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Detection methods for COVID-19 in Nasal Swab samples: A study

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Abstract: *COVID-19, CoronaVirus Disease 2019 has devastated the population worldwide since late 2019. This pandemic has affected huge economic and social disruption in the majority of Countries on theglobe. At International level R&D work is being set off in the direction of production of vaccines injections and therapeutics for forestalling and cure of COVID-19, to stabilize the circumstances. We present our study on challenges and opportunities for devising schemes for SARS-CoV-2 detection. Swift and not so complicated effective approaches at reasonable cost are required for the clinical analysis of COVID-19. Diagnostic processes followed by conventional techniques for SARS-Cov-2 detection exhibit their own limitations, whereas Biosensors-based diagnostics have some advantages. Scientists are working on an easily portable and effective detection method to diagnose CoronaVirus.*

Keywords – *Nasal Swab, PCR, COVID-19, Biosensors, SARS-CoV-2*

I.Introduction

The sensitive respiratory condition virus SARS-CoV-2, that forms the basis for COVID-19 gets into the weak cells mainly through endocytosis by means of its Spike protein (S) by binding to human Angiotensin Converting Enzyme protein (ACE2). Upon getting in, Virus begins to multiply inside the human cell. The nucleocapsid (N) protein of SARS-CoV-2 is the prevalent structural protein of the virus that surrounds the bulky genomic RNA asshown in the fig. 1 and hence responsible for helical structure. Among the two viral proteins S and N, protein N is more significant and measured at high levels during infection.

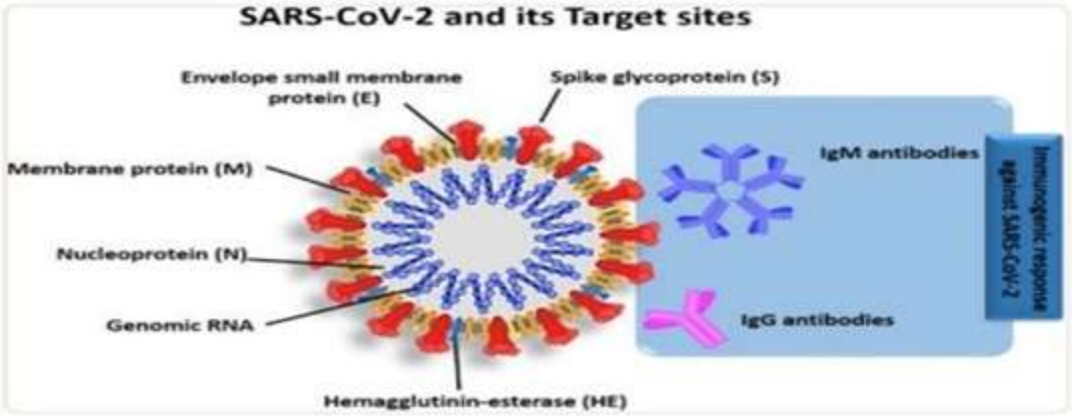

Fig. 1 Structure of SARS-CoV-2 virus (COVID-19)

Fig. 2 Nasal swab Test

The majority of the community infected by the virus has sensible respiratory infection and gets cured up on their own without particular treatment. On the other hand, some people get extremely sick and need medical treatment, for Elder citizens and people with medical history for heart disease, Asthma conditions, diabetes, etc. Virus infection may lead to severe sickness. Corona virus spreads through air passing through our nose when weinhale small liquid particles, sneezed or coughed out by an infected person at a close distance.

In our study, we focus on the discovery of COVID-19 present in the mucus samples collected through Nasal swab test, during the test a mild nasal swab about 5 inches or so, goes into the nasal cavity, the swab is rotated in each nostril for 15 seconds to collect the mucus. Fig. 2 shows Nasal swab test, it is painless and is easily performed by trained sample collection supervisors.

At present, two main techniques are used for the finding of COVID-19 namely; Reverse Transcriptase (RT) quantitative Polymerase Chain Reaction (RT-qPCR) a real time approach which identify the presence of the corona virus RNA in a patient nasal swab sample. Another one is an Immunological test which identifies the viral protein antigens or serum antibodies secreted in reaction of the human immune system to virus infection. PCR approaches identify the Virus during its vigorous phase, whereas the immunological tests identify the persons who have gained antibodies to fight the virus. Fig. 3 shows the treatment procedure of the Patient affected by COVID-19

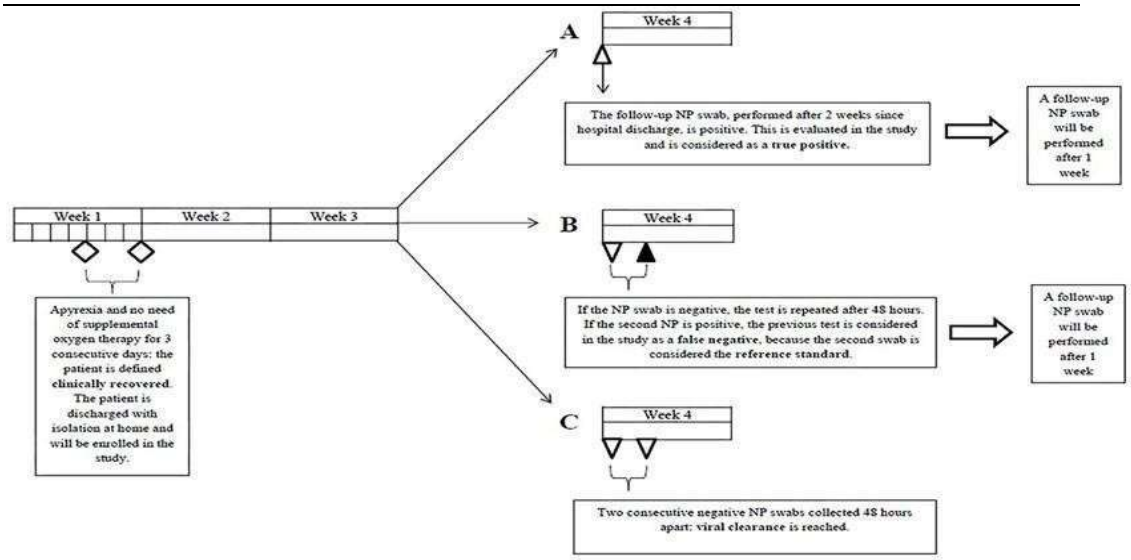

Fig. 3 Course of action for the handling of Patient affected by SARS-CoV-2

II. Literature Review

Literature division shows various analysis and studies in the area of interest, as well as in published results. Research is mainly made to analyze the context of the proposals and help to uncover vulnerabilities in existing systems, and guide how we can resolve issues. In our study we have reviewed some of the COVID-19 detection methods and Nasal swab collection approaches. COVID-19 detection methods are as follows

- **Polymerase Chain Reaction (PCR) variants**

For detection of SARS-CoV-2, quite a lot of researchers have attempted to propose PCR-based variants with reduction in time and to announce accurate results. RT-qPCR is regarded as a typical standard for carrying out clinical diagnostics. At the same time, there is a demand for schemes that are quick, precise, easy, and portable. A nucleic acid-based detection method for COVID-19, Reverse Transcriptase Loop-mediated isothermal AMPlification (RT-LAMP) is another long-duration test, but it does not have need for RNA extraction kit. Another new variant of PCR for COVID-19 diagnostics is RT-PCR technology; this has reduced the viral detection time to 15 minutes. RT-PCR method is comparatively costly and needs the presence of an expert technician as it is included in a single device plasmonic thermo cycling with fluorescent signal detection.
Up-coming methods other than PCR variants are also promising to offer quicker and cost-effective alternative for COVID-19 bio-molecular diagnostics, one of them is Gene-based CRISPR technology (Clustered Regularly Interspaced Short Palindromic Repeats), employ fluorescence-based recognition that are fixed to smart-phones to generate results within 15-60 minutes. Tests are approved for emergencies, number of steps to realize

detection are reduced, tests are portable.

[1] An Opto-electrical method for measuring the interaction between nucleocapsid protein and antibody anti-N to decide among positive and negative SARS-CoV-2 in the Nasal Swab sample. When the patient NS sample mixed with anti-N antibody is exposed to Light, Charge Transitions (CT) in the modified protein folds are initiated between side chain moieties of amino acids and the peptide backbone which acts as both donor and recipient. A commendable number Figure of Merit (FOM) is assigned to determine the disparity of sample with and without antibody at two voltage levels. Through experiments it was shown that within 2 minutes presence of CoronaVirus in the Swab sample was detected, if the recorded threshold of FOM is greater than one otherwise, the patient is considered as negative for virus.

- **Biosensors utilization for COVID-19 detection**

Furthermore, many researchers have investigated the use of biosensor devices to detect COVID-19. [6] Biosensors after working jointly with bio-molecules generate the results in a measurable form such as, enzymatic, electrical, optical, etc.[2] FET transistor-based biosensor need fine concentration of antibodies to boast a really reactive detection of coronavirus in clinical swabl samples. Detection arrangement is non portable; it wants trained personnel to run and regular calibration.

Projected techniques stress upon surface functionalization and viral series detection that need compound sample preparation and management. Researchers have proposed the deployment of Membrane-engineering for Cells having the human chimeric spike S1-antibody; the method has no practical solution as it tries to utilize Electro- insertion of CoronaVirus spike S1 antibody into cell membrane. Biosensors can offer real-time determination and require no-specific binding but they too encounter some challenges like immobilization of bio-molecules on
sensing surface.

- **Nasal swab collection approaches**

Nasal swab test is for viruses and bacteria that cause respiratory Infections . Nasal swab tests can help patients and respective Physicians to assess the type of infection caused and prescribe the suitable treatment. Swab Test is performed by collecting samples of mucus cells from patient nostrils. Nasal swab sample collection and storage has challenges and issues, researchers have done excellent work in this field also.

[4] Polyester nasal swabs stocked up in dry storage tubes present an inexpensive self-collection method for CoronaVirus load testing, as virus RNA continues to stay constant under circumstances necessary for home-collection and shipment to labs. Polyester swabs stored up at dry demonstrate comparable results with foam swabs in favor of discovery of small and modest SARS-CoV-2 viral loads.

[5] NasoPpharyngeal (NP) swabs are regarded as the better Samples for testing of viruses related to respiratory diseases, including Corona Virus also. To boost the ability for SARS-CoV-2 test in a range of settings, with scarcity of sample gathering provisions, this has motivated research for options for sample variety with good sensitivity. Results of collective Oropharyngeal and NS swab study on alternative specimen blend are helpful.

Table 1, Summary of research work done in regard to COVID-19 detection methods & Nasal sample collection

Sl. no.	Salient features of the Proposed work	Observation and Results
1.	[2] FET based Biosensor for the detection of CoronaVirus in clinical samples. Sensor is created by coating the sheets of graphene of FET by a definite antibody beside the SARS-CoV-2 spike protein. Sensor's effort was checked using antigenic protein, cultured virus and nasopharyngeal swab Samples from Covid19 victims. FET device detects COVID-19 spike protein	SARS-CoV-2 in culture media and clinical trial, was successfully detected by FET sensor, it is shaping as promising biosensor
2.	[3] NasoPharyngeal Aspirate (NPA) and Nasal Swab (NS) of 475 kids admitted in hospital with severe Respiratory infections have been considered for detection of Influenza virus, multiplex PCR test and virus culture. Generally sensitivity of virus recognition with NasoPharyngeal samples is better than that obtained with Nasal Swab samples.	NS is an insufficient Model if only diagnostic test are performed to detectcommon respiratory viruses.
3.	[6] Biosensor-like dual-functional plasmonic that unite the effect of PPT and LSPR sensing transduction offer a substitute product for clinical CoronaVirus diagnosis. 2-D gold nano islands (AuNIs) functioning with complementary DNA receptors can achieve a responsive discovery of the	The thermo plasmonic effect removes the un-matching hybridization rapidly and encourages the selective detection of the final sequence, to realize correct detection of nucleic acid and virus demarcation.

	targeted series right from Corona Virus to nucleic acid hybridization	
4.	9] SARS-CoV-2 infected Patients; his small viral load from later stages ofinfection simply can lead to the results of false negative nucleic acid testing, based on RT-PCR. With challenges for prevention and control of pandemic. To estimate specimen collection time on the +ve detection of coronavirus	Detection ratio of nasopharyngeal swab95% and nasal swab 95 %.
5.	10] Insight of two representative false-negative cases and converse role of experimental information with rRT-PCR, Co-infections with corona virusand other viruses are conferred.	Laboratory test results and tomographyfeatures.
6.	11] Ag-RDTs spot the majority of COVID-19 infected people in the initial stage like the first week of sickness and persons with viral load. Hence, this method has good service for diagnosis at the initial stage of disease. A promising tool for controlling the increase of CoronaVirus. Studies on Standardizing conduct manner and reporting of medical precision would extend use of data.	Testing symptoms at an early stage resulted in considerably high sensitivity(83.8%, 95% CI 76.3% to 89.2%).
7.	12] An organized assessment and meta-analysis of the study to compare Respiratory sampling approach for detection of CoronaVirus. The basis for inclusion in review was papers that evaluate at least a pair of respiratory samples of participants with COVID-19. Positive tests percentage was evaluated among sampling modalities by performing Z-test and utilizing the standard errors obtained from random outcome of meta-analysis.	To decrease the danger of the spread ofCOVID-19, criteria that must be followed while discharging the patients is the assessment of virus RNA tests ofinduced sputum should be considered not the throat swab sample
8.	[13] Combined swab is a suitable specimen for COVID-19 monitoring and diagnosis with rRT-PCR, This method helps physicians to assess virus clearance, while looking for evidence-based decision prior to discharge a patient. Implementation of combined swabs will certainly facilitate in managing and controlling epidemic	Combined swabs expressed positive cases 100% accurate, sputum (63%), OPS (72.3%), NPS (91.5%), whereas nCoV was undetected in specimens ofserum, urine, plasma

III. Sars-Cov-2 Detection Methods

Recently, Countries all over the world came across pandemic due to COVID-19; a number of detection methods are proposed and implemented. Literature related to detection methods for COVID-19 is categorized into following groups [14]. Fig. 4 explains the classification plan of Detection approaches. Schemes in First part, utilize biomolecules as targeted analytes. Methods in the second part are reviewed based on the process and technologies used for sensing viruses. Third part presents Sampling methods. Various types of nanoparticles are used by each type of target analytes of detection methods.

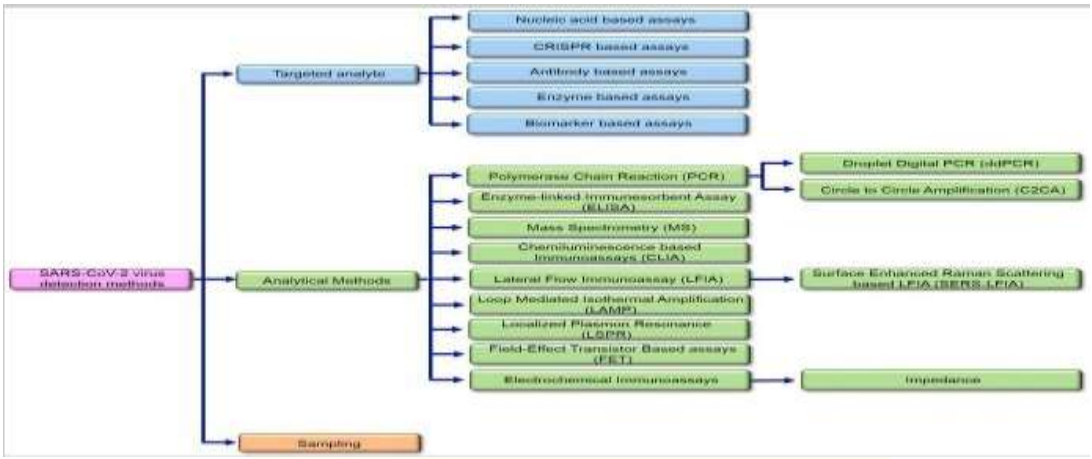

Fig. 4 Classification of detection methods for SARS-CoV-2

- **Usage of Biosensors for SARS-CoV-2 Detection**

Biosensors are better when compared with conventional methods, they require RNA extracts only; biological sensors are highly selective, sensitive and accurate one-stage sensors [7]. Biosensors are exact and precise in marking virus infections, so they are employed to discover the latest infectious SARS-CoV-2 virus. fig. 5 is a Chart of Biosensor types used for the detection of Coronavirus some of them are, CRISPR-Cas-based RNA detection; Electro-chemical method for detection of virus RNA based on AuNPs, Detection of virus mutant proteins based on graphene FET biosensor, silicon Nanowires for virus detection; Label free plasmonic procedure like Surface enhanced Raman scattering (SERS), Surface Plasmon Resonance (SPR), and Quartz crystal microbalance (QCM) based biosensors.

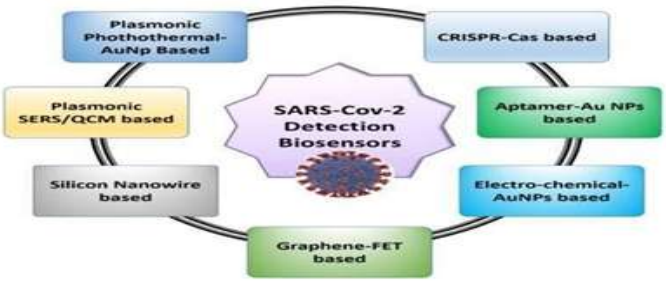

Fig. 5 Assorted Biosensors for COVID-19 virus detection

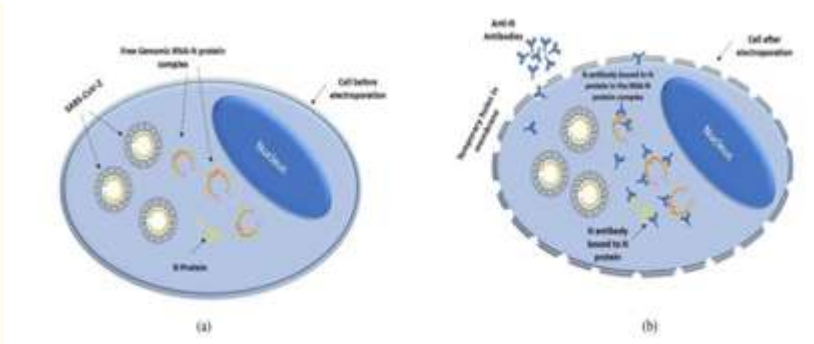

Fig. 6 Schematic diagram of the binding of anti-N with SARS-CoV-2 virus N- protein (a) Cell with no electrical biasing (b) Cell with electrical biasing [1]

IV. Experimental Setup

To improve our understanding, Schematic illustration or experimental set up of some of the Corona Virusdetection methods are exhibited as follows, [1], Electro-insertion method permits antibodies to break into the cell to be expressed out of the cell. Fig. 6(a) and (b), shows the cell status with and without being exercised by electric field. When Electric pulse is appliedto the cell, it causes pores in the cell membrane. The antibody anti-N pierces into the cell via the pores and binds along with SARS-CoV-2 N protein. Virus's N protein and anti-N binding be facilitated electrically thenidentified optically in this experiment.

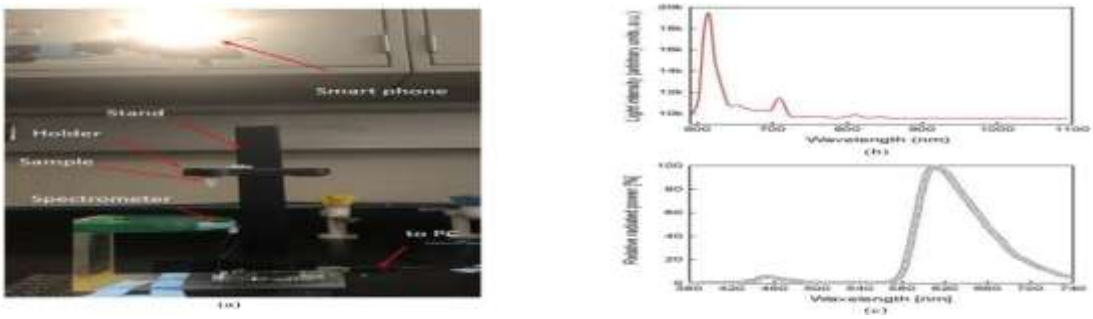

Fig. 7(a) Experimental setup for the concept of Optical detection (smart phone as source of light and spectrometer to collect light signal passing thro' the sample on holder) b.Plot of Light intensity falling on Swab sample (c) plot of Power intensity of Smartphone [1]

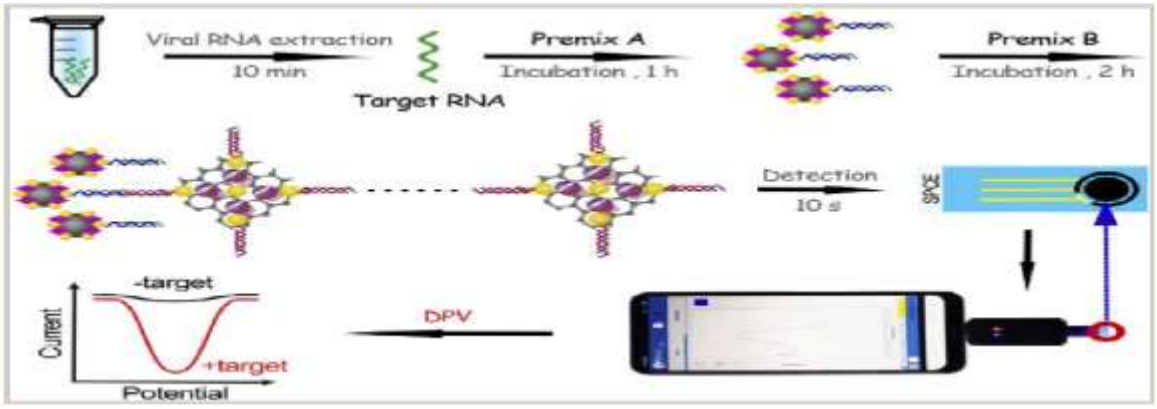

Fig. 8 Systematic process for COVID-19 detection by means of Electro-chemical biosensor (a). premix A and B preparation ; (B) procedure of Electro-chemical method using smart-phone [14]

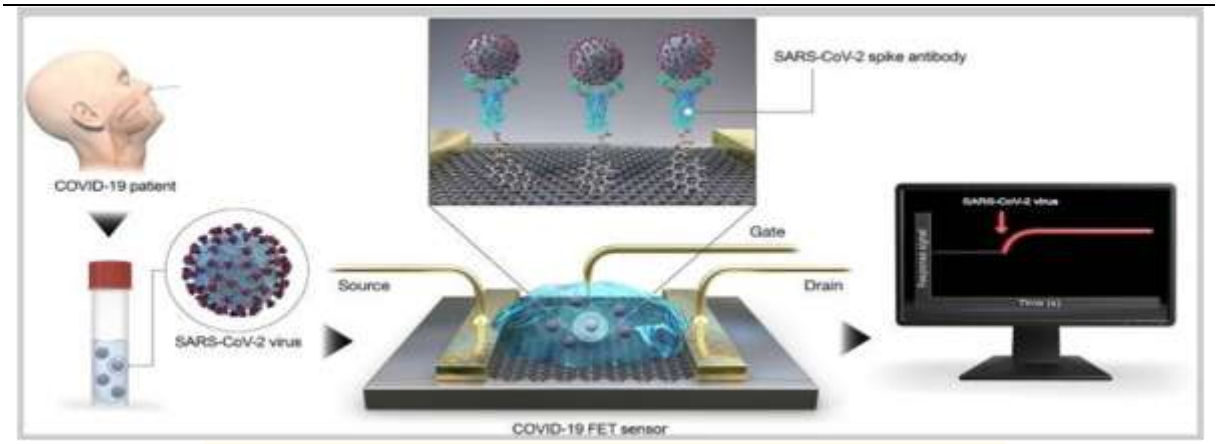

Fig. 9 Systematic process of FET based sensor for COVID-19 detection [2]

As shown in Fig. 9, Graphene is used as the sensing material; Corona Virus antibody is conjugated on graphene pane through 1-pyrenebutyric acid N-hydroxysuccinimide ester, being a probe linker [2]

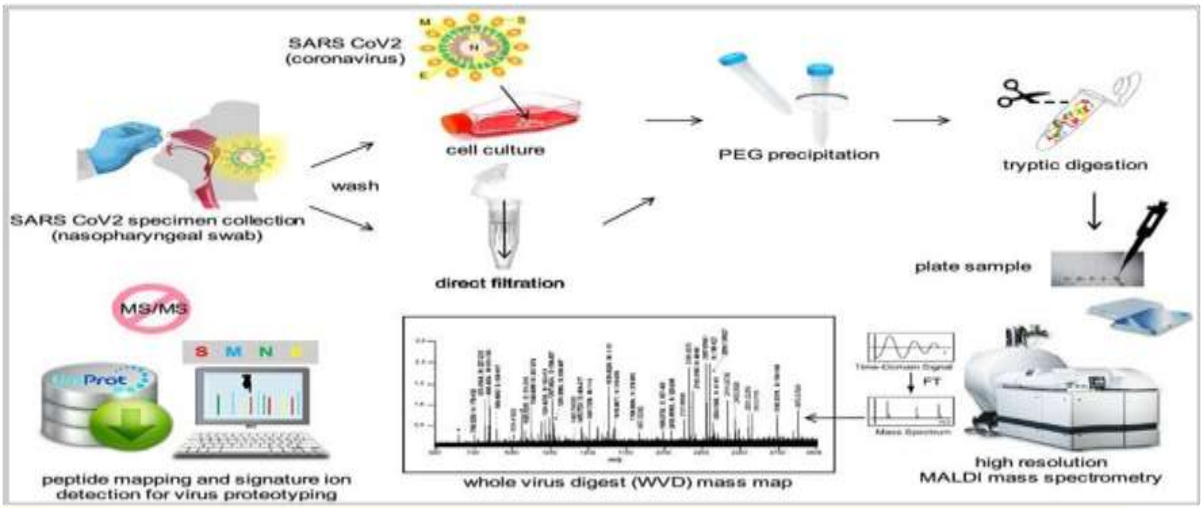

Fig. 10 Schematic diagram of procedure followed to detect Corona Virus by high resolution Spectrometry [15]

V. Result Analysis

Although there are some 4044 methods for detecting Virus particles, detection methods come up with some problems like, Lower accuracy, elongated time, high expense for equipment, maintenance, requirement of truly skilled technical staff, etc. Among the various methods and strategies for detecting SARS-CoV-2, antibody- based diagnostics and PCR methods are admired for sensitivity and correctness. Table 2, shows the Comparison of the COVID-19 detection techniques, targeted analytes used and their advantages

VI. Conclusion

Through this paper, we attempted to study the detection methods for COVID-19 classified under different categories, like the bio-molecules employed as analytes, and sampling methods. We noted the comparison of the latest technologies with the existing ones which are currently in use. During pandemic, Diagnosis for disease is important for prevention and controlling of the increase of SARS-CoV-2 virus. It is remarkable to mention that Corona virus and its variants are spreading very fast, and the pandemic has not yet come to an end. Therefore, developing an easy, fast, inexpensive, accurate detection method for CoronaVirus at present is an utmost challenge.

Methods	Analyte	Detection time	Sensitivity	Accuracy	DOL	Advantages
RT-PCR	RNA	75 min	–	–	10 copies/reaction	Specific
PCR	DNA	–	91%	93%	2 copies/reaction	specific
RT-PCR	C2CA	100 min	100%		0.4 fM	specific
ELISA	IgG/IgM	–	89.6%	–	–	Portable, fast
LFIA	IgG/IgM	–	100%	–	–	sensitive
LAMP	DNA	–	97.6%	–	0.2–47 ng/μL	Specific, portable
LSPR	IgG/IgM	2 h	–	–	0.08 ng/mL	sensitive
LSPR	RNA	–	3.2 copies	–	0.22 pM	Specific, sensitive
FET	antibody	–	–	–	16 Pfu/μL	Specific, sensitive
electrochemical	DNA	–	–	–	200 copies/mL	Sensitive, fast, portable, specific

Table 2, Comparison of the COVID-19 detection techniques with targeted analytes used

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