analogue porosity.

For the coke analogue to represent metallurgical coke, there should be similarities in the key characteristic properties such as porosity, carbon bonding, and general reactivity with CO_2 gas and so on of both materials. Studies using the coke analogue with added mineral matter, of a composition chosen to mimic industrial coke, showed similar dissolution behavior in liquid iron to that of industrial coke. Use of the coke analogue allows control of the porosity, mineralogy, mineral particle size and distribution and the general reactivity with CO_2. This similarity and the control of those properties make the coke analogue a useful laboratory tool in coke studies.[3]

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Student Corner

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RT PCR

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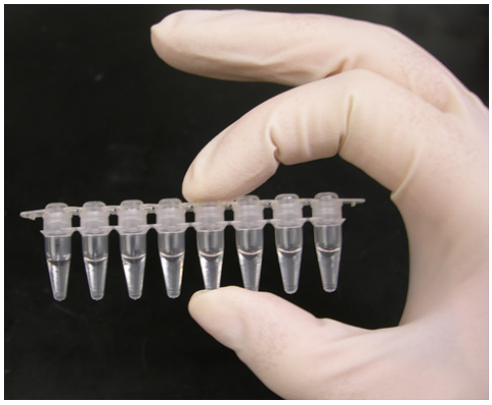
The whole world is engulfed with uncertainty, emptiness, lack of hope and pretty much one-third of the world is under some sort of lockdown due to the Corona Virus threat spreading across the world. While there are many preventive methods such as social distancing, hygienic practices, washing hands, using sanitizers, etc, a viable method of identifying the patients still at the top of the list. Identifying and isolation cut down the community spread of the disease significantly, so highly populated regions can be controlled and prevent the spreading of the virus. Unlike other diseases, COVID-19 seems to hide inside a person for quite a long time before showing symptoms, where he/she can spread across the community unknowingly.

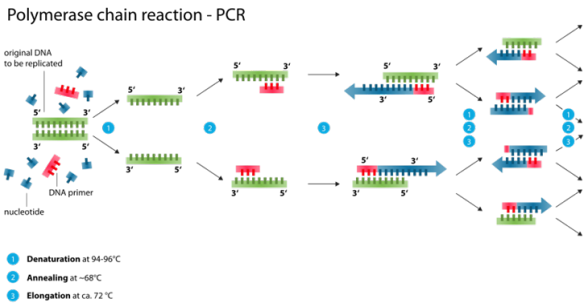
It all comes down to the availability of facilities to handle the pandemic situation. Developed countries have their own strategies in handling the situation, but one thing is common for all. Testing the suspected patients and infected using a PCR Test. Test, Test, Test!

PCR, as it stands for Polymerase Chain Reaction, which is self-explanatory among the chemical community. The goal of general PCR or Polymerase Chain Reaction is to amplify a target region of DNA using the DNA polymerase enzyme for analysis and further studies. In living organisms, DNA is replicated to increase the number of copies. Since the replication process takes place under biological conditions, many enzymes are involved. However, in PCR this replication is done in a test tube using DNA polymerase as the only enzyme and other ingredients. The majority of PCR methods depend on thermal cycling, which involves exposure of reactants to repeated cycles of heating and cooling to initiate a specific reaction at each temperature. The reaction mixture contains a DNA template, two primers, dNTPs (deoxy-nucleotide triphosphate), $MgCl_2$, DNA polymerase, and sterile water or buffer. In all PCR reactions the DNA polymerase that has been used is a heat-stabIe enzyme. In a general PCR cycle, there are three main steps 1. Denaturing 2. annealing of primers and 3. Extension.

Denaturation of DNA

The structure of DNA is a double-stranded helical form; therefore, the two strands have to be separated for amplification. This is done by heating the reaction mixture that contains the template DNA (original DNA) to about 98 ^{0}C. At this temperature, the two strands will be separated and this is called the melting of DNA strands. In traditional PCR there is an initial denaturation for about one minute and at each cycle, denaturation is done for about 30s.





Primer Annealing

All DNA polymerases can only add dNTPs (deoxynucleotide triphosphate) to an existing DNA strand. Therefore, in order for the DNA polymerase to act it needs a starting point called a primer, which is a short sequence of deoxy-nucleotides (about 20 bases in length). Since DNA is double-stranded each PCR mixture needs two deoxy-primers. The primers bind to the template DNA by complementary base pairing. Primer annealing takes place at a lower temperature than melting, usually around 68 ^{0}C for about 30s.

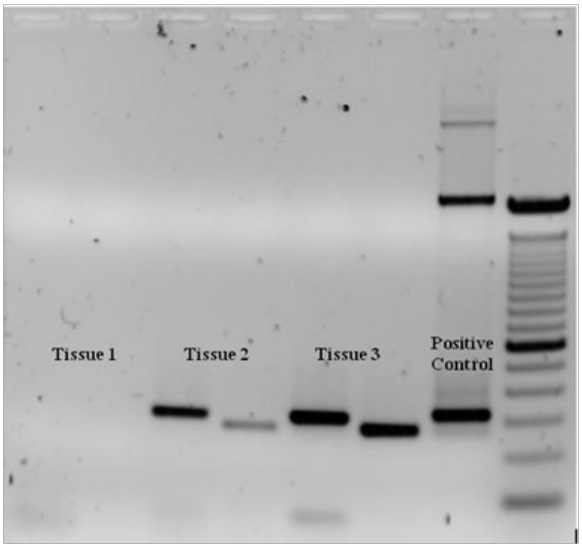
Extension/Elongation

In this step, dNTPs are added to the primer by DNA polymerase. The temperature at this step depends on the thermostable DNA polymerase used and the commonly used temperature is around 72 ^{0}C. In this step, DNA polymerase synthesizes a new DNA strand, which is complementary to the DNA template strand by adding free dNTPs from the reaction mixture. The precise time needed for elongation depends both on the DNA polymerase and length of the amplifying region of the target DNA. At their optimal temperature, most DNA polymerases can add thousands of bases per minute. At this step, the amount of DNA is doubled. With each successive cycle, the original DNA strand and all the newly generated strands become template strands for the next round. In most cases, about 35-40 cycles (denaturation, primer annealing and, extension) are carried out to obtain high enough concentration of DNA for further studies.

After this cycling routine, there is the final elongation step, where the reaction mixture is allowed to undergo the last extension step for about 5-15 min at the same extension temperature used in order to make sure that any remaining single-stranded DNA is fully elongated.

Finally, there is a cooling step (cools to around 4 ^{0}C) where the samples can be stored until they are used for further analysis.

The purity of the PCR products is checked using DNA agarose gel electrophoresis using ethidium bromide as the staining agent. A DNA ladder (a mixture of DNA fragments with different sizes) is used to determine the size of the PCR products. Picture of an agarose gel containing ethidium bromide- stained PCR products is shown below.



Ethidium bromide-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs.

Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

There are several advantages of doing PCR. It is fairly simple, easy to use, and understand, produce rapid results and the technique is very sensitive for producing billions of copies of DNA fragments.

This technique can be used in high sensitivity even when only RNA is present in the sample. However, in such cases, RNA code has to be first converted to its complementary DNA (cDNA) molecule and this process is called reverse transcriptase reaction. This is followed by a normal PCR procedure where billions of copies of cDNA can be obtained for further analysis. This process is called Reverse transcriptase PCR or RT-PCR. There are two ways for quantification of cDNA present, which are endpoint analysis or real-time analysis. Endpoint analysis could take an average of 6-8 hours, whereas real-time RT-PCR could only take about three hours. Also in real-time RT-PCR, a specific fluorescence marker is used to observe the development of the desired cDNA. Therefore, real time RT-PCR technique is highly sensitive, specific, and fast. Compared to other available methods, the real-time RT-PCR has a lower potential for contamination or errors as the entire process can be done within a closed tube.

Thus, PCR tests can be done to identify possible infected patients for COVID-19, however, there's only a 70% success rate in PCR tests in the identification of Positive results.

The accuracy of a medical test is determined by measuring two things: sensitivity and specificity.

- A sensitive test will correctly identify people with the disease. Sensitivity measures correct positive results.

  If a test is 90% sensitive, it will correctly identify 90% of infected people – called a true positive. However, 10% of people who are infected and tested would get a false negative result – they have the virus, but the test indicates they don't.

- A specific test will accurately identify people without the disease. Specificity measures the correct negatives.

  If a test is 90% specific, it will correctly identify 90% of people who are not infected – registering a true negative. However, 10% of people who are not infected will test positive for the virus and receive a false positive.

Most experts believe that problems with sample collection are the main culprit behind inaccurate testing. False negative results are likely occurring because health care providers aren't collecting samples with enough of the virus for the tests to detect.

This can happen because the person who collects the sample doesn't insert a swab deep enough in the nose or doesn't collect enough of the sample. False negatives could occur if a person is tested too early or too late during their infection and there isn't a lot of virus in their cells. And finally, errors can happen if a sample sits too long before being tested, which allows the viral RNA to break down.

However, there are many fully automated PCR's equipped with cutting edge technology, where everything except sample collection can be completely automated. The above script is just a general description of most common practices in the PCR technique and individual requirements and specific testing procedures may vary.

Nonetheless, it's important to conduct PCR tests in this type of outbreak and a pandemic situation to control community spreading. Lack of trained personnel and price of Testing materials, chemicals are always challenging to an upper-middle-income country like us, but it's vital to invest in these not only for the current situation but for future possibilities as well.