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Review Article

PCR Technology and Its Importance In Covid-19 Pandemic

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ABSTRACT

Abstract:

Since the discovery of the PCR technology, its application in the various fields is increased gradually. Based on to this principle, many variations of the PCR have been established. Year by year, it is upgraded very much. It is established as a most common and accurate technique for the detection of the various diseases in the field of medicine. Now it is a 'Gold standard' for the detection of covid-19 also, which is much needed to contain the spread of the virus. Though various detection techniques are there for detection, but real time RT-PCR (variation of PCR) is most reliable. Viral detection is based on a simple principle of nucleic acid (viral) amplification. Various manufacturing companies are manufacturing the PCR instrument. Though the accuracy of the instruments are slightly differ to each other.

Keywords: COVID-19, RT-PCR, Viral Detection.

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INTRODUCTION

In the end of 20th century, one of most significant invention was the real time PCR method by Kary Mullis. It can be easily said that, this technique gives a revolution in the biological field. In the time of early 21st century, this technology used in various field like biotechnology, gene cloning, detection of various diseases, forensic field. Since November 2019, we are going through the pandemic called covid-19, which was originated from Wuhan city, China. SARS CoV-2 is the virus which is responsible for the disease.

In this time, to counter the pandemic situation, accurate and mass testing is very important. There are several laboratory testing methods are available to confirm infections of the individuals, these are real time RT-PCR, rapid antigen test, rapid antibody test, CT scan. From all methods, real time RT-PCR technique is considered as 'Gold Standard' in the diagnosis of the infection. Normally polymerase chain reaction is based on amplification of a desired gene or DNA fragments. But in case of real time RT-PCR, the template is an RNA, which is then amplified by reverse transcriptase enzyme.[1-2]

HISTORY OF PCR

- **1967-** Thomas Brock reports on the isolation of extremophilic bacterium *Thermusaquaticus.*
- **1971-** Kleppe and co-workers first describe a method using an enzymatic assay to replicate a short DNA template with primers in vitro.
- **1976-** Taq polymerase is isolated(a thermostable DNA polymerase named after the thermophilic bacterium *Thermusaquaticus*)
- **1977-** Fredrick Sanger and colleagues introduce the 'dideoxy' chain termination method for sequencing DNA(also known as 'Sanger sequencing'). It utilizes DNA polymerase, nucleotide precursors and oligonucleotide primer.
- **1983-** Working for Cetus Corporation, Kary Mullis discovers that using two oligonucleotides instead of one- on opposite strands- enables DNA to be

synthesized from a single, specific location in the genome. Technique for PCR was created.

- **1985-** PCR technique is described in an article published in science.
- **1988-**Patent for Taq DNA polymerase is filed by Mullis. The first automated PCR cycle is introduced to the market by Parkin, Elmer and Cetus(Joint venture)
- **1989-** Science magazine named Taq polymerase its first 'molecule of the year'.
- **1992-**Initial work by Higuchi and first demonstrated the simultaneousamplification and detection of specific DNA sequences in realtimeby simply adding ethidium bromide (EtBr) to the PCRreaction so that the accumulation of PCR product could bevisualised at each cycle. (Higuchi et al., 1992).When EtBr is bound to double stranded DNA and excited byUV light it fluoresces.
- **1993-**Kinetc PCR= Continuously measuring the increase in EtBrintensity during amplification with a charge-coupled devicecamera (Higuchi et al., 1993).
- **1993-** Kary Mullis received the Nobel prize. The first real time PCR instrument is described.

VARIATIONS OF PCR[3-5]

1. ***Conventional PCR:*** In vitro amplification of gene of interest or DNA fragments.

2. ***Real time PCR(qPCR):***Based on PCR, which used to amplify and simultaneously quantify a targetedDNA molecule. The products are analysed and visualized by gel electrophoresis.Various fluorescent dyes are used.

3. ***Reverse Transcription PCR(RT-PCR):***This method is based on reverse transcription, which reverse transcribes RNA into cDNA.It is of two types:

a) One-step RT-PCR=

All reaction components are mixed in one tube prior to initiation of the reaction.Although one-step RT-PCR offers simplicity and convenience and minimizes thepossibility for contamination, the resulting cDNA cannot be used for detecting multiplemessages from a single RNA sample as in two-step RT-PCR.

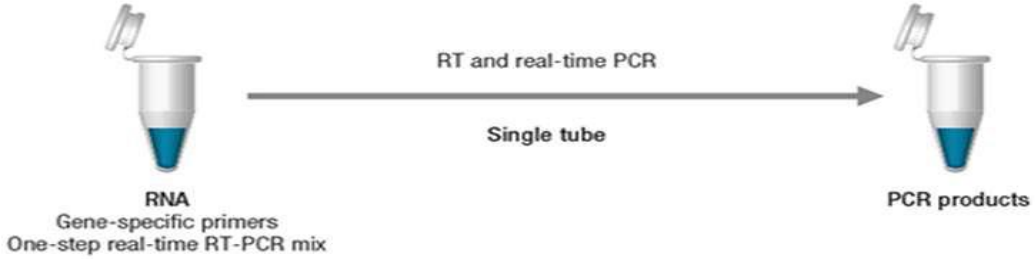

Figure 1: One-step RT-PCR

b) Two-step RT-PCR=

Traditionally, RT-PCR involves two steps: the RT reaction and PCR amplification whichcan be simple PCR or qPCR.RNA is first reverse transcribed into complementary DNA(cDNA) using an enzyme,reverse transcriptase.The resulting cDNA is used as templates for subsequent PCR amplification usingprimers specific for one or more genes.

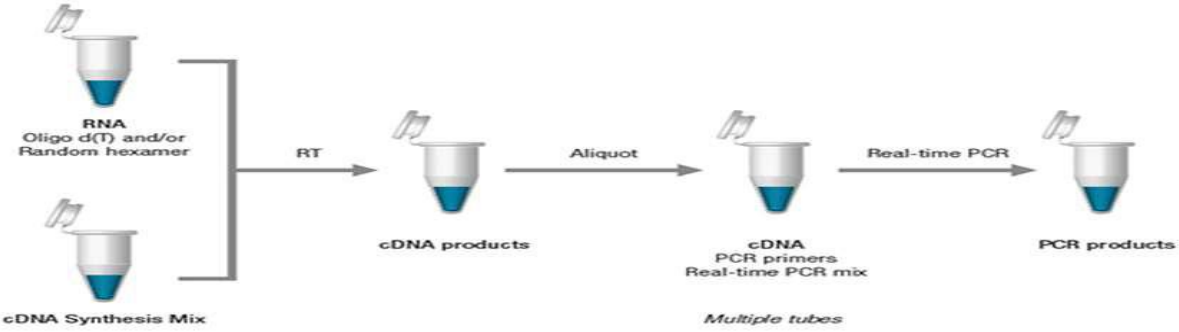

Figure 2: Two-step RT-PCR

1. ***Colony PCR:*** It is used for the screening of bacterial or yeast clones for correct ligation or plasmid products.
2. ***Nested PCR:*** Here two pairs (instead of one) are used to amplify a fragment DNA.
3. First step is to amplify a fragment similar to a standard PCR. Then second pair of primer (Nested primer)bind inside the first PCR fragment product fragment.
4. ***Host start PCR:*** This technique reduces non-specific amplification of during the initial step of PCR.
5. ***Asymmetric PCR:*** It is used to preferentially amplify onestrand of the original DNA more than

the other.PCR is carried out as usual, but with a great excess of one primer for the chosen strand.

6. ***AFLP PCR:*** This method is used for detecting polymorphisms inDNA. It is highly sensitive.
7. ***In Situ PCR:*** In Situ PCR (ISH) is a polymerase chain reaction that actuallytakes place inside the cell on a slide. In situ PCR amplificationcan be performed on fixed tissue or cells.
8. ***Long PCR:*** It is used for extended or longer than standard PCR, meaning over 5kilobases (frequently over 10 kb).Long PCR is useful only if it is accurate. Thus, special mixturesof proficient polymerases along with accurate polymerasessuch as Pfu are often mixed together.

INSTRUMENTATION

A modern PCR instrument composed of:

A. Thermal Cycler-

It is a computer controlled system, where actual amplification takes place. It has several parts:

- Reaction Module Bay-Holds the inserted reaction module.
- Air Vents- Allow the thermal cycler to cool.
- Status LED- Turns on to indicate that the block is running.
- LCD display- Displays the home and other screen.
- USB A port- Connects a USB key, computer mouse or other USB devices.
- Connecter Port- Connects to the host machine and reaction module.
- Ethernet Port- Connects the thermal cycler to a computer.

Ex:-LightCycler 480 II(LC480II, Roche)

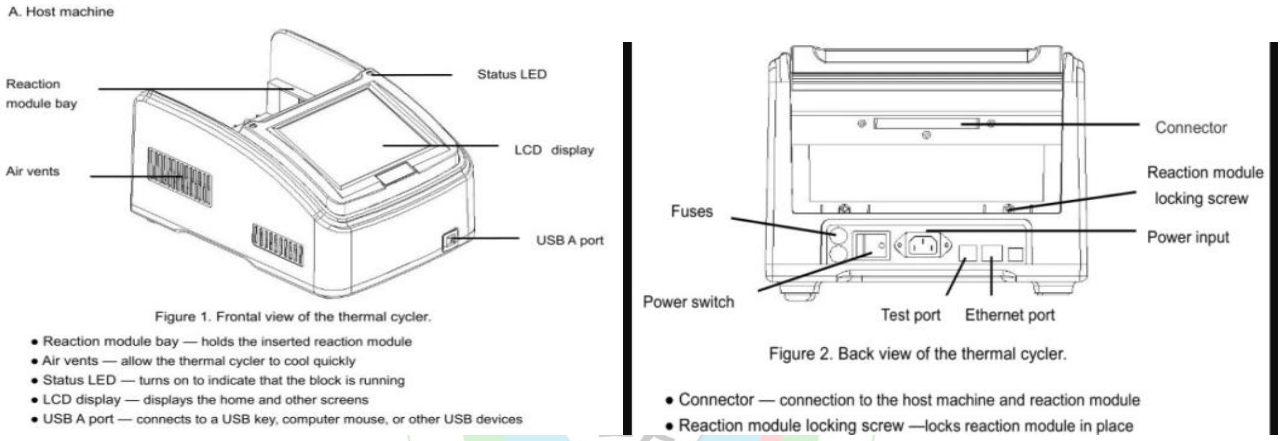

Figure 3: Front and Back view of a thermal cycler

B. Detectors-

Several detectors are available for different varieties of PCR. Some commonly used detectors are: Gel Electrophoresis, Southern blotting, Flourimetry etc.

In real time PCR technique, flourimeters are used to analyze and visualize the product. The components are:

- **Light Sources-**
- **Light emitting diodes:** There can be individual or multiple LEDs present in a shuttle mechanism, which is positioned above each well so that each one is independently illuminated, or these can be arranged in a stationary array that excites multiple array at a time.
- **Halogen Lamp:** Emits broad spectrum white light, which is then filtered to excite specific flourophore.
- **Laser:** It emits high intensity light, but of a narrow band width, confining its use to collecting data from asmall set of flourophores, limiting its use in multiplexing.

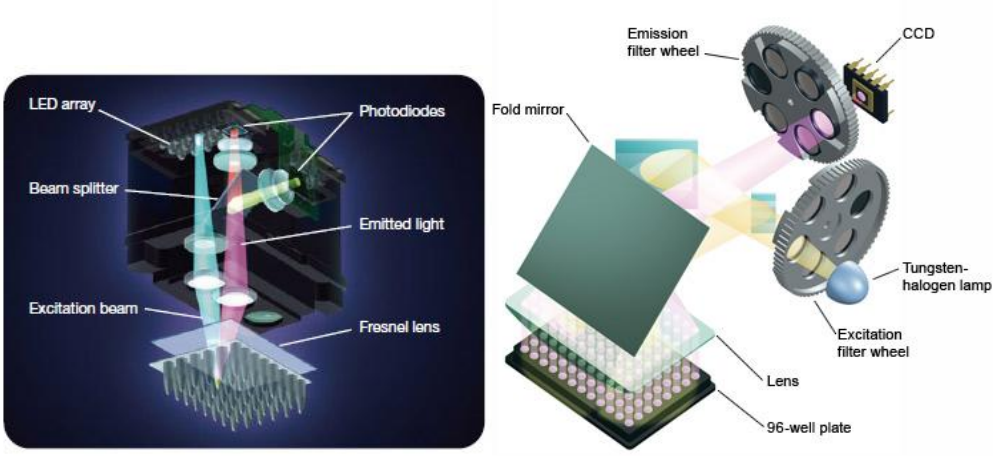

Figure 4: Light emitting diodes and Halogen lamp containing detection system

- **Detection of emitted radiation-**
  As it is a flourimeter, the emitted radiation measured at 90° angle (Pathlength is 10 mm or 1 cm).
- **Photo voltaic cell:**A type of photodetector that, when exposed to light, causes current to flow. It is composed of two electrodes- thin metallic layer coated with silver or gold, metal base plate made of iron. Electrodes are separated by semiconductor layer of selenium. When light falls into the semiconductor layer, it emits electron and current flows.
- **CCD(Charge- coupled Device):** It converts the light that it captures into digital data. The quality of image captured is determined by resolution (megapixels). Typically used to capture an image of the reaction plate, whose content is then interpreted by instrument software.
- **Photomultiplier tube(PMT):** It is most sensitive, expensive. Principle is multiplication of photoelectrons by secondary emission of electrodes. Can detect very weak signals.

COMPONENTSNEEDEDFORREACTION

I. ***Sample to be amplified:*** It can be one DNA containing region to be sequenced(conventional PCR, qPCR, AFLP PCR etc.) or RNA(In real time RT-PCR). Generally,upto 3 kbp long.

II. ***Primers:***2 sets of primers generally needed. Generally, 20-30 nucleotides long. Synthetically produced. Complementary to the 3´ ends of target DNA. They are not complementary to each other. Not containing inverted repeat sequences to avoid formation of internal structure. 40- 60 % GC content preferred for better annealing. Primer pairs should have compatible melting temperature within 5° c.

- **Melting temperature(T_m)** of primer can be calculated to determine annealing temperature(T_0)
- **T_m of primers:**

Two standard approximation calculations are used:

For sequence less than 14 nucleotides the formula is-

T_m= (wA + xT)2 + (yG + zC)4

Where, w,x,y,z are the no of the bases A,T,G,C in the sequence.

For sequence larger than 13 nucleotides, the formula is:

T_m= 64.9 + 41* (yG + zC – 16.4)/(wA + xT + yG + zC)

When degenerative nucleotides are included in the primer sequence, nucleotides will be internally substituted prior to minimum and maximum T_m calculations.

- DNA primers can be designed by using software. E.g.- Primer3

a) ***Enzyme:*** Taq polymerase, a thermostable DNA polymerase, which is stable at temperature upto95°C. Taq polymerase directs 5´ to 3´ only. They have no proofreading activity.RNA dependent DNA polymerase or Reverse transcriptase, isolated from retrovirus, which is used in RT-PCR.

b) ***Nucleotides:*** dNTPs(ATP, GTP, CTP, TTP). In equimolar ratios, 200 µM each dNTPs.

c) ***Reaction buffer:*** Tris-HCl, ammonium ions, KCl, magnesium ions, bovine serum albumin.Buffers provide the ionic strength and buffering capacity needed during the reaction.

d) ***Divalent cations:*** Mg^{2+} or Mn^{2+}. Mn^{2+} better for PCR based mutagenesis.\

e) ***Monovalent cations:*** K^+ ions.

WORKING PRINCIPLE

1. It occurs in 3 steps:

a) **Denaturation or Melting of DNA-**In this step the DNA templates separated to each other by heating. It mimics the function of helicase in the cell. It can be done by heating the mixture containing DNA template at 90- 95°C for 30 to 45 seconds.

b) **Annealing or Primers Binding**-Primers bind to the complimentary sequence on thetarget DNA. Primers are chosen such that one iscomplimentary to the one strand at one end of thetarget sequence and that the other is complimentaryto the other strand at the other end of the targetsequence. Two primers are needed: forward primer, reverse primer. In this step, generally temperature is lowered(50- 60°C for 45-55 seconds).

c) **Extension/ Polymerization/ Renaturation**-DNA polymerase catalyzes the extension of thestrand in the 5-3 direction, starting at theprimers, attaching the appropriate nucleotide (A-T, C-G). Occurs at approx 72°C and p^H at 8.0.

Time required for one cycle- 2 to 5 mins and 25 to 30 cycles- less than 3 hrs.

2. End point Analysis:

For the detection of the products, many methods can be used like gel electrophoresis, southern blotting, Flourimetry etc.

For southern blotting and Flourimetry, some fluorescent dyes and probes are used. They react with the amplified product and can be measured by Flourimeter.

Flourescent signal is directly proportional to the number of PCR product (amplicons) generated.

Concept of Flouroscent dyes:

When a population of fluorochrome molecules is excited by light of anappropriate wavelength, fluorescent light is emitted. The light intensitycan be measured by Flurometer or a pixel-by-pixel digital image of thesample.

Excitation and Emission: Fluorodyes absorb light at one wavelength &thereby boosts an electron to a higher energy shell.

- The excited electron falls back to the ground state and the Flurophorere- emits light but at longer wavelength.
- This shift makes it possible to separate excitation light from emissionlight with the use of optical filters.
- The wavelength (nm) where photon energy is most efficiently capturedis defined as the $Absorbance_{max}$&

the wavelength (nm) where light ismost efficiently released is defined as the Emission$_{max}$.

- The range for which Flurodyes absorb light is small (~ < 50nm)and light outside this range will not cause the molecule to fluoresce.

***Linearity*:** The intensity of the emitted fluorescent light is a linearfunction of the amount of fluorochrome present. The signalbecomes nonlinear at very high fluorochrome concentrations.

Brightness:Fluorochromediffers in intensity.Dull fluorochrome is a less sensitive probe than a brightfluorochrome. The brightness depends on two properties of the fluorochrome:

Its ability to absorb light (extinction coefficient), The efficiency with which it converts absorbed light into emittedfluorescent light (quantum efficiency).

Environmental factors: Environmental conditions can affect thebrightness or the wavelength of the absorption or emission peaks.

Example of fluorescent dyes:
SYBR Green I-

It is mostly used fluorescent dye. Advantages are Stronger signal, Higher selectivity for dsDNA, Lesser sequence dependent, Higher stability, Lesser inhibitory for Taq, Higher resolution in melting curves, less hazardous and mutagenicity.

Other examples are SYBR Green II, EVA Green, LC Green, BEBO, YO-PRO, SYTO family.

Example of fluorescent probes:

Table: 1 Example of fluorescent probes

NAME	DESCRIPTION	ADVANTAGE	DISADVANTAGE
Hydrolysis probe/Taq Man probe	When intact, the fluorescence of the reporter is quenched due to its proximity to the quencher. Probe hybridizes to the target. dsDNA-specific 5'—>3' exonuclease activity of Taq or Tth cleaves off the reporter. Reporter is separated from the quencher. Fluorescent signal generated. Signal is proportional to the amount of amplified product in the sample.	Highly fluorogenic; Easy PCR setup; Sequence-specific detection, multiplexing	Expensive; Probe design and positioning challenging; Similar conditions for primers and probes; Elevated background(Quenching capacity); Probe degraded: no end-point analysis
Hairpin Probes/ Molecular Beacons	MB(Molecular Beacon) is non-fluorescent due toclose proximity of the non fluorescent quencher (Q) and the fluorescent Reporter; The probe denatures and the loopanneals to the target sequence of the amplicon; Separating the quencher from the fluorophore and thereby producing fluorescence which is proportional to the amp icons produced during PCR; MB is displaced not destroyed during amplification, because a DNA polymerase lacking 5' exonuclease activity is used.	High specificity, low background; Post PCR analysis; PCR multiplex; Allelic discrimination (greater specificity than linear probes).	Challenging design; Long probes – less yield; Intramolecular competitive binding; Low signal levels (proximity of reporter and quencher)
Hybridizati-on Probes	These assays use two sequence-specific oligo nucleotide probes in addition to two sequence specific primers. The two probes are designed to bind to adjacent sequences in the target. The probes are labeled with a pair of dyes that can engage in FRET. The donor dye is attached to the 3' end of the first probe, while the acceptor dye is attached to the 5' end of the second probe. During real-time PCR, excitation is performed at a wavelength specific to the donor dye, and the reaction is monitored at the emission wavelength of the acceptor dye. At the annealing step, the probes hybridize to their target sequences in a head-to-tail arrangement. This brings the donor and acceptor dyes into proximity, allowing FRET to occur. The increase in PCR product is proportional to amount of fluorescence.	Probe with only one fluorophore. Easy synthesis and quality controls. Reduced background fluorescence. High specificity.	Strict compatibility between donor & acceptor fluorophores.

3. Parameters used in detection process are:

- **Threshold Cycle (C_T)** is defined as the fractional cycle no at which the fluorescence passes the fixed threshold. It detects the accumulation of the PCR product.
- **Amplification plot** is the plot offluorescence signal versus cyclenumber. In initial cycles of PCR, there is little change in fluorescence signal. This defines the **baseline** of amplification plot.
- $\mathbf{R_n+}$ is the $\mathbf{R_n}$ value of a reaction containing all components (the sample of interest).
- $\mathbf{R_n-}$ is the $\mathbf{R_n}$ value detected in NTC (baseline value).
- $\mathbf{\Delta R_n}$ is the difference between $\mathbf{R_n+}$ and $\mathbf{R_n-}$. It is an indicator of the magnitude of the signal generated by the PCR.
- **Flourescence Resonance Energy Transfer:** It is a distance dependent interaction between the excited states of 2 dye molecules in which excitation is transferred from a donor molecule to a acceptor molecule without emission of a photon.

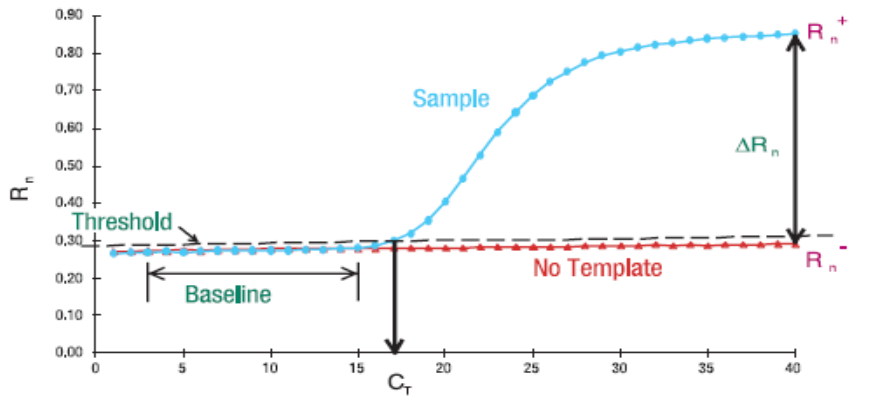

Figure 5: Graph representing the C_T value in PCR amplification

IMPORTANCE OF RT-PCR IN COVID-19 PANDEMIC

Real time RT-PCR test (Nucleic Acid Amplification Test) currently is 'Gold Standard' for the detection of the virus. Efficiency is almost 100%, but some false negative result may occur. It is also called as Nucleic Acid Amplification Test (Cartridge based).[6-10]

Structural representation of the virus (SARS CoV-2):

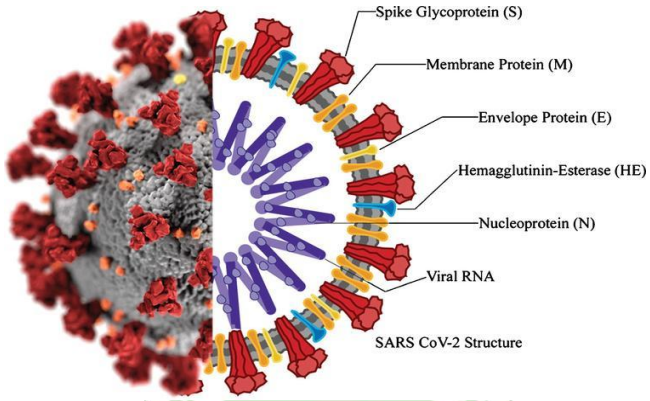

Figure 6: Structure of SARS CoV-2

SARS CoV-2 virus is a single stranded, positive(+) sense RNA virus belonging to Family- Coronaviridae; Subfamily- Orthocoronavirinae and causes the disease called covid-19 in humans.

The virus consists of a single stranded RNA, nucleocapsid (Nucleoprotein,N) covering the RNA and a viral envelop made of lipid bi-layer. In the surface of lipid bi-layer, membrane protein (M), envelop protein(E), Hemaglutinin-Esterase(HE), spike glycoproteins (S) are attached. The genome of SARS CoV-2 is approximately 30,000 nucleotides length.

Molecular targets for conducting of PCR:

Some molecular targets are identified in the viral RNA, these are helicase (Hel), nucleocapsid (N), transmembrane (M), envelope (E), spike protein(S), haemaglutinin-esterase(HE), open reading frames ORF1a and ORF1b, RNA dependent RNA polymerase(RdRp).

At least two molecular targets should be used to avoid the potential genetic drift of SARS-CoV-2 and cross reaction with other endemic corona virus.

The WHO recommends the E gene and RdRp gene assay for screening of the virus. In the US, the CDC recommends two nucleocapsid (N1 and N2) proteins for assay.

Protocol for testing:

Lab premises:-

SARS CoV-2 is airborne, highly transmissible virus. To protect the laboratory personnel and to prevent the spreading of the virus from the lab a minimum bio-safety level required. According to the CDC, the routine diagnostic test should be done in a bio-safety level 2 laboratories. Laboratory with bio-safety level 3 is needed for isolation or culture or research purpose.

Sample collection:-

For the detection of this disease, sample collection and its preserving method is very important, otherwise we can get false negative result. The persons who are collecting the sample, should be skilled personnel. Sample collection can be done from two major sources: upper respiratory tract (Nasopharyngeal swab and Oropharyngeal swab) and lower respiratory tract (bronchoalveolar lavage, tracheal aspirate, sputum).

Samples can be stored in 4°C for 5 days(Nasopharyngeal swab and oropharyngeal swab) or 2 days(Bronchoalveolar lavage, Tracheal aspirate, Sputum) in a buffer solution or Viral Transport Medium(VTM).

Sample preparation for reaction:-

At first, 100 µL of viral transport medium from aswab is added to 100 µL lysis buffer containing guanidium to inactivate the virus. The elution volume was set to 60 µL, and 10 µL of the purified RNA was added to the PCR.

Then, 10 µL of the extracted sample is diluted in a mix of 3.2 µL of DEPC-treated water, 2 µL ofRTBuffer (× 10 concentrate), 2 µL of RT Random Primers (× 10concentrate), 0.8 µL of dNTP mix (100 mM), 1 µL of RNaseinhibitor (20 U/µL), and 1 µL of Reverse Transcriptase (50U/µL) and 5 µL TaqMan probe.

RT-PCR:-

The reaction is occurred in a thermal cycler. The cycling is performed at 56°C for 15 min for reverse transcription. Then, the formed cDNA is again denatured by elevating the temperature at 95°C for 20 sec. The annealing of primers is occurred at 60°C for 50-55 Sec. then polymerization occurred at approx 70°C.

Detection of sample:-

Sample detection is done by flourimetry. The C_T(Cycle Threshold) value of the sample is responsible, if the sample tested positive or not. Generally, the C_T value for positive result should be in the range of 17.25 to <40.In case of the value exceeds 40 or more, the sample will be considered as negative. Some C_T values of USFDA approved instruments are (in case of positive result):

Table: 2 Types of Kit

Name of Kit	Manufacturer	Target Genes	C_T Value
Allplex2019-nCoV assay	See gene	E, N, RdRp	C_T< 40 positive
FOSUN COVID-19 RTPCR Kit	Fosun	E, N, ORF1ab	C_T< 36 positive
TRUPCR SARS-CoV-2 RTqPCRkit	Black Biotech	E, N, RdRp	C_T< 35 positive
TaqPath COVID-19 Combo Kit	Thermo Fisher Scientific	S, N ORF1ab	C_T< 40 positive
Lab Gun Real-Time PCR Kit	Lab Genomics	E, RdRp	C_T< 40 positive
Real-Time Fluorescent RTPCR Kit for 2019- nCoV	BGI Genomics	ORF1ab	C_T< 37 positive

Comparison between RT-PCR, Rapid Antigen Test, Serology based Antibody test:

Several detection tests can be performed for the diagnosis of the disease. These three tests are mostly used. These tests are based on different principles. Some of the characteristic comparisons are enlisted below:

Table: 3 Comparisons between RT-PCR, Rapid Antigen Test

Characteristics	RT-PCR	Rapid Antigen Test	Serology based Antibody Test
Main Principle	Amplification of viral genetic material(Nucleic acid amplification)	Antigen-antibody reaction.	Antigen-antibody reaction.
Technology used	PCR(Polymerase Chain Reaction)	Biosensor technology	ELISA(Enzyme linked immune sorbent assay)
Detection	Detects the presence of specific viral target genes(E, N,RdRp etc.)	Detects the presence of Viral proteins(Nucleocapsid and Spike protein)	IgM antibody in the blood.
Sample used	Upper or Lower respiratory tract swabs.	Upper respiratory tract swab.	Blood sample.

Sensitivity and specificity	Almost 100%	Varies from 34-80%(device to device)	98-100%
Specific feature	Early detection of infection.	Detected when the virus is actively replicating.	Detected after the 7 to 10 days infection.
Advantage	Confirmatory assessment possible.	Rapid detection of the disease.	Possible to detect immunity against the disease in a population.
Limitation	High cost.	Medium to low cost.	Low cost.

Comparison of some common PCR machines available for testing:

Right now, several PCR machines are available for laboratory diagnostic purpose. Thermo Fisher, Quiagen, Bio-Rad, Roche, Abbott etc. are some manufacturer brands for PCR machineries. Some of the popular instruments details are given below:

ABI QuantStudio 3D Digital PCR system=

It is manufactured by Thermo Fisher. It is available with 96 welled plates. Capacity is: 0.1 ml block(10-30 µL), 0.2 ml block(10- 100 µL). Dyes used are SYBR Green, FAM, TET, HEX. It is used for RT-PCR.It can analyse highly sensitive data and can be used for broad applications such as gene expression, regulation, and variation.

Advantages are easy to use, suitable for sensitive data, small footprint.

Disadvantages are reaction blocks are not interchangeable, systems can't be upgraded, medium throughput.

QuantStudio 5=

It is manufactured by Thermo Fisher. Available with 96 and 384 welled plates. Capacity is: 96 well (0.1 and 0.2 ml block) and 384 well (5-20 µL). Dyes used are SYBR Green, TET, HEX, Texas red. It performs well under high-pressure conditions.

Advantages are easy to use, more user interactive, have locked flow and paused features.

Disadvantage is reaction blocks are not interchangeable.

RotorGene 6000=

It is manufactured by Qiagen. Available with 36, 72 and 96 welled plates. Dyes used are FAM,SYBR Green, HEX,TET, JUN, Texas Red™. Light source is LED and used are 2 PMTs.

Problems regarding the testing of covid-19 by RT-PCR methods(Nucleic Acid Amplification Test):

A major drawback of the test of the disease by RT-PCR method is false negative or false positive results of sample. The reasons behind these variations are listed below:

- Faulty sample collection, storage and handling:

It is one of main factor for false negative results. To avoid these, sample collection should be done by skilled professional. Proper storage condition should be maintained till the sample tested.

- Sample collected very early or lately of the infection.

Advantages have extensive optical range, wide range of application, minimal maintenance and longevity, unique rotary design.

Disadvantage is bit slower than other PCRs.

BioRad CFX96=

It is manufactured by Bio-Rad. It is a 6 channelled real time PCR. Have high optical activity with 6 filtered diodes. Dyes used are SYBR, FAM, HEX, TE, TA, VIC. Available with 96 welled plates.

Advantages are easy to setup and run, minimal sample and reagent used, fast data analysis.

Disadvantage is medium throughput.

Roche LightCycler 2.0=

It is manufactured by Roche. Available with 32 welled plates. Capacity is: 20 µL and 100 µL. Dyes used are SYBR Green I, HEX, LC Red.

Advantages are suitable for in vitro diagnosis, high temperature homogeneity.

Disadvantage is low throughput.

Roche LightCycler 480=

It is manufactured by Roche. Available with 96 and 384 welled plates. Dyes used are SYBR Green I, HEX, Light Cycler red.

Advantages are sturdy and has high-performance hardware, innovative and high-efficient, high sensitivity.

Disadvantage is software is not user-friendly.

Abbott m200 real time system=

It is used for in vitro diagnostic use under FDA emergency use authorization only. It is manufactured by Abbott. Available with 24-96 welled plates. It targets RdRp and N genes of SARS CoV-2 virus.

- Mutation of the virus(Mutation in the genetic target sequence):

Virus can mutate for its own survival, thus it can posses better transmissibility, better penetration into the cells, achieve more virulency. This happens by altering the gene sequence of spike, envelop, membrane, nucleocapsid proteins. For this reason, it became difficult to identify the mutated gene sequence in nucleic acid amplification test. To counter this problem primers and probes should be redesigned according to the mutation of the virus.

- Presence of PCR inhibitory compound:

Sometimes, during testing some inhibitory compound can be present with the reaction mixture. This can alter the test

result. Inhibitory compounds can be present are haemoglobin, urea, heparin, organic or phenolic compounds, calcium ions, fats, glycogens etc.

Some enhancer can be used like DMSO, glycerol, BSA, Formamide, PEG, special commercial enhancers etc.

CONCLUSION:

Since the development of PCR technology, it becomes an essential and integral part of molecular biology as well as biotechnology. Through this technology, human genome project has been established. Based on this technology various applications are performed in medical science also. It can be said that, in this pandemic situation accurate detection of the disease is one of the main weapons to combat the disease and RT-PCR test is the game changer in this purpose. Though it is costly enough, but it can detect the disease accurately in an early stage and helps the patient to get an early treatment.

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