UNDERSTANDING THE FUNCTIONALITY OF MULTIPLEXED SENSORS IN ORDER TO AID DESIGN AND ENHANCE PERFORMANCE

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ABSTRACT

Multiplexed sensors enable simultaneous detection of disease biomarkers and environmental contaminants using a single test. Test results feature multiple signals, which if unclear, can result in misdiagnosis. This work studies the phenomenon of weakening signal intensities on multiplexed lateral flow tests. Results indicate that physical analyte entrapment has minimal impact on this phenomenon, which is influenced more, by the quantity of analyte in the sample and the positioning of the test line. Recommendations are made on the maximum number of test lines that should be employed to avoid reduced signal intensities, and the benefits of paper based microfluidics are discussed.

KEYWORDS: Multiplexed, Lateral flow tests, Paper based microfluidics

INTRODUCTION

The use of multiplexed paper sensors for health and environmental monitoring applications continues to rise. However, challenges, such as the misinterpretation of test results, difficulty in signal quantitation and cross reactivity continue to exist. Misinterpretation is often due to the presence of multiple test lines (TLs), especially since the standard is that two lines indicate a positive test result (such as the home pregnancy test). Weak signals exacerbate the problem further, being misinterpreted as false positive or negative signals. In this work and that of others [1], the signal intensity of TLs on a multiplex lateral flow test (LFT) were found to reduce the further away it is positioned from the colorimetric reporting element. To investigate this, a multiplexed LFT for Salmonella and Escherichia coli was used to study aspects such as bacteria entrapment by paper fibers or preceding test lines, cross reactivity of target bacteria with preceding test lines, and inadequate labelling of target analyte. Little work has been found on this subject matter [2,3].

EXPERIMENTAL

Colorimetric paper based immunosensors for Salmonella and *E. coli* were manufactured (Fig.1). The test lines and conjugate for each architecture contained anti-*E. coli* and anti-Salmonella antibodies (AbD Serotec and KPL). Colloidal gold was used as the colorimetric reporting element. Each test constituted a sample and conjugate pad, a membrane and a wick assembled on a common backing card, cut into 5mm test strips. Flow through and paper based microfluidic test formats utilized the same reagents. Grey scale measurements (Image J) were used to assess signal intensities (where 0=black and 255=white). ATCC strains of *E. coli* and Salmonella were used for sample preparation.

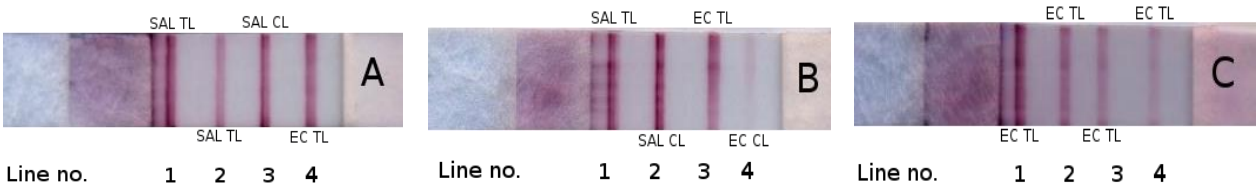

Figure 1: Lateral flow test architectures investigated; types A to C. (SAL=Salmonella; EC=E. coli)

RESULTS AND DISCUSSION

To analyse bacteria entrapment by paper fibers, bacteria suspensions of varying concentrations were flushed through lateral flow tests having a single *E. coli* test line. Thereafter, the LFTs were separated into their constituent sections (sample pad, conjugate pad, and membrane) and passed through an elution process. Eluted bacteria were cultured in nutrient media and counted. When bacterial samples of the order 10^8 to 10^6 cfu/ml are employed, the same order of magnitude of bacteria was found to reach the furthest end of the membrane (Fig.2). SEM analysis (image inserts in Fig.2) demonstrate however, that some bacteria randomly lie in the membrane, sample pad and conjugate pad after signal development. As these bacteria do not contact the TL antibodies, they do not participate in the detection process. The bacteria are lost either due to entanglement within paper fibers or cease to flow once the carrier fluid has depleted.

To determine if this negatively impacts the detection limit of the LFT, a flow-through test was performed. Flow through tests eliminate the sample and conjugate pads, forcing most, if not all the bacteria to contact the TL. The detection limit of the flow through and lateral flow tests were found to be the same, indicating that the bacterial losses due to physical entrapment are insignificant. Therefore, these losses cannot be entirely responsible for weakening signal intensities on multiplexed lateral flow tests.

Physical entrapment at preceding test lines was investigated by using LFT architectures A, B and C. For each sample concentration investigated using LFT type C, the signal intensity of the TLs decreased the further away it was positioned from the sample pad (Fig.3). The intensity of the test lines were strongly influenced by the bacteria concentration of the sample (arrows in Fig.3). At all bacteria concentrations, it becomes difficult to distinguish the test lines located further up the length of the test.

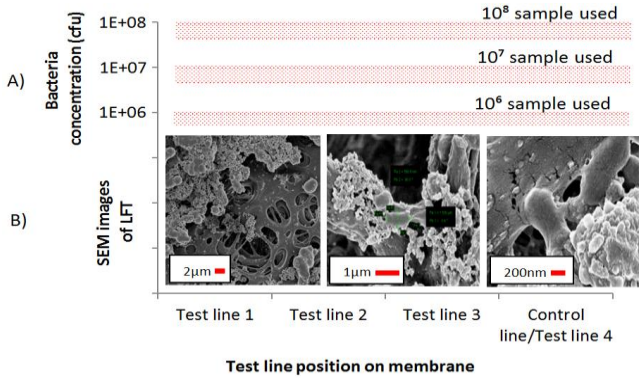

Figure 2: Plot A) bacteria count on LFT membrane, *B) SEM images of entrapped E. coli* in membrane.

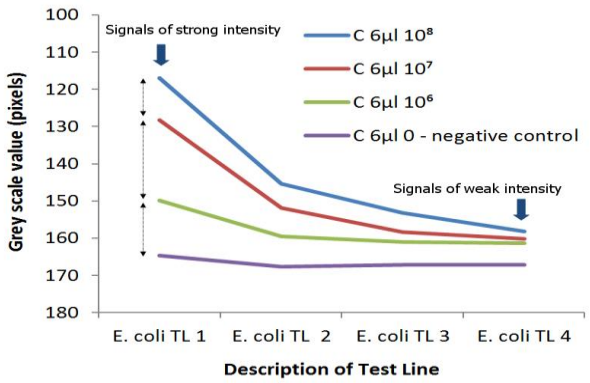

Figure 3: Signal intensities of test C with 6µl conjugate and 10^8-10^6 cfu/ml bacteria.

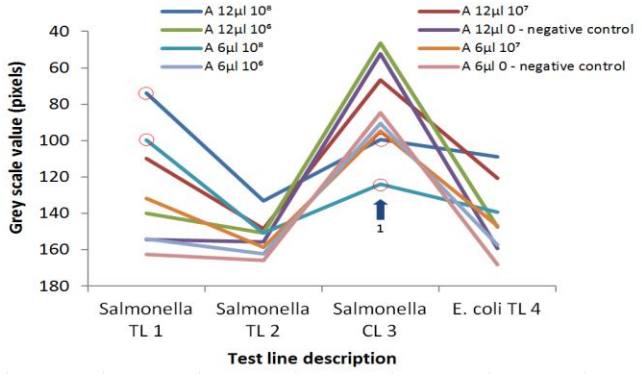

Figure 4: Signal intensities of test A with 6µl and 12µl conjugate and 10^8-10^6 cfu/ml bacteria.

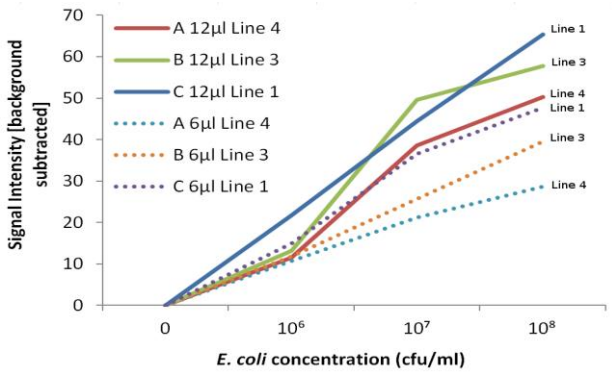

Figure 5: Signal intensities of tests A and B with 6µl and 12µl conjugate and 10^8-10^6 cfu/ml bacteria.

This indicates that the number of **labelled bacteria** available for capture at these positions is limited. As physical entrapment by paper fibers can be ruled out, either the quantity of conjugate or the number of bacteria in the sample was insufficient. Fig.4 demonstrates the signal intensities of LFT type A. Control lines (no. 1, Fig.4) form for each bacteria concentration investigated, and its intensity is dependent on the bacteria concentration (see red enclosed areas, Fig.4). This is expected since the more bacteria present, the fewer free conjugate antibodies available to bind at the control line (CL). The formation of both a control line and an *E. coli* test signal (positioned 4th on this test) indicates that there is sufficient conjugate available. Therefore in LFT types A and C, the number of test lines that form and their relative intensity to each other is dependent on the bacteria concentration of the sample. As further confirmation, Fig.4 shows a comparison of signal intensities obtained on test strips made with either 6µl or 12µl of conjugate. A similar trend of reducing signal intensities was observed for both tests. Another important finding from test type A (Fig.4), is that on a multiplexed test, wherein each test line detects a different target bacteria, the final (4th) test line is able to produce a positive test signal for its target (*E. coli* in this case). Ideally, all labelled *E. coli* should pass over the Salmonella TLs and bind only to the *E. coli* TL. The *E. coli* test line intensity however, is weaker than its preceding test lines for all bacteria concentrations investigated. To explain this further, Fig.5 compares the *E. coli* signal intensities of test types A, B and C, wherein the *E. coli* test line is positioned as line 1, 3 and 4 respectively. When the E .coli test line is positioned 1st, it produces the highest signal intensity, reducing as its position changes from 3rd and then to 4th. Seeing as all other parameters were kept constant, this is almost solely due to its positioning on the multiplex test. This could be due to *E. coli* cross reacting or becoming physically entrapped by preceding Salmonella test lines. Fig.5 therefore indicates that to aid semi-quantitation, and to improve signal intensity, the maximum number of test lines should be limited to 3 on a lateral flow test. When more analytes are required to be detected, paper based microfluidic sensors may prove a better option. The *E. coli* signal at line 3 on the lateral flow test was found to be weaker than the test signal for the same analyte on a paper based sensor (test type D, data not shown). This may be due to the architecture allowing for reduced contact and therefore reduced cross reactivity between the target analyte and other detection zones.

CONCLUSION

Physical bacteria entrapment within paper fibers and at preceding test lines on a multiplexed lateral flow test is insignificant, having minimal impact on signal intensities. It was found that signal reduction increases the further away the TL is positioned from the reporter element. This is likely due to non-specific interactions between the target analyte and preceding test lines, and the number of bacteria in the sample. To aid semi-quantitation, the maximum number of test lines should be limited to three.

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