Using Lyophilization To Make Reagent Pellets For Rapid Covid 19 Testing.

Kayla Karl, BASIS Primary School, Ryan Flores, Michael Hansen, Jennifer Blain Christen, SenSIP Center, School of ECEE ASU

***Abstract*—With the widespread infection of Covid 19, development of rapid Covid 19 tests is essential. Lyophilization is a drying process that allows for long term stability. Many factors come into play when trying to successfully Lyophilized reagents including the ingredients of the reagent. If the proteins of the reagent are broken down in the process, the reagent is no longer effective in detecting Covid 19 in a patient. Once lypolization is successful you must look at how tests can be distributed. While determining the best distribution method, a Point of care testing device is required.**

Keywords: Lyophilization, Covid 19, Reagent, CRISPER, Trehalose, Xylitol

Project Description

COVID-19 has spread across the globe and infected millions of humans. Developing countries lack the technology to detect and treat COVID-19. To prevent the spread of disease, there is a critical need for quick, sensitive, reliable, point-of-care and economical methods for the laboratory diagnosis. [1] In this study, we are looking at reagent mixtures to lyophilize and create a point (PoC) of care device.

Figure 1:

1. Reagent

Effective reagents are critical for the detection of Covid-19 (figure 1). Reagents contain biological components which must be preserved during lyophilization. Reagents include enzymes, primers, nucleotides, probes and 4 biomarkers. At high temperatures, the enzymes in the reagent will break down and become ineffective.

A reagent is a chemical substance that is utilized to facilitate a chemical reaction. Reagents can be composed of many substances. We will focus on the enzymes in the reagent that are vital in detecting the RNA strands found in Covid-19. The multitude of mechanisms involving enzymes that are utilized to catalyze chemical processes is extensive. We will look at some of the common processes that can be manipulated to generate signals in order to detect the presences of diseases such as Covid-19. Research on the best lyophilization reagent is continuous (figure 2)

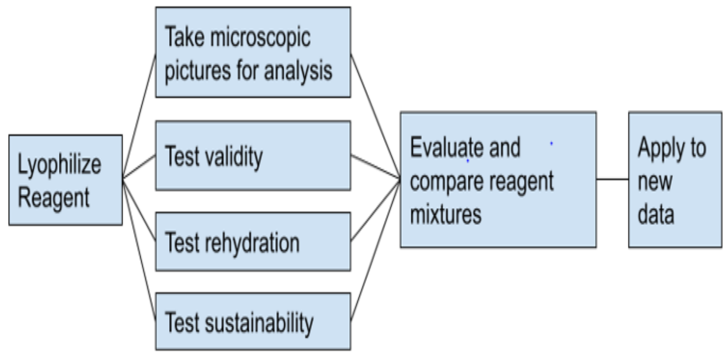

Figure 2:

1.1. CRISPR-Cas Enzymes

Clustered regularly interspaced short palindromic repeats are also known as CRISPR. CRISPR is a family of a DNA sequence that was discovered in the genomes of prokaryotic organisms. Multiple Cas proteins are used to detect prokaryotic organisms. Cas9 is RNA guided to detect and cleave dsDNA. Cas 12 and 14 cleave ssDNA and is followed by collateral trans-cleavage of ssDNA molecular beacon probes to generate fluorescence. Cas 13 ssRNA and is also followed by collateral trans-cleavage of ssRNA probes to generate fluorescence.

1.2. Redox Enzymes

Redox enzymes are often used due to their expedited results and easily detectable signal. The signal is initiated from the oxidation or reduction of substrates. The most common oxidase enzyme is glucose oxidase. In the process of oxidizing glucose, hydrogen peroxide is produced. Electrochemiluminescence is used to detect redox reactions.

1.3. Peroxidase

Peroxidase enzymes catalyze the splitting of peroxidases into either water or alcohol in the presence of an electron donor. The most popular and commonly employed peroxidase is horseradish peroxidase, a metalloenzyme that derives its redox capacity from an iron-containing heme group.

2. Point of Care

Point of care testing (PoCT) is essential in underdeveloped countries where technology, medical professions, and financial stability are scarce. PoCTtechnologies can be separated into two categories. The first is a large table-top device that is used for analysis. While the devices are still large, they have been reduced in size and complexity. These include critical care analysers and, more recently, small haematology and immunology analysers [2]. The second category that was the focus of the study are small smaller handheld devices that provide qualitative and/or quantitative data.

2.1. Point of Care testing device

Point of care testing devices (figure-3) must follow guidelines set by the World Health Organization (WHO). ASSURED guidelines were developed to ensure that the technology can address the needs of the user in a clinically and cost effective manner and avoid the introduction of possibly expensive devices which fail to deliver the required outcomes. [2] ASSURED is an acronym meaning affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users. The device must be affordable to those at risk of becoming infected. [3]

Fig. 2: Schematic showing different technical components that could combine to form the ideal diagnostic test.

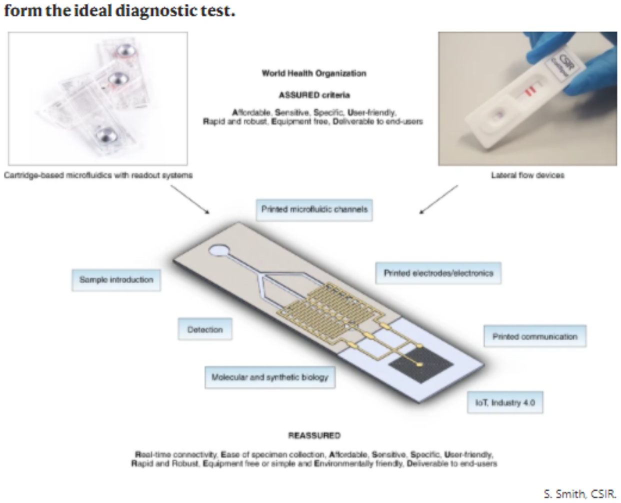

Figure 3: [3]

2.2. Affordable

In underdeveloped countries, financial resources are limited. Affordable PoCT devices are essential for early detection of infection. Economies in underdeveloped countries are lacking the resources to budget a reasonable percentage of gross domestic production that is allocated to health care. To determine affordability, each health programme performs an economic analysis based on the cost data for specific countries. Cost data includes staff, implementation, patient cost, etc. The more cost-effective and affordable, the greater amount of accessibility for possible infectious patients to become diagnosed.

.

2.3. Sensitive

The test should be sensitive enough to prevent a false negative. A false negative will prevent patients from getting the care they need and will also help to spread the infectious agent. To help with sensitivity, we plan on adding a control step to allow confirmation that the test was administered properly and valad.

2.4. Specific

The test should be specific and avoid a false positive. When a false positive occurs, this could result in a shortage of medicine. False positives could double the cases and medicine needed to deter the spread of the infectious agent. The medicine administered could also harm the patient by killing good bacteria in the stomach. The financial impact for medicating uninfected patients could affect the economy negatively.

2.5. User-friendly

The test should be easy to administer and diagnose. Tests should be easy to perform in 2–3 steps and require minimal user training with no prior knowledge of diagnostic testing. A patient should administer and understand the results with confidence and certainty. Patients' ability to diagnose will allow for early treatment, quarantine and preventative actions for close contacts.

2.6. Technology accessibility

New advancements in technology (figure 4) have made it possible to utilize smart phones for diagnosis in diseases. Smartphone technology is expanding exponentially throughout the world. A handheld digital microfluidic device with bluetooth capability has been used for detection of diseases. [4] While technology is scarce in underdeveloped countries, this could be an option for more advanced countries for early detection and treatment.

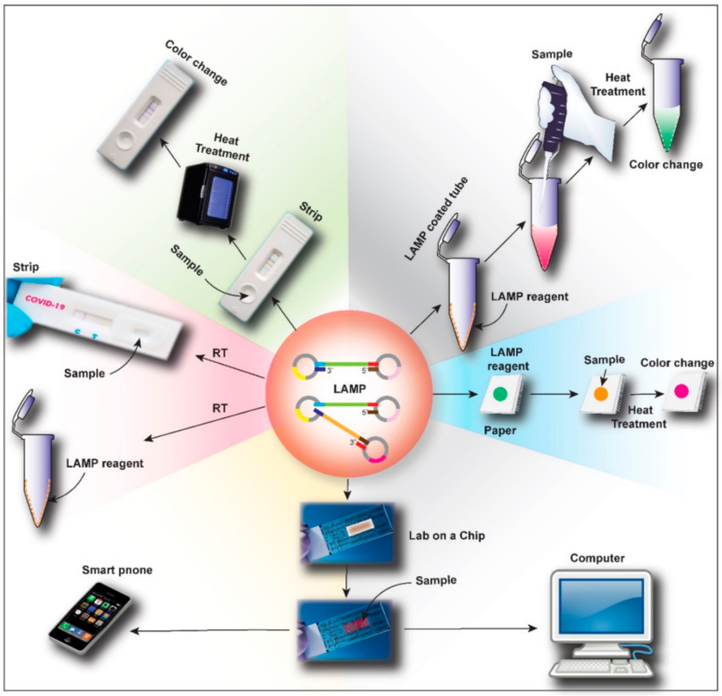

Figure 4: [8]

Schematic representation of the future possible advances in RT-LAMP based point-of-care test (PoCT) for novel coronavirus disease (COVID-19). LAMP reagents can be loaded in a paper-based system for easy use, which can be heated after sample loading to get quick results. Another advancement will be the coating of the LAMP reagent on the walls of a reaction tube where a simple mixing of the sample with the buffer and heating will give the results. Similarly, an RT-LAMP test-strip, like a conventional pregnancy detection kit, can also be developed. The researchers will also explore the possibility of developing an RT-LAMP master mix that can react at room temperature, which will fully transform the current concept of RT-LAMP based diagnosis. Most advanced development will be a lab on a chip (or LAMP on a chip) with advanced microfluidics, sensors, and computer integration, which can also be connected with an electronic device like computer or mobile phone to analyze the results. [8]

Acknowledgements: This research is sponsored in part by NSF RET Award number 1953745

References

[1] Parsons LM, Somoskovi A, Gutierrez C, et aal. Laboratory Diagnosis of Tuberculosis in Resource-Poor Counties: Challenges and Opportunities. Clinical Microbiology Reviews. 2011;24(2):314-350.doi:10.1128/CMR.00059-10

[2] St John A, Price CP. Existing and Emerging Technologies for Point-of-Care Testing. Clin Biochem Rev. 2014 Aug;35(3):155-67. PMID: 25336761; PMCID: PMC4204237.

[3] Land, K.J., Boeras, D.I., Chen, XS. et al. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol 4, 46–54 (2019). https://doi.org/10.1038/s41564-018-0295-3

[4] Infectious and Tropical Diseases Research Group (e-INTRO), Biomedical Research Institute

of Salamanca-Research Centre for Tropical Diseases at the University of Salamanca (IBSAL-CIETUS),

Faculty of Pharmacy, University of Salamanca, 37007 Salamanca, Spain; juanbernalt95@usal.es (J.G.-B.D.);

ama@usal.es (A.M.)

[5]

[6] Kumar, K. N., Mallik, S., & Sarkar, K. (2017). Role of freeze-drying in the presence of mannitol on the echogenicity of echogenic liposomes. The Journal of the Acoustical Society of America, 142(6), 3670–3676. https://doi.org/10.1121/1.5017607

[7] Valera, E., Jankelow, A., Lim, J., Kindratenko, V., Ganguli, A., White, K., Kumar, J., & Bashir, R. (2021). COVID-19 Point-of-Care Diagnostics: Present and Future. ACS Nano, 15(5), 7899–7906. https://doi.org/10.1021/acsnano.1c02981

[8] Augustine, R., Hasan, A., Das, S., Ahmed, R., Mori, Y., Notomi, T., Kevadiya, B. D., & Thakor, A. S. (2020). Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic. Biology (Basel, Switzerland), 9(8), 182–. https://doi.org/10.3390/biology9080182

[9]

[10]

[11]

[12]