Team Literature Review:

RADx-rad: Digital Antigen Test for COVID-19

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Background

Identifying what is happening in someone's body is obviously important. When one is feeling sick knowing if they have a particular virus or infection can help a person in knowing how they should help themselves to get better. If this test can be done at home instead of at the doctor, all the better and more convenient for the sick patient. Thus, the topic of immunoassays and point of care tests has been an area heavily researched in order to develop tests that patients can purchase and take at home to get rapid, affordable, and convenient results. Literature compares different types of testing kits advantages and disadvantages. For instance, viral culture tests that are more accurate, lack the affordability and speed of multiplex assays. Furthermore, in the wake of the pandemic, the push for improving the technology of current take home tests was bolstered. All of a sudden the need for fast results that were affordable, accurate, and could be used at home (due to lockdowns) outweighed all and COVID tests were flying off the shelves of the local CVS. Worldwide, government initiatives funded new technologies to make testing for COVID easier. For instance, smartphone compatible tests, like the ones administered at the UCSD vending machines, that showed patients their results in an easily understandable format were created. Today, though the pandemic has lessened in severity, new issues with current at home tests have been identified. When one today gets sick with cold-like symptoms it is hard for one to tell: is it the flu? Covid? A sinus infection? Or simply the common cold? Therefore the need for creating a multiplex test which could test a patient for multiple viruses, bacteria, or infections at once has become paramount. In the quest to create the multiplex test it is also important to ensure that the test is easy to use at home, accurate, and affordable.

Lateral Flow Immunoassay

A lateral flow immunoassay (LFIA) is a simple diagnostic test employed for detecting the presence or absence of a target within a sample. This platform works as a sample is placed on one end of a strip which is allowed to migrate along the complex through capillary action. In a general lateral flow assay there is a sample pad acting as a filter allowing it to flow efficiently. The sample will then flow to the conjugate pad

filled with nanobodies or antibodies specific to the target antigen where it will bind if the antigen is present, creating an antigen-antibody complex. The particles continue to move along the strip where the antigen-antibody complex will bind to another set of antibodies on the test line specific to the complex. This detection portion can develop a signal from these immobilized molecules that can be seen through a colored dot or line, indicating a positive qualitative result [12]. Tests come with a control line and an absorbent pad to maintain accuracy.

These assays, which became available in the 1980s, are used for a wide range of diagnoses including HIV, AIDS, and Covid-19. These lateral flow assays are commonly used and have become a reliable tool in everyday diagnostics as they are found to be one-step, fast, accurate, and affordable. For example, conventional laboratory-based analytical methods such as mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA) and real time polymerase chain reaction (qPCR) require a long procedure which takes hours to get results. Expected results from an ELISA takes up to twenty four hours minimum, when a LFIA can obtain answers from five to ten minutes [8]. Thus, the quickness in results allows for immediate action and response to the problem in issue, making it the most common method to detect SARS-CoV-2 or Covid-19 during the recent pandemic. In a study conducted by WHO, it was found that approximately 70% of LFIAs were used for infectious diseases over a ten year period up until 2020 [12]. This trend shows the increase of the global LFA market, which places a need for advancements in accuracy of the results as these tests come with some limitations. Limitations of LFIA include the sensitivity to their specific antigen dependent on their antibodies as some may not detect low levels of the target. Furthermore, LFIAs are qualitative in which they may not provide precise measurements of the antigen concentration. A study conducted by ACS Omega compared results of an LFIA and an ELISA to detect SARS-CoV-2 in their sensitivity. The LFIA results were generally consistent with ELISA, but some false positives and false negatives were observed. The sensitivity of LFIA for detecting SARS-CoV-2-N protein-specific IgM antibodies was 93% for IgM and 97% for IgG, while the test specificity was 97% for IgM and 100% for IgG antibodies. They also tested standard LFIA strips developed without the methods proposed in the study, and these had lower sensitivity and specificity, with 80% for IgM and 83.3% for IgG antibodies sensitivity and 86.6% for IgM and 90% for IgG antibodies specificity. In summary, the study showed that the LFIA test with the proposed methods had enhanced sensitivity and specificity compared to standard LFIA strips, making it a promising tool for detecting SARS-CoV-2 antibodies although with some imperfections [3]. Detection and accuracy can be enhanced by improvements in its choice of label that can affect the overall performance as different types of visual particles and their effectiveness, particularly those utilizing europium labels for precise measurement and heightened analytical sensitivity, have been increasingly adopted and have been

previously discussed [4]. Overall, a lateral flow immunoassay is a simple tool commonly used to diagnose diseases such as Covid-19, with limited restrictions and ease of use that can be further developed to be even more reliable and useful.

Point of Care Device

Point-of-care (POC) testing refers to conducting diagnostic assessments away from a traditional laboratory setting, yielding fast and dependable results. This approach enables disease diagnosis directly at the patient's location. These tests for detecting antigens employ various methods, including chromatographic digital, microfluidic immunofluorescence, and lateral flow immunoassays, and they can be conducted either at home or in a laboratory. Each testing kit is evaluated for both sensitivity (its ability to detect COVID-19 infection) and specificity (its ability to rule out other infections in the body) [9]. Some tests measure the total antibody count, while others specifically target IgG and IgM antibodies. The majority of these testing kits utilize techniques such as ELISA, Digital Lateral Flow, photometric immunoassay, lateral flow, or enzyme-linked fluorescent assay (ELFA). Point-of-care tests (POCTs) and rapid diagnostic tests (RDTs) primarily depend on the detection of antigens or antibodies and can be carried out in a matter of minutes without the need for a laboratory.

For example, the Abbott's BinaxNOW COVID-19 Test is a home-use lateral flow immunoassay designed for detecting the nucleocapsid protein antigen of COVID-19 [9]. It involves an immunochromatographic membrane containing specific SARS-CoV-2 antibodies and a control antibody on a test strip. To perform the test, an individual collects a nasal swab from both nostrils and adds six drops of reagent to the top well. After gently rotating the swab, it is brought into contact with the test strip. Once the card is sealed, a QR code is scanned, and the results are displayed on the card. The test results, which include pink/purple-colored positive lines, become visible within 15 minutes. This test can be delivered to a home address and, with the guidance of a trained telehealth professional via a video call, individuals can self-administer the BinaxNOW test and view the results on the NAVICA app.

Similarly, the Quidel's QuickVue At-Home Over the Counter (OTC) COVID-19 Test also tests using a lateral flow immunoassay. QuickVue's positive results show an 83% similarity with PCR findings and a 99% similarity for negative results, based on several systematic reviews [9]. Quidel recommends re-testing within 24-36 hours for confirmatory results. It's important to note that QuickVue cannot distinguish between SARS-CoV-2 and other coronaviruses. The issue of determining which variant the patient contracted is what we are trying to target in our project through our multiplexing testing.

Both of these POC tests demonstrate different elements that we look forward to including in our own test that will be more all-inclusive with the smartphone compatibility as well as the multiplexing component.

Relevant Patents

To use most of these at-home tests, a patient's swab sample is added to a reagent tube and rolled three times within the reagent solution. Afterward, a test strip is placed in the reagent tube, and results can be interpreted within 10 minutes using a color change key, similar to a home pregnancy test.

To develop our device, we will be using a pregnancy test as the foundational model and basis for how we build the hardware for the POC COVID test. For example, a relevant patent we found was the "Pregnancy Test Device and Method" that was published in 2015 by SPD Swiss Precision Diagnostics explains how a lateral flow immunoassay strip test is used to detect pregnancy [11]. With these similar methods, we can detect COVID.

Multiplex Test

Multiplex assays are an exciting area of research interest. These tests could let a patient know if they are positive or negative for multiple viruses at once. This test would be particularly useful when it comes to helping patients identify if they have flu, RSV, or the Coronavirus. In 2020, the CDC even authorized emergency authorization of the progression of research into biodetection devices and research. There was a push to make a multiplex assay as well [7]. Even out of the pandemic, the push for the best multiplex assay continues. A recent comparison study was published in April 2023. The study compared current multiplex assays using their usability and accuracy as key comparison points. Three multiplex assays were compared in the study: Taqpath, XpertXpress, and PowerCheck [5] . The study found that all assays were able to detect influenza and COVID. The PowerCheck is arguably the best of the three multiplex assays due to its low price, low training needed to use, and the fact that it can work with any PCR instrument. In the search to find the most effective multiplex assay it is also important to be weary of the potential issues using multiplex assays. One key disadvantage of using multiplexed PCR devices is that using the assay runs the risk for DNA contamination [6]. If DNA is contaminated in collection the test results may be inaccurate. Another pitfall in using PCR multiplex assays actually occurs to one of their advantages. The advantage being that these assays are very sensitive. The downside though of this sensitivity is that the multiplexed assays may "lead to detection of non-relevant co-infections" [6]. While these concerns with the abilities of multiplexed assays are valid, the multiplex assay should still be pursued as a useful testing agent due to its potential and shown success of newly developed multiplexes. For example, the FilmArray® system is a new nested multiplex PCR technology showing promising results [10]. In clinical investigations the new assay was tested against reliable viral culture. After processing 61 samples from ill patients with upper respiratory infection systems, of the amount of viral pathogens that the viral culture yielded, the multiplexed assay was shown to be able to detect 94.5% of them with a much faster turn-around time [10]. Multiplex assays are clearly an exciting and helpful tool that should be explored further.

For patients who are wondering if they have the flu, covid, or something else the multiplex assay would ease a lot of questions.

Smartphone Compatibility

Since the recent pandemic of *SARS-CoV-2*, the use of compact, portable PCRs have become popularized and commonplace due to its portability and convenience. The reason for this is that dPCR can absolutely quantize DNA/RNA without the tedious standard quantification process of quantitative PCRs (qPCRs), and, additionally, dPCR enables multiplex PCR to increase the quantity of DNA samples with undesirable ratios of abundant and uncommon DNAs [13]. In particular, there has been an increased number of studies and implementations of digital PCRs (dPCRs) paired with smartphone-based mobile devices.

In a recent study published in the journal *Biosensors and Bioelectronics*, a smartphone-based mobile dPCR device has been developed to enable accurate DNA quantitative analysis in laboratories with limited infrastructures. The device integrates thermal cycling control, on-chip dPCR, data acquisition, and result analysis using customized Android software [14]. Results showed that this device could accurately quantify copies of human RNA and detect a single cancer biomarker gene within the biosample, making it a low-cost, portable, and robust tool for DNA quantitative analysis.

In another study, scientists developed an ultrasensitive saliva based COVID-19 test using CRISPR- Cas12a activity to amplify the viral amplicon signal which is stimulated by a laser diode in a smartphone based fluorescence microscopy device. This device quantifies viral load over a wide linear range (1-105 copies/ µl) and has a detection limit of 0.38 copies/ µl below that of the reference real time PCR (RT-PCR) assay [15]. The researchers found that CRISPR-read *SARS-CoV-2* RNA levels were comparable in patient saliva swabs and nasal swabs. Thus, RT-PCR viral loads measured by smartphone-read CRISPR assay showed good correlation.

With recent advances in the integration of smartphone-based devices with PCR technology for POC analysis, its modifications can be used in innovative biomedicine areas.

Conclusion

In the article, “COVID-19 Point-of-Care Diagnostics: Present and Future”, molecular diagnostics tests like reverse transcription-polymerase chain reaction (RT-PCR) are considered the “gold standard” for SARS-CoV-2 virus detection due to their high sensitivity and reliability (Valera). However, they still take longer than ideal. RT-PCR tests typically require a minimum of 60-90 minutes, not including data analysis and reporting time. In contrast, antigen tests deliver results in as little as 5 minutes but are approximately 100 times less sensitive [16]. Therefore, a primary objective for point-of-care (POC) devices is to develop a molecular amplification-based test that can provide results in 5 minutes, similar to antigen tests, while maintaining high sensitivity. This would offer a faster

alternative to antigen tests with improved accuracy. COVID-19 will establish itself as a persistently occurring disease. Consequently, there will be a demand for multiplexed point-of-care (POC) devices capable of both testing and distinguishing between various coronavirus variants and other common respiratory infections like influenza. To ensure their effectiveness, these devices should undergo validation testing with consideration for all existing variants, as well as potential future ones, as advised by the FDA.

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