspheres deposited in PBS (a), and buffer with a low (b), medium (c), and high (d) concentration of sucrose, BSA, and Tween-20.

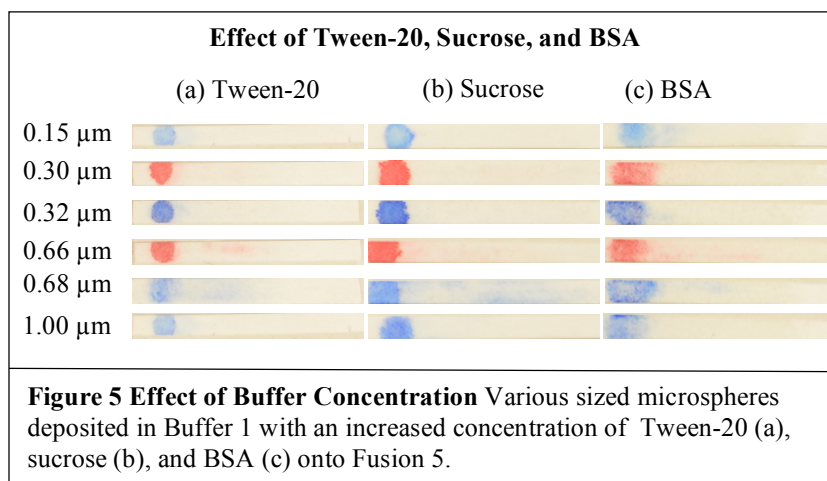
buffer, buffer 3, seems to block the pores of the Fusion 5 (**Fig 4d**). Additionally, the high concentration of sugar created a glaze, causing the test strip to appear shiny.

3.4 Effect of Microsphere Size on Wicking Distance

Our quantitative DNA detection method utilizes the fact that larger microspheres travel shorter distance; this inverse wicking relationship is central to our detection mechanism. To analyze this trend in nitrocellulose,

High Tween-20 concentration	High sucrose concentration	High BSA concentration
1mM PBS	1mM PBS	1mM PBS
0.5% PVP40	0.5% PVP40	0.5% PVP40
1% PVA	1% PVA	1% PVA
1.0% Tween-20	0.1% Tween-20	0.1% Tween-20
1% BSA	1% BSA	20% BSA
15% Sucrose	25% Sucrose	15% Sucrose

filter paper, and Fusion 5, 50 μL of 0.1% solid microspheres, from 0.15 μm to 1 μm , were deposited onto a test strip made of each material (**Fig 5**).

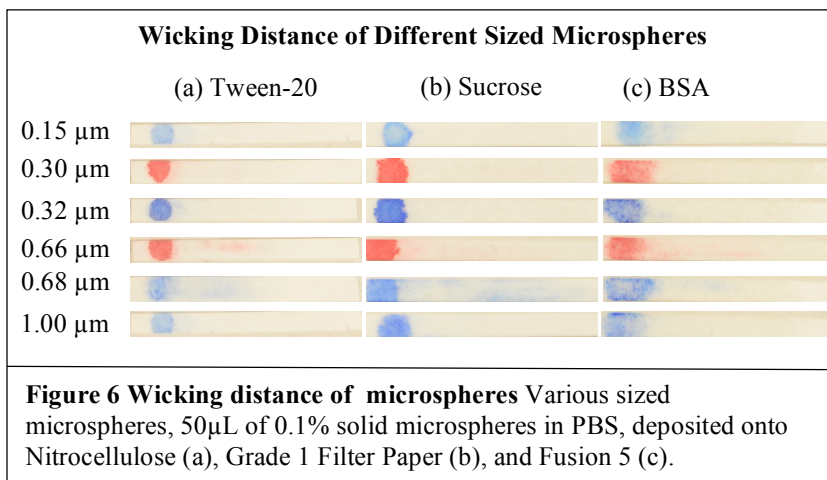


We expect to see that the smallest (0.15 μm) microspheres travel farther than the largest (1 μm) microspheres. In nitrocellulose and filter paper this trend is observed (**Fig 5a-b**). In Fusion 5, all of the microspheres travel a very short distance with no significant difference

between the smallest and largest microsphere. This may be due to the PBS buffer the microspheres were deposited in or the pH of the microsphere solution. Further investigation must be done to determine if different-sized microspheres wick different distances in Fusion 5 to ensure it can be used in our assay.

3.5 Effect of Surfactant and Blocker Concentrations on Microsphere Re-Hydration

The effect of Tween-20, sucrose, and BSA in the conjugate release buffer was studied by increasing the concentration of a single component in three different conjugate release buffers (**Table 2**). Tween-20 was increased from 0.1% to 1%, sucrose was increased from 15% to 25%, and BSA was increased from 1% to 25%. These values were chosen based on recommended conjugate release buffers for polystyrene latex microspheres [11]. The low concentration of each component in Buffer 1 and the high concentration in these buffers represents the entire range of recommended values.



Each condition improved the conjugate release in Fusion 5. The concentration of all components of the conjugate release buffer were identical to Buffer 1 (Fig 4b). Although there was a slight improvement in each case, increased Tween-20 (**Fig 6a**) did not significantly increase the amount of microspheres that re-hydrated and wicked through the

test strip. Increased sucrose (**Fig 6b**) had a more noticeable effect than the increased Tween-20. Tween-20 and sucrose mainly improved conjugate release for the red 0.66 μm and blue 0.68 μm microspheres. Increasing the amount of BSA (**Fig 6c**), improved conjugate release for each size. This suggests that BSA is extremely important in blocking the microspheres from binding to each other. However, the amount of BSA was increased 25X, while the amount of sucrose was only increased 1.7X, and Tween-20 10X. The observed increase in conjugate release in the buffer with increased BSA may be due to the greater percent increase of BSA as opposed to the functionality of BSA itself.

4. CONCLUSION AND FUTURE WORKS

The aim of this project was to develop a method of depositing microspheres onto a substrate such that they quickly re-hydrate and wick through the test strip upon addition of a sample solution. This would simplify in user-input, an important aspect for developing practical assays for point-of-care testing. Different substrates, conjugate release buffers, and microsphere sizes were tested in this paper. Common materials used in LFAs, Nitrocellulose, filter paper, and Fusion 5, conjugate release buffers with varying concentrations of surfactants and blockers, and a range of microspheres were tested.

In all trials in nitrocellulose and filter paper, the microspheres permanently aggregated when dried. Fusion 5 showed a small amount of microsphere re-hydration in each case compared to the PBS control, though only one condition provided a significant amount of conjugate release. The 0.68 μm microspheres in the medium-strength buffer deposited on Fusion 5 appears to successfully rehydrate upon addition of buffer and wick through the test strip. However, upon further investigation, it is not clear the Fusion 5 is an adequate substrate for our method of quantitative DNA detection. When 50 μL of 0.1%

solid microsphere solution in PBS was deposited onto Fusion 5, the wicking distance was not affected by the size of the microspheres. The 0.15 μm and 1 μm microspheres travelled similar distances. Further investigation needs to be done to determine if deposition in different buffers or with different pHs have the same effect.

5. REFERENCES

- [1] Assadollahi, Saied, et al. "From lateral flow devices to a novel nano-color microfluidic assay." *Sensors* 9.8 (2009): 6084-6100.
- [2] Chan, Cangel PY, et al. "Development of a quantitative lateral-flow assay for rapid detection of fatty acid-binding protein." *Journal of immunological methods* 279.1 (2003): 91-100.
- [3] Smits, Henk L., et al. "Lateral-flow assay for rapid serodiagnosis of human leptospirosis." *Clinical and diagnostic laboratory immunology* 8.1 (2001): 166-169.
- [4] Danks, C., and I. Barker. "On-site detection of plant pathogens using lateral-flow devices." *EPPO Bulletin* 30.3-4 (2000): 421-426.
- [5] Hossain, SM Zakir, et al. "Reagentless bidirectional lateral flow bioactive paper sensors for detection of pesticides in beverage and food samples." *Analytical chemistry* 81.21 (2009): 9055-9064.
- [6] Tang, D., et al. "Magnetic nanogold microspheres-based lateral-flow immunodipstick for rapid detection of aflatoxin B 2 in food." *Biosensors and Bioelectronics* 25.2 (2009): 514-518.
- [7] Posthuma-Trumpie, Geertruida A., Jakob Korf, and Aart van Amerongen. "Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey." *Analytical and bioanalytical chemistry* 393.2 (2009): 569-582.
- [8] Jauset-Rubio, Miriam, et al. "Ultrasensitive, rapid and inexpensive detection of DNA using paper based lateral flow assay." *Scientific Reports* 6 (2016).
- [9] Niemz, Angelika, Tanya M. Ferguson, and David S. Boyle. "Point-of-care nucleic acid testing for infectious diseases." *Trends in biotechnology* 29.5 (2011): 240-250.
- [10] Mao, Xun, et al. "Disposable nucleic acid biosensors based on gold nanoparticle probes and lateral flow strip." *Analytical chemistry* 81.4 (2009): 1660-1668.
- [11] O'Farrell, Brendan. "Lateral flow immunoassay systems: evolution from the current state of the art to the next generation of highly sensitive, quantitative rapid assays." *The immunoassay handbook: theory and applications of ligand binding, ELISA, and related techniques*. Oxford: Elsevier (2013): 89-107.