

# The Evolution of Influenza Point of Care Testing: A Novel Mobile Phone App for the Reading of BD Veritor™ Flu A+B Tests



EPSRC IRC in Early Warning Sensing Systems for Infectious Diseases

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## Influenza Infection

Influenza is a formidable public health threat worldwide, causing significant human morbidity and mortality each year (approximately, 3 to 5 million infections result in severe illness each year) (1). The precise global impact of influenza is difficult to determine due to incomplete and non-standardized collection practices of global surveillance systems (2). With the constant threat of newly emerging viruses, mobile connected point of care testing (POCT) linked into surveillance systems offers greater access to diagnosis and treatment, whilst alerting public health authorities to areas of heightened incidence/outbreaks in real time.

The 2009 flu pandemic highlighted the shortcomings of visually read flu tests in terms of their sensitivity and usability (3). Consequently, there is a need to improve the sensitivity of POCTs and make the results easier to interpret.

## Evaluation of the BD Veritor Influenza A/B System

The Becton Dickinson (BD) Veritor Flu A+B System is a rapid immunochromatographic assay for the qualitative detection of influenza A and B viral antigens in clinical specimens. The system uses a reader to interpret the test strips, providing an objective result.

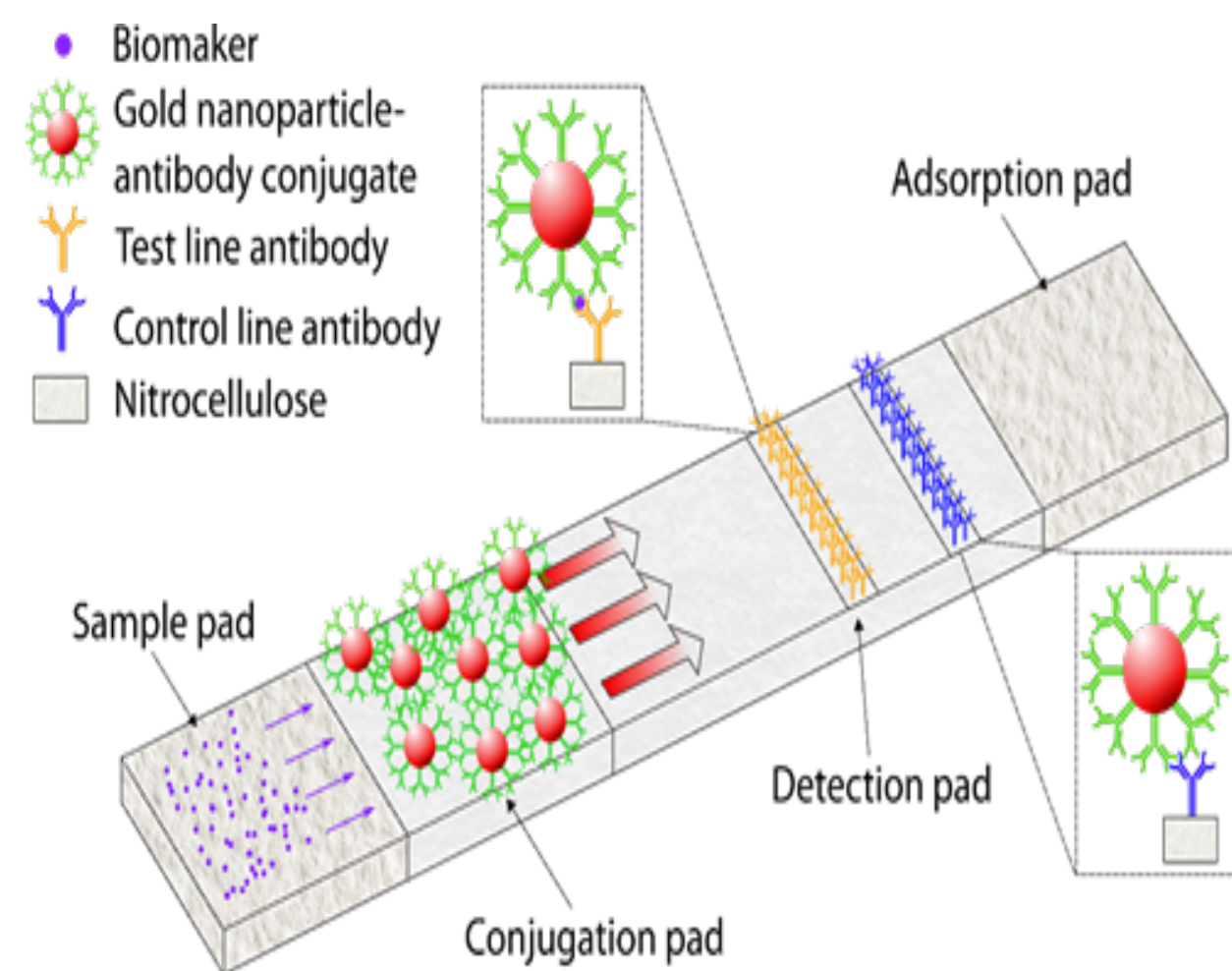
## Aim

A proof of principle study was conducted to evaluate the performance of the BD Veritor Flu A+B test system in 29 nose and throat swab samples, and explore whether a mobile phone app (i-sense Flu App) could be used as a cheaper alternative to the BD Veritor reader for reading lateral flow test strips.

## Method

- 15 positive and 14 negative combined nose and throat swab samples were obtained from patients with influenza like illness and tested using real-time reverse transcription PCR, which was used as a reference test for analysis.
- Samples were then tested using the BD Veritor Flu A+B test strips, and processed according to the manufacturer instructions. The test strips were read after 10 minutes using the BD reader and the i-sense Flu App (Figure 3 and 4).

## Immunochromatographic assay for Flu detection



- Fluid from a respiratory specimen is added to the sample pad and migrates in the direction of the adsorption pad.
- The biomarker (nucleoprotein) in the respiratory sample binds to antibodies conjugated to gold nanoparticles. An immunosandwich is formed with the nanoparticles/nucleoprotein and test line monoclonal antibodies in the detection pad.
- After about 10 minutes a red line is formed at the control and test line in a positive sample. Only a red line is formed at the control line in a negative sample. (Figure 2).

## Reading Veritor Test Strips

### 1) Veritor Reader

The BD Veritor reader uses a reflectance based method to evaluate the intensity at each spatially defined test and control line positions on the test strip. The results are interpreted using a scoring algorithm and reported as 'positive', 'negative' or 'invalid' based on pre-set thresholds (Figure 3).

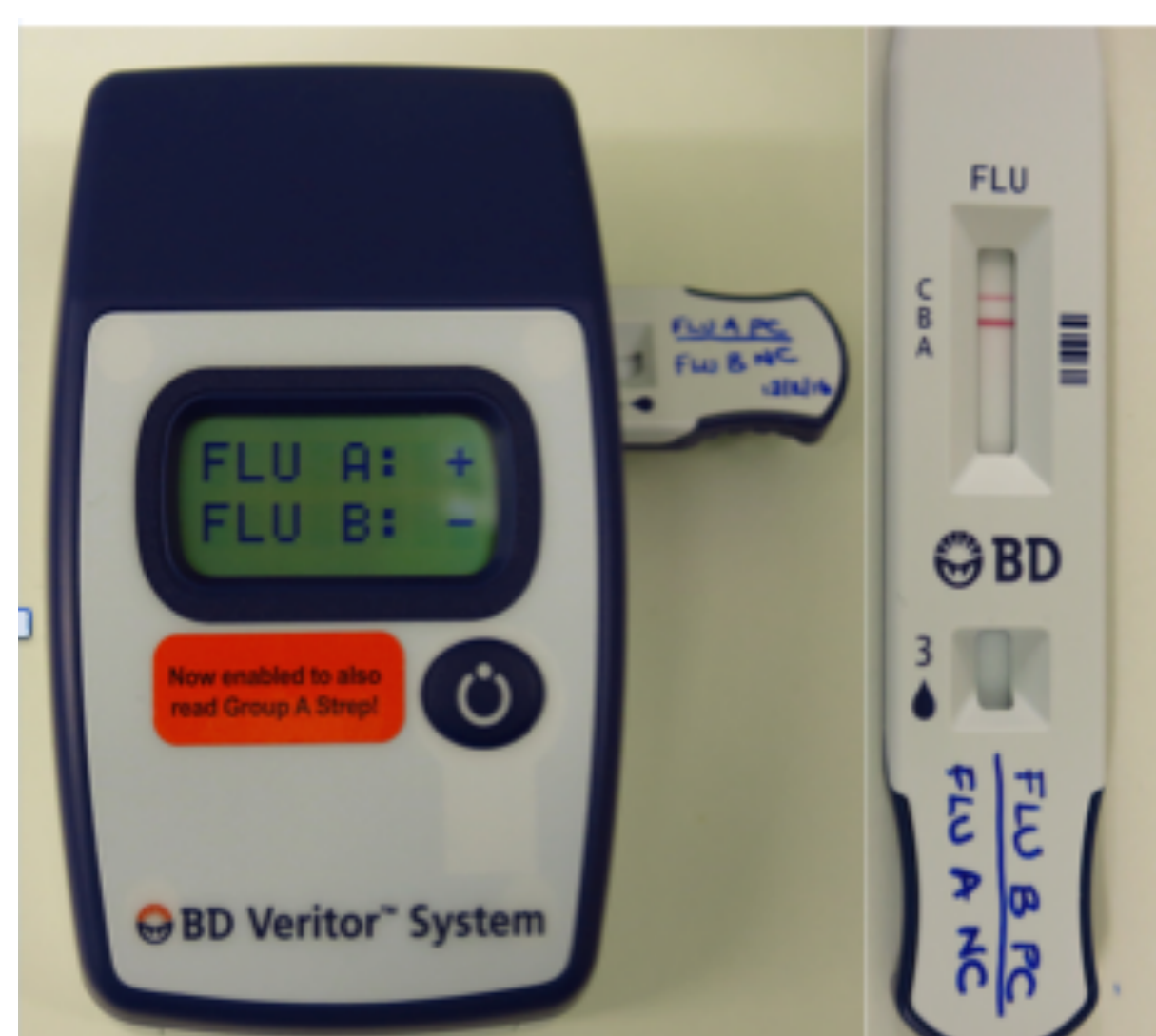


Figure 3: Shows the BD reader and a Flu A+B test strip

### 2) i-sense Flu App

- The test strip is visualized on the screen of the smartphone i.e (HTC One\_M8) using the phone's camera.
- The test strip is aligned and i-sense Flu App captures the region of interest (ROI) using the phone's camera.
- An image of the ROI is saved.
- The App measures the intensity down the length of the test strip in the green channel and subtracts the background (between the control and test). The app extracts the signal intensity at the control and test lines and outputs a quantitative result (Figure 4).

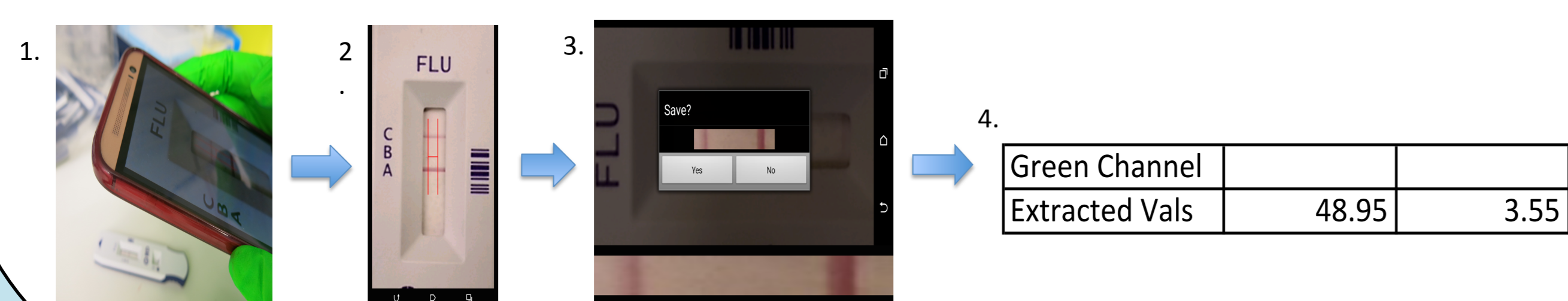


Figure 4: Workflow for reading the BD Veritor Flu test strips using the i-sense Flu App

## Symptoms of Flu infection

- Patients can be asymptomatic
- Present with a range of symptoms (Figure 1) (2), similar to other respiratory infections.

## Common Diagnostic Strategies

- Clinically- based on symptoms (non-specific)
- Molecular Assays (expensive, requires expertise and time consuming)

## Influenza in the Community

The majority of influenza cases occur in the community and traditional surveillance systems underestimate influenza infection and disease in the community (4). Smart phone-connected rapid tests will increase access testing and therefore help us to understand the real-time burden of flu in the community.

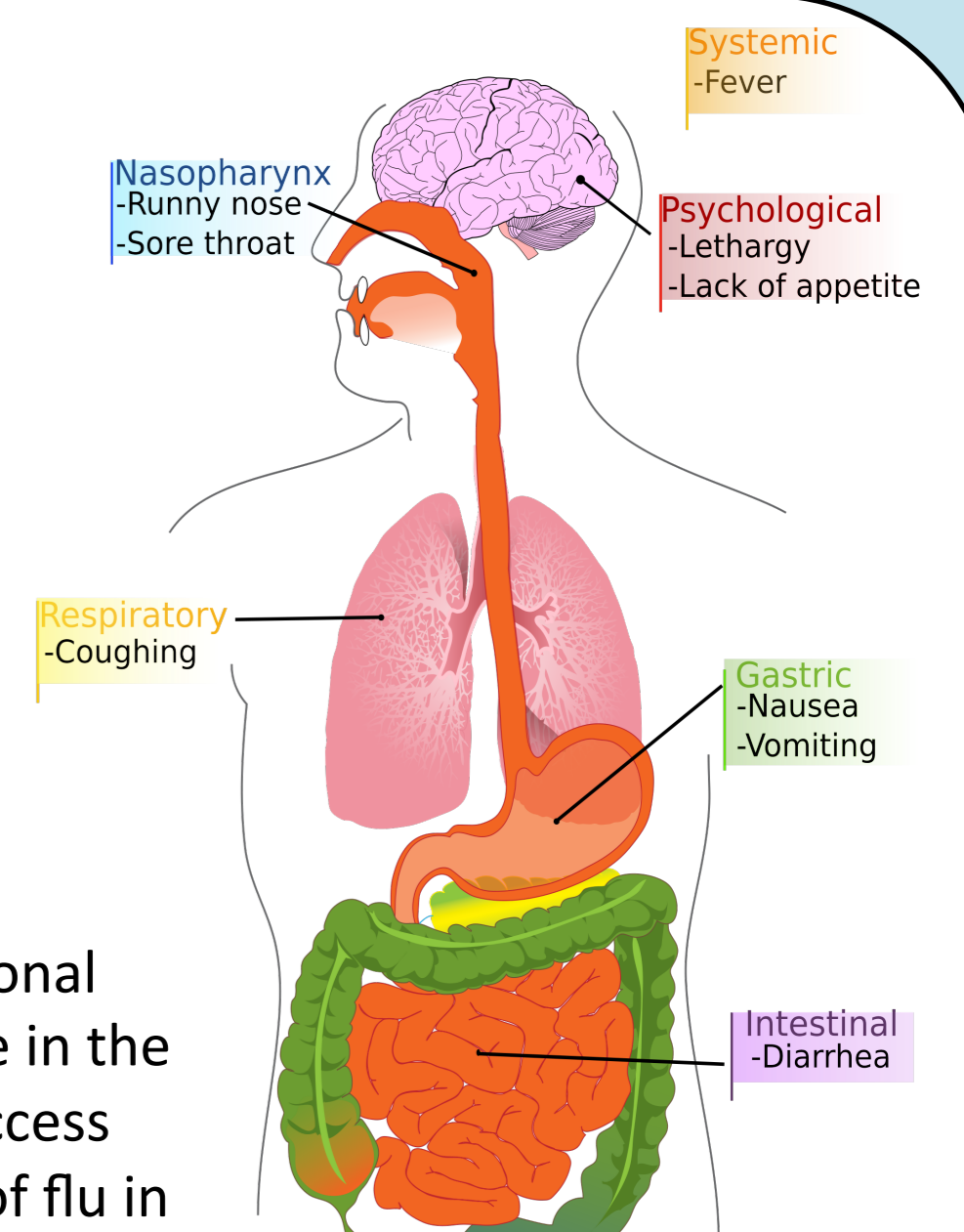


Figure 1: schematic showing common influenza symptoms

## Results

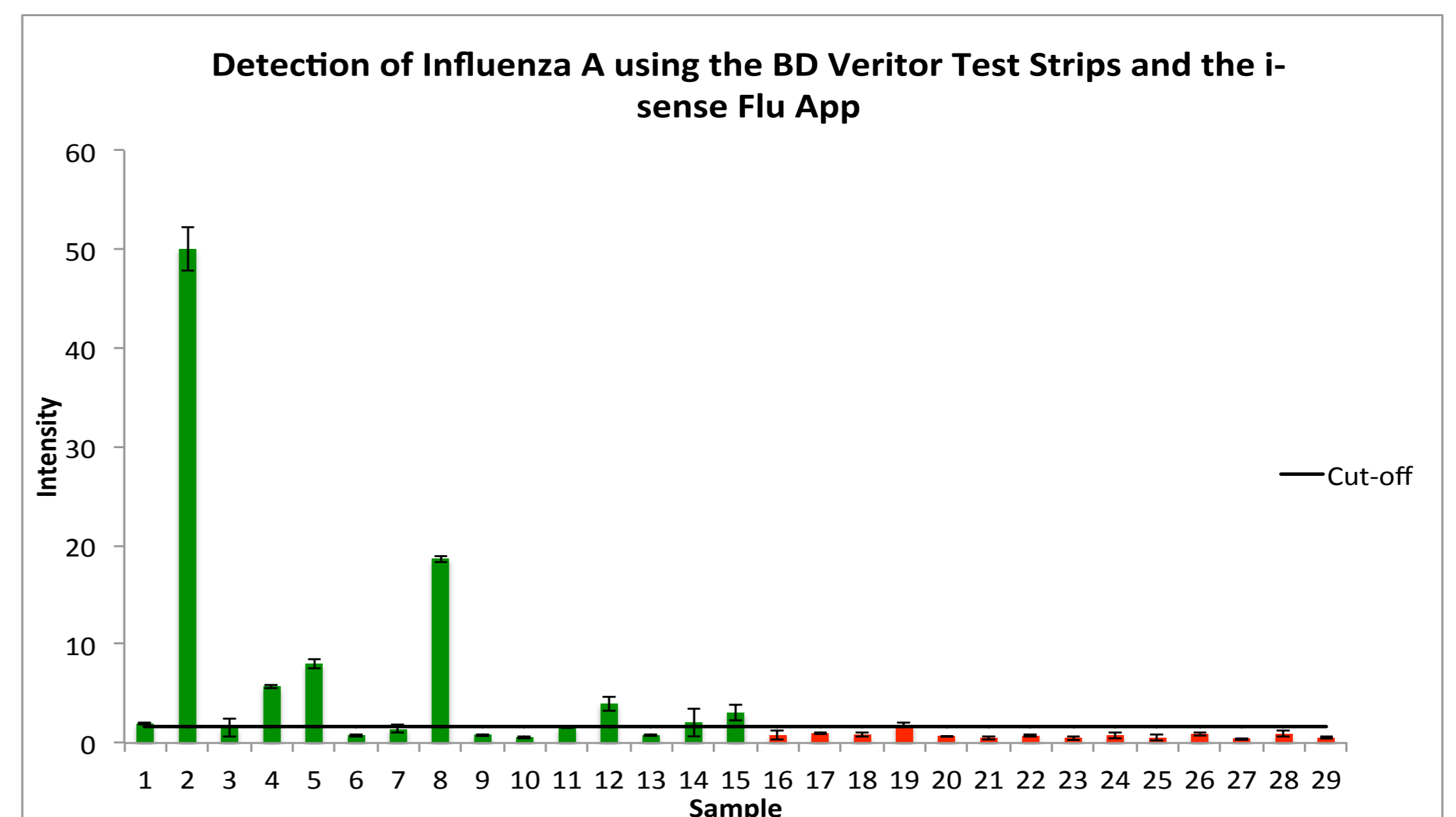


Figure 1: shows the signal intensity reading of the test line on the Veritor Flu A+B test strips using the i-sense Flu App with additional linear regression for background subtraction using Mathematica. The bars in green signify positive samples and the red bars denote negative samples. The error bars represent the standard deviation of each test strip captured by the i-sense Flu App three times. A cutoff 1.6 was calculated using the means of the negative + 2 times the standard deviation.

|                       | 1    | 2    | 3      | 4      | 5      | 6     | 7      | 8      | 9      | 10   | 11    | 12   | 13    | 14     | 15    | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|-----------------------|------|------|--------|--------|--------|-------|--------|--------|--------|------|-------|------|-------|--------|-------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Veritor Result        | -    | +    | +      | +      | +      | -     | +      | +      | -      | -    | +     | +    | -     | +      | +     | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| App Result            | +    | +    | +      | +      | +      | -     | +      | +      | -      | -    | +     | +    | -     | +      | +     | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| PCR Result            | +    | +    | +      | +      | +      | +     | +      | +      | +      | +    | +     | +    | +     | +      | +     | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| PCR Result (C. value) | (33) | (23) | (30.3) | (28.4) | (20.2) | (n/a) | (27.1) | (27.2) | (25.1) | (29) | (n/a) | (33) | (n/a) | (26.3) | (n/a) | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |

Table 1: Shows interpretation of the Veritor reader and i-sense Flu App compared to the PCR results using a cut off of 1.6

### BD Veritor system compared to rt-PCR

| BD Veritor Reader | RT-PCR   |          |
|-------------------|----------|----------|
|                   | Positive | Negative |
| Positive          | 10       | 1        |
| Negative          | 5        | 13       |

Table 2: 2x2 table comparing the results of reading the BD Veritor Flu A+B test strips using the BD reader against rt-PCR

### Veritor strips with i-sense App compared to rt-PCR

| i-sense Flu-App | RT-PCR   |          |
|-----------------|----------|----------|
|                 | Positive | Negative |
| Positive        | 8        | 1        |
| Negative        | 7        | 13       |

Table 3: 2x2 table comparing the results of reading the BD Veritor Flu A+B test strips using the i-sense Flu App against rt-PCR

### Concordance between Veritor and i-sense App

| i-sense Flu-App | BD Veritor Reader |          |
|-----------------|-------------------|----------|
|                 | Positive          | Negative |
| Positive        | 8                 | 1        |
| Negative        | 3                 | 17       |

Table 4: 2x2 table comparing the results of reading the BD Veritor Flu A+B test strips using the BD reader against the i-sense Flu App

- Initial data has highlighted good concordance in results between the Veritor reader and the i-sense Flu App (86.2%)

## Conclusions

- The high number of false negative results indicates the need to improve the sensitivity of the BD Veritor Flu A+B test (Table 2 and 3).
- Good concordance in results between the Veritor reader and the i-sense Flu App (86.2%) (Table 4) was observed.
- With further work to improve concordance and additional evaluation with a larger sample set, the downloadable i-sense Flu App could potentially be an affordable alternative to the Veritor reader for reading BD Veritor test strips.
- A smartphone connected flu test will widen access to objective rapid influenza testing and enable results to be fed into surveillance systems with geo-location information which can be used for public health purposes.

## References

- WHO. Influenza (Seasonal) [Internet]. Fact sheet N°211. 2014. Available From: <http://www.who.int/mediacentre/factsheets/fs211/en/>
  - Image source: [https://commons.wikimedia.org/wiki/File:PD\\_Diagram\\_of\\_swine\\_flu\\_symptoms\\_EN.svg](https://commons.wikimedia.org/wiki/File:PD_Diagram_of_swine_flu_symptoms_EN.svg)
  - FDA. Executive Summary Proposed Reclassification of the Rapid Influenza Detection Tests CDRH Microbiology Devices Advisory Committee Meeting Gaithersburg, Maryland. 2013
  - Hayward AC, Fragaszy EB, Bermingham A, Wang L, Copas A, Edmunds WJ, et al. Comparative community burden and severity of seasonal and pandemic influenza: Results of the Flu Watch cohort study. (2014) Lancet Respir Med. 2 (6):445–54.
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