Most Effective Testing Method for COVID-19- PCR Testing and How it Works

Sera Brady

Faculty Mentor: Dr. Cubon, Department of Chemistry, Kent State University Trumbull Campus, Warren, OH

Abstract

There are several different testing methods for COVID-19 including antibody testing, antigen testing, and molecular testing. This poster focuses on polymerase chain reaction (PCR) testing. PCR has been used in many studies, due to it being nearly impossible to study segments of deoxyribonucleic acid (DNA) without it. DNA is a molecule that is found in all living organisms that contains genetic coding. Real-time reverse transcription polymerase chain reaction (RT-PCR) is a type of PCR testing used to detect COVID-19. RT-PCR takes ribonucleic acid (RNA) and creates a complimentary DNA strand. The background, important chemicals, production of tests, administration, effects on health, and statistics on PCR and RT-PCR testing will all be discussed within this poster.

Background

- COVID-19 is a virus that affects the respiratory system, which can be fatal where underlying conditions are apparent.[1]
- Three possible testing methods for COVID-19:
  - Antibody Testing
    - Determines if a person has previously had COVID-19 [2]
  - Antigen Testing
    - Determines if a person currently has COVID-19 [2]
    - Has a high false negative rate [2]
  - Molecular (PCR) Testing
    - Determines if a person currently has COVID-19[2]
    - Food and Drug Administration deemed this to be the most reliable test [2]
- PCR was first discovered in 1982 by Kary Mullis to study sickle cell anemia. It can also be used for DNA fingerprinting, diagnosing genetic disorders and detecting bacteria/viruses.[3]

Chemical Components

- Reverse transcriptase- enzyme that converts DNA to RNA.[4]
- Polymerase- enzyme that copies DNA.[4]
- Template RNA- ribonucleic acid sample taken from patient via throat or nasal swab.[5]
- Primers- determines region of DNA to be copied.[5]
- Buffer- a stabilized environment for tests.[5]
- Nucleotide- creates copies of DNA strands.[5]
- Taq polymerase- builds new strands of original DNA.[5]
- Figure 1.1 contrasts DNA and RNA

Chemical Components

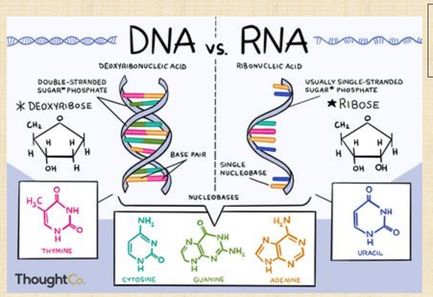

Figure 1.1: Shows how DNA and RNA differentiate through the different nucleotides[6]

- DNA: a self-replicating material that is present in nearly all living organisms as the main constituent of chromosomes.
- RNA: also present in all living cells, acts as a messenger

Production of Tests

- Tests are produced by:
  FDA and Center for Disease Control and Prevention[2]
- At-home tests contain:
  sterile swabs and sterile containers in an insulated package to keep the virus alive until it reaches a lab.[2]
- Average costs:
  For hospitals, $100-150 per test[7]
  For patients, free at any health center and covered by most insurances for at-home testing[2]

Administration

- How Tests are Ordered:
  Health professional must order PCR COVID-19 tests.[2]
- RNA Samples taken from:
  Nasopharyngeal, oropharyngeal, saliva.[8]
- Proper temperature for the sample to be contained in is -70°C.[9]
- Licensed pharmacists analyze the PCR tests. It can take a couple of minutes to get a result at testing sites, but due to delays and the amount of people getting tested, it takes around two to four days to receive results.
- There are no known complications to taking the test.
- Figure 2.1[10] shows how nasal sample is taken and figure 2.2[11] shows how the throat sample is taken.

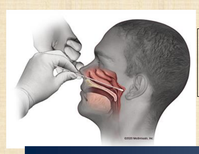

Figure 2.1: A long swab is inserted through the nostril take a sample from the nasopharynx

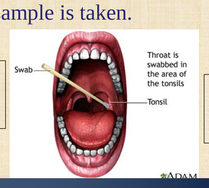

Figure 2.2: A swab is inserted through the mouth to take sample from the tonsils

How the Tests Work

- COVID-19 is an RNA virus, RNA must be extracted from patient and then converted into DNA.[9]
- Must use real-time reverse transcription polymerase chain reaction (RT-PCR) test.
- Sample of RNA is taken from area on patient that COVID-19 affects (nasal or throat swab).[12]
- Sample is reverse transcribed into DNA using reverse transcriptase, and nucleotides, primers and fluorescent dyes are then added to the DNA.[12]

How the Tests Work

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Process is shown in Fig 3.1 and described below.

A. Sample Collection:
   Sample of RNA is taken from area on patient that COVID-19 affects (nasal or throat swab).[15]
B. RNA Extraction:
   Chemicals like guanidine salts or phenol-based compounds are used to extract RNA without degradation occurring
C. Reverse Transcription:
   Sample is reverse transcribed into DNA using reverse transcriptase, and nucleotides, primers and fluorescent dyes are then added to the DNA.[15]
D. RT-PCR Amplification:
   Nucleotides build upon the DNA strands while primers build on the DNA for amplification and fluorescent dyes are used as markers.[5] DNA polymerase is used to determine the sections of the strand that needs to be amplified.
E. Analysis:
   i. Mixture is placed in a RT-PCR machine (buffer) which makes billions of copies of the specific region of DNA taken.[15]
   ii. Taq polymerase binds to copies of DNA strands and release fluorescent dyes. A computer tracks amount of fluorescence in the sample, once fluorescence reaches a certain level, confirms that the virus is present.[15]

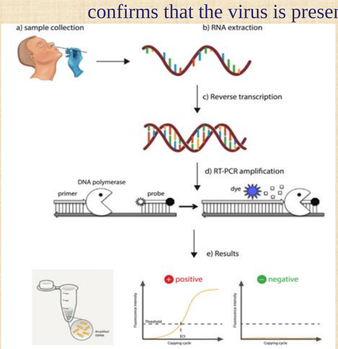

Figure 3.1: The image shows the RNA sample being collected. The small tube is the buffer, with the primer, nucleotides and fluorescent dye are put with the transcribed sample. Graph shows how results should look[13]

Statistics

- PCR testing is most accurate for COVID-19 testing, but there could be complications. Examples including if the sample was kept at a proper temperature, the timing of when the patient took the test, or trouble shipping the test.[17]
- The false negative rate depends on timing of how long the virus is present in the patient. If a patient goes to get tested and the virus has been active for less than five days, a study shows that the rate was at 100%. If the patient gets tested after having the active virus for more than five days, the rate drops to 20%.[17] With the antigen tests, 1 in 2 people are incorrectly told that they don't have the virus.
- False positive rate is close to 0 percent.[17]
- Due to rapid results of antigen tests, most people take them. However, they have a high false negative rate, so doctors will order a PCR test if result is negative.[2]
- Figure 4.1 shows a map of the percentage of COVID-19 tests that come back positive.
- Figure 4.2 shows the amount of COVID-19 tests that are taken daily.

Statistics

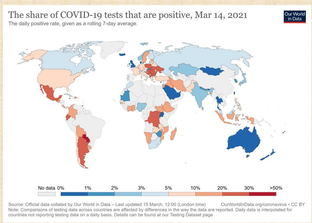

Figure 4.1: This figure shows the scale of testing relative to the size of the outbreak. According to Ritchie, any region below a five percent positive rate shows that the country has the virus under control.[18] The data included in the map is for all testing methods, not just RT-PCR testing.

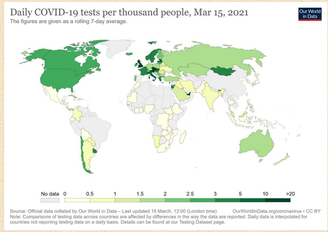

Figure 4.2: Each country reports the amount of cases differently. For example, some countries report number of people tested, while others report number of tests.[18] This shows the data for all testing methods and not just RT-PCR testing.

Conclusion

There are multiple ways to test for COVID-19, but PCR testing is the most reliable. PCR tests create multiple copies of DNA segments in order for researchers to study it. PCR can be used for many things, including identifying DNA fingerprints and diagnosing genetic diseases. RT PCR is used to identify if a person has COVID-19, due to it being a RNA virus. RT PCR takes the RNA template from a patient and transcribes it into complimentary DNA. RT PCR is the most reliable way for testing for COVID-19 shown through multiple studies of its low false negative rate (if timing is right) compared to other testing methods.

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