Rethinking Aptamers as nanotheranostic tools for SARS-COV-2 and COVID-19 infection

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Abstract

The novel coronavirus named by WHO and Coronavirus Study Group (CSG) as SARS-COV-2 is the etiological agent of the newly emerged Coronavirus disease (COVID-19). COVID-19 has become a pandemic threat as the WHO declared it a public health emergency of international concern. Early and precise detection of the virus is important for effective diagnosis and treatment. Various testing kits and assays, including real-time reverse Transcriptase PCR, thermal screening guns, ELISA-based immunoassays, and Point-of-Care (POC), have been implemented or are being explored to detect the virus and/or characterise cellular and antibody responses to the infection. However, these approaches have inherent limitations such as non-specificity, high cost, characterize by long turnaround times for test results, and can be labour intensive. Aptamers, which are single-stranded oligonucleotides, generated artificially by SELEX (Evolution of Ligands by Exponential Enrichment) may offer the capacity to generate high affinity bioprobes for monitoring relevant SARS-COV 2 and COVID-19 biomarkers. This article discusses the prospects of implementing aptasensing technologies for rapid point-of-care detection of SARS-COV-2.

Keywords: Aptamers; Theranostics; SARS-COV-2; COVID-19; Bioaffinity

1.0 COVID-19, SARS -CoV 2 Structure and Interaction

Severe acute respiratory syndrome-coronavirus-2 (SARS-COV-2) is the pathogen that is currently causing a worldwide public health threat. The infection caused by the virus is

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named by WHO as coronavirus disease 2019 (COVID-19). COVID-19 started in China, in a city called Wuhan located in Hubei province. The disease starts feverish symptoms, progresses to severe respiratory challenges, and finally pneumonia-like symptoms [1]. According to WHO situational report-70, as at April 27, 20020, a total of 2, 858 635 cases had been confirmed globally with 196 295 deaths. The mortality rate stands at 7.2%. globally. Since the emergence of the 2019 novel coronavirus outbreak, laboratories across the globe are sequencing the full genome of SARS-CoV-2 virus at extraordinary rate. This has enhanced real-time progress and research in the comprehension of the virus origin and genetic evolution, virulence, epidemiology, and transmission pathway. Also, it has fast tracked diagnostics design and development, treatment regime and provisional therapies. Apparently, over 11,000 viral genome sequence of new virus has been shared via GenBank and Global Initiative to Share All Influenza Data (GISAID) platform[2]. Currently, the information from genomic sequencing and analysis shows that SARS-CoV-2 is highly linked to a familiar or common bat SARS-like coronavirus, suggesting bats as the origin or reservoir [3].The available genome sequences SARS-CoV-2 do not vary and proves their appearance and escalation in the human populations in late December 2019 [2, 3]. A collection of the related SARS-CoV-2 infections indict human-to-human transmission and provide further information on the virus evolution[4]. Despite the bat origin, the mutational variants identified from the genomic sequencing are know to infect other animals such as ferrets and pangolin[5], which are sold illegitimately in china as ingredient to make traditional medicine. Thus, pangolin is suspected to mediate SARS-CoV-2 transmission between bats and humans. There is also some conjecture on the emergence of SARS-CoV-2, which was created or synthesized from coronaviruses in existence, however there is no supportive proof for this theory[6].

The pathogen causing COVID-19 has been identified to be similar to the virus that caused Severe Acute Respiratory syndrome (SARS) in 2002 with about 800 people losing their life worldwide[7]. The SARS coronavirus, usually referred to as SARS-COV, is an RNA virus that is positively stranded in nature with about 29727 nucleotides in the genome and possesses structural proteins. The structural proteins are made of an inner nucleocapsid (N) surrounded by an outer layer of envelope (E) and membrane_(M) proteins with glycoprotein spike (S) protruding from the surface[1]. A structural and

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functional analysis of the N, E and S proteins indicated that SARS-COV and SARS-COV-2 have significant similarities and differences, and the S and N proteins provide stability to the virus. Both SARS-CoV-2 and SARS-CoV belong to the coronavirus family, specifically from β-coronavirus genera. A study by Xu, et al. [8] showed that, two viruses share almost same gene sequence with 85% similarity, especially at the nucleotide level. Their genomic sequence containing six region of difference (RD) partially code for both *orf* (open reading frame) lab and S gene sequences. These RDs serves as biomarkers for the virus identification and drug targets. Additionally, proteomic comparison demonstrates that both SARS-CoV-2 and SAR-CoV protein are highly identical (95%). However, SARS-CoV-2 proteins (*orf8 and orf10*) were unavailable in SARS-CoV. Furthermore, the viral drug targets including protease and polymerase enzyme are highly conserved in both SARS-CoV-2 and SARS-CoV[9]. Thus, potential drugs or treatments against SARS-CoV protease and enzymes may demonstrate activity against similar SARS-CoV-2 enzymes. The critical surface protein found in SARS-CoV-2 and SARS-CoV differ and are only 75% identical, hence suggesting their mechanism to survival in the human host[10, 11]. The According to Lu, et al. [10], SARS-CoV S protein is shorter than SARS-CoV 2 S protein. Despite this, the spike S protein stalk in both viruses are highly homologous with 99% identity[12]. As the less scarce protein in Coronaviruses, the coding amino acid sequence of both SARS-CoV-2 and SARS-CoV N protein are 90% identical[13], indicating the probability of having similar antibodies. SARS-CoV N protein is able to neutralize the host immune response, a characteristic which have not yet been identified in the SARS-CoV-2 N protein[14]. From structure studies and biochemical tests[1, 15-17], SARS-CoV -2 possess high binding affinity to the host cell than the SARS-CoV. This was ascribed to mutation of amino acid residues located in the Receptor binding domain (RBD) of the spike S protein. Also, the SARS-CoV 2 has a unique polybasic cleavage site and three adjacent predicted O-linked glycans, which are unavailable in SARS-CoV and related family B of betacoronaviruses[6].

The angiotensin-converting enzyme 2 (ACE2) is the receptor in the human host cell that interacts with the S protein of COVs through affinity binding to facilitate infection [16, 18-21]. The S protein is used by the virus for entry into the host cell and subsequently fusing into the cell membrane. These two steps are very important in the viral pathogenesis and

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infection[22]. The S protein is made of three protein macromolecules referred to as trimeric proteins and has a flexible structure that enables it to undergo structural rearrangements after entry into the host cell[20, 21]. The ease of human-to-human spread of COVID-19 has been associated with the spike protein having a high affinity for the ACE2 receptor[23]. Figure 1 shows the schematic representation of SARS-COV-2 and its interaction with ACE2.

Coronavirus infection stimulates immune response in the host using the N protein. During the early stage of infection, the N protein is predominantly expressed, and this has been a target for diagnostics development[24]. N protein is a highly phosphorylated and abundant protein in CoVs that induce strong immune response after infection[25-28]. The viral N protein is an important structural protein responsible for transcription and assembling of the viral particle. It is also involved in the formation of helical ribonucleoproteins, regulation of RNA synthesis and transcription and metabolism modulation of infected cells[29-32]. Primarily, the CoV N protein binds and packs the viral genomic RNA into a nucleocapsid protein complex. All these attributes and functionalities have been demonstrated in *ex vivo* and *in vivo* biochemical studies[31, 32]. Further studies revealed that, N protein regulated interactions between the host and virus[33-35]. Additionally, it possess binding affinity for nsp3 located on the replicase complex and the M protein[36], leading to transition from viral genome to replicase transcriptase complex, and finally packaging of genomic nucleocapsid into a viral particle.

The N protein consist of two separate domains (Amino and carboxy terminus domain)[37]. These domains connected via short Serine-Arginine linker are able to bind RNA via contrasting mechanism. The amino and carboxy terminus domain undergo RNA binding and oligomerization respectively[38-42]; and the linker is responsible for phosphorylation. The amino terminal – genomic RNA binding is possible via electrostatic interactions. Also, relevant amino acids residues in the amino terminal domain are responsible for the viral RNA binding and infection[43-45]. These features of N protein have assisted in the development of diagnostic assays and several certain candidate antibodies and vaccines have been emerged against SARS-CoV virus [26, 27]. In a recent study, [46]demonstrated novel promising RNA binding pocket on SARS-CoV 2 virus, a domain absent in other coronaviruses. The authors said this finding would speed up the design/development of

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new antiviral drugs for COVID-19. Also, the pocket would be a target site for blockers/inhibitors that will hinder or hamper SARS-CoV 2 N protein replication.

The S protein is a fusion protein consisting of S1 and S2 units, predicted to form α-helical secondary coiled coil structure[47-49]. Within the S1 unit, is a receptor – binding domain (RBD) which recognize and interact directly with the human ACE2 receptor. S1 unit consist of N and C terminal domains (NTD, CTD). S2 helps in the virus – cell membrane fusion and anchorage[6, 17]. The S2 domain harbours other protein such as putative fusion peptide and heptapeptide repeats (HR1, HR2) domains. In final stages of S2 protein mediated membrane fusion, these peptides of SARS-CoV fold into anti-parallel six-helix bundles[50, 51].The Membrane anchored S2 subunit is supported and stabilized by the S1 unit. In SARS-CoV 2, Both S1 and S2 subunits are separated by a furin cleavage site[6]. Furin is a host protease which resides in the Golgi. The removal of this cleavage site affects the virus entry into VeroE6 and BHK cells. Primarily, the spike protein induces immune responses and neutralizes antibodies against virus infections, suggesting that therapeutics (vaccines and antiviral blockers or inhibitors or prophylactics), if developed against S protein could induce certain antibodies. These antibodies may either inhibit the viral binding and fusion or neutralize the virus infection[52]. A collection of these candidate therapeutics and vaccines for SARS-CoV can be seen in this review article [53].

Membrane (M) protein helps in the assembly and formation of the viral core and envelope via interaction with the N protein[54]. The envelope (E) protein is a transmembrane helix protein of 76 – 109 amino acids[55-57]. It is comprised of three domains; amino terminal, transmembrane and carboxy terminal[58-61]. The amino and carboxy terminal consist of short and long hydrophilic peptide respectively. The transmembrane domain are hydrophilic in nature and consist α – helix secondary structure. The E protein is involved in host cell recognition and viral assembly[55]. However, the function in coronavirus infection remains elusive. Hence, becoming difficult to consider this as important target for the development of therapeutics. All Together, in an antibody profile studies on SARS patients' sera, both M and E protein lack neutralizing or antagonistic antibodies as these proteins located within the viral envelope[62, 63]. However, development of antagonist for these M and N protein would contribute to relevant alternative therapeutic for coronavirus.

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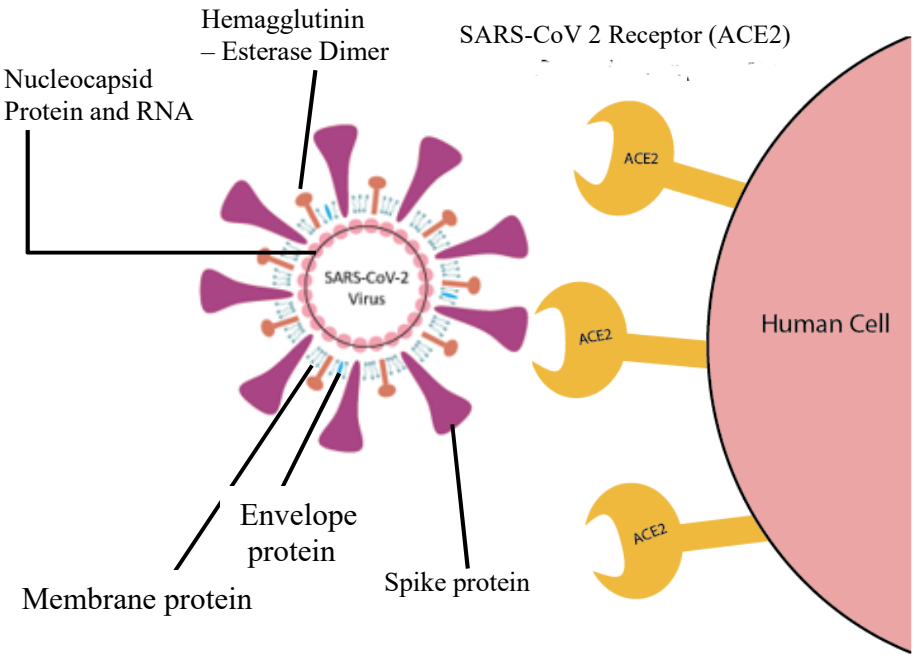

Figure 1 Schematic representation of the structure of coronavirus and the ACE2 host cell receptor. ACE2 receptors interact with SARS-COV-2 spike protein S. Adapted from [64]

2.0 Emerging *in vitro* Detection and Diagnostic tests for COVID-19

Several COVID-19 *in vitro* diagnostic kits have been manufactured by many companies, and these require approvals from FDA and other regulatory bodies. These testing assays and kits differ in the biochemistry and turnaround time. Most of the new testing assays and kits developed to support COVID-19 mitigation efforts have received Emergency

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Use Authorisation (EUA) from FDA[65], and are limited to the laboratories that invented them.

Currently, the diagnostic techniques approved for the detection of SARS-COV-2, and in fact COVs in general, include Reverse-T Polymerase Chain Reaction (RT-PCR)[66], Isothermal Nucleic Acid Amplification, and Blood-Based Serological Test [67, 68]. Table 1.0 shows the list of approved products and tests published by FDA[69] .

Majority of this test and kits are based on the polymerase Chain Reaction (92% - figure 2.0). In this technique[65], deactivated virus RNA is extracted and purified from nasopharyngeal swab collected from patient. The purified RNA is then reverse transcribed and amplified to DNA. Through repeated heating and cooling, millions copy of DNA are made. These virus DNA is then mixed with fluorescent dyes, which give off more light after binding to the virus DNA, an indication of the presence of the virus in the collected sample

The ease of use, specificity and high sensitivity of RT-PCR makes it an effective method for SARS-COV-2 detection[70, 71]. It detects the virus from sputum, blood, urine, saliva, pharyngeal swabs, nasal, anal swabs and stool specimen. RT-PCR kits may have three different assays with each associated with a separate SARS-CoV-2 gene target. Hence, the probability of mutation is minimal. The RT-PCR has shown to detect 4-8 viral copies by amplifying targets in Orf1ab, E and N viral genes at a 95% confidence level [72-74]. However, RT-PCR identified 47-59% of the positive cases in COV tests and 75% of results initially indicated as negative were found positive on repeated run[73, 75, 76]. Another short fall of RT-PCR assay relates to heat inactivation of the samples, and this may lead to inactivation of the viral particle, hindering effective downstream diagnostic evaluation[77].

The isothermal nucleic acid amplification method for detecting nucleic acid targets is void of limitations associated with thermal cycling[78]. The isothermal amplification method uses only one temperature and includes techniques such as helicase-dependent amplification, recombinase polymerase amplification and loop-mediated isothermal amplification (LAMP)[70]. All these techniques can be incorporated into a multiplex system during amplification stage.

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The LAMP technique has been proven for COVID-19 detection [67, 79-81]. In LAMP test, DNA polymerase and ~ 6 primers are used. These primers bind to six different regions on the target genome. Similar to PCR, collected nasopharyngeal or oropharyngeal samples from patient is added to the tube, followed by the DNA amplification and detection via either turbidity, colour or fluorescence. In a recent study by Park, et al. [82] , a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was designed and evaluated. The investigators detected over 100 copies of genomic RNA of SARS-CoV-2 using the colorimetric approach. The assays were void cross-reactivity with other human coronaviruses. This RT-LAMP are promising point -of – care test for COVID-19 but RNA extraction method needs to be optimized. Compared to PCR, RT-LAMP requires no centralized laboratory testing or facilities. Hence, it can conducted with simple instruments (for example, drying oven or water bath) and have similar sensitivity and specificity[83]. The limitations associated with this method include challenges of improving primer and reaction conditions[70]. Examples of these testing kits include Real Time SARS-CoV-2 assay and ID NOW COVID-19 produced by Abbott Diagnostics Scarborough, Inc[69]. SARS-CoV-2 nucleic acid extraction, isolation and purification from swabs specimens are required prior to the Abbott assay. The ID NOW assay involves target amplification, heating, mixing and detection of nucleic acids. The heating step in ID NOW system could inactivate collected samples[73].

The blood-based serological test involves qualitative detection of antibodies (IgM,IgA and IgG) linked to SARS-CoV-2 to assess individuals exposed to COVID-19[84]. The detection of the antibodies via this assay indicates the immune response to SARS-CoV-2 virus in suspected patients who have been infected previously or with recent COVID-19 infections. These antibodies can be detected approximately 14 days after infections. This test kit is produced by Cellex Inc. Prior to this development, quantitative immunoassay tests such as Dual ELISA were performed[85]. It detects different antibody types (IgA, IgM and IgG) against SARS-CoV in the blood of suspected individuals. Also, an ELISA kit assay for the detection of SARS-COV-2 nucleoprotein has been developed. These tests are for research use only and have currently not been approved by FDA Emergency Use Authorization.

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Despite being an accurate, rapid and simple essential tool for elucidation of interactions between several reported cases, antibody-based detection methods are associated with sensitivity and specificity limitations, and target types are scarce[86]. Moreover, antibody detection may not be suitable for early-stage infection as the immunoassay could generate negative results since antibodies may still be the development stage. Another problem could be potential positive results for formerly infected patients. Hence, this calls for a huge scrutiny about the accuracy, reliability and uncertainty of the serological test.

Additionally, a PCR test used for screening COVID-19 can cost up to $51 whiles antibody test cost less than $10 under Medicare[87]. On the frontline of turnaround time, whiles it takes 15 minutes to get serological test result, PCR runs last for about 4 – 6 hours. Due to the back and forth shipment and transfer of samples, results from PCR are procrastinated. Patient receive results in several days after test run. However, all this has been rectified through the design and invention of portable point – of – care test and results are received in less than an hour (table 1.0).

PCR testing is still considered to be highly accurate and embraced by the most hospitals, recommended by most doctors for mass screening. This test shows whether a person is still struggling with the virus and can transmit to others.

According to the FDA, over 5,000, 000 test are reported in a week via the PCR technique and predicts over 1000,000 tests in the coming months for the antibody-based test[69].

NGS is a new technique with high accuracy, used for the identification and characterization of pathogen quickly and result in rapid treatment. NGS based test involves a metagenomic sequencing assay which detects the SARS-CoV-2 virus with a sensitivity of 500 copies per milliliter[88]. Drawbacks of this technique include high instrument cost, requirement of bioinformatics expert, Difficulty in mass screening and its test time. It takes at least 2 days to get the test result (table 1.0). According to the manufacturers, they currently focused on the European market. Currently there only two approved tests using this technique which is approximately 3% (Figure 2.0) of the FDA approved test with CE marking.

From the above-mentioned limitations of existing and emerging diagnostic tests and kits, it is critical to continue the development of rapid, sensitive, inexpensive, specific, robust point-of-care diagnostics for COVID-19 to enable mass screening exercises especially in

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low resourced communities. A surveillance diagnostic test that can easily be carried out in different laboratories and outside healthcare settings is urgently needed to prevent the wide and rapid spreading of COVID-19. It is important that this test can distinguish between COVs and specifically target SARS-COV-2 virus. This will help individuals with suspected symptoms to rapidly check if they have been infected with the virus.

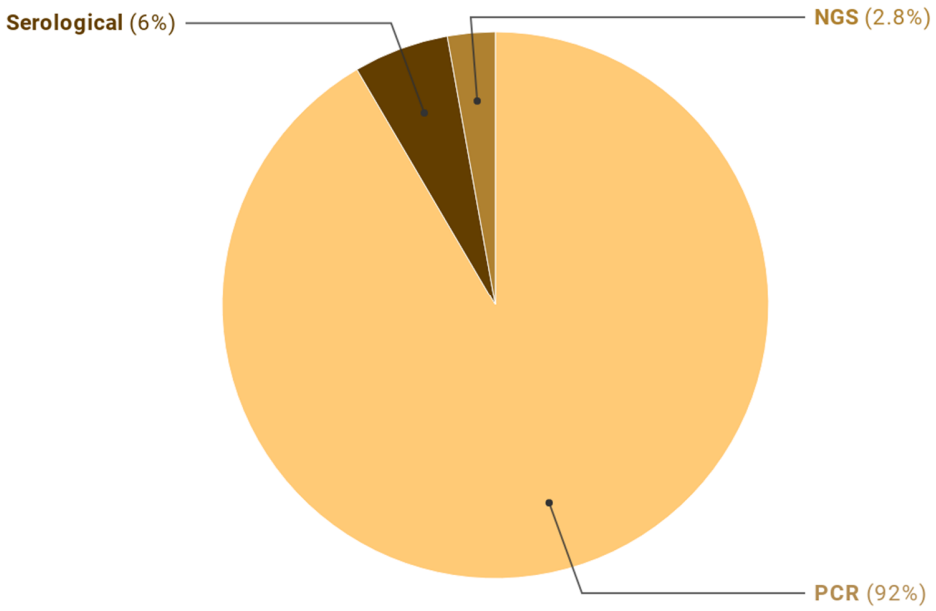

Figure 2.0: Distribution of Diagnostic test and kits based on Techniques or test type. Figure created with *Datawrapper*.

Table 1.0 FDA Approved Diagnostic test kits for COVID -19 infections

PRODUCT	*Manufacturers*	*Test Type*	*Result Time (hr)*	*Approval Status*

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1.	*RealTime SARS-CoV-2*	Abbott	PCR	4-6	FDA-EUA
2.	*ID NOW COVID-19 test*	Abbott	Isothermal amp-PoC	<1	FDA-EUA
3.	*AvellinoCoV2*	Avellino Labs	PCR	24-48	FDA-EUA
4.	*BioGX SARS -CoV-2Reagents*	BioGX,BD	PCR	2-3	FDA-EUA
5.	*Real-Time Fluorescent RT-PCR Kit*	BGI	PCR	3	FDA-EUA
6.	*BIOFIRE COVID-19 test*	BioMerieux-BioFire Defense	PCR	<1	FDA-EUA
7.	*2019-nCoV Real-Time RT-PCR Dx Panel*	CDC	PCR	24-72	FDA-EUA
8.	*qSARS- CoV -2IgG/IgM Rapid Test Kit*	Cellex	Serological	<1	FDA-EUA
9.	*COVID-19 ELISA IgG Antibody test*	Mount Sinai Laboratory	Serological	<1	FDA-EUA
10.	*DPP COVID-19 IgM/IgG system*	Chembio Diagnostic System, Inc	Serological	<1	FDA-EUA
11.	*VITROS Immunodiagnostic Products Anti – SARS – CoV-2 Total Reagent Pack*	Ortho Clinical Diagnostic, Inc	Serological	<	FDA-EUA
12.	*Xpert Xpress SARS -CoV-2 test*	Cepheid	PCR-PoC	<1	FDA-EUA
13.	*Logix Smart Coronavirus COVID -19 Test*	Co-Diagnostics	PCR	1-2	FDA-EUA
14.	*Simplexa COVID-19 Direct*	DiaSorin Molecular	PCR	1	FDA-EUA
15.	*ePlex SARS -CoV-2 Test*	GenMark Diagnostics	PCR	2	FDA-EUA
16.	*COVID -19 RT-Digital PCR Detection Kit*	Gnomegen	PCR	4-6	FDA-EUA
17.	*Panther Fusion SARS -CoV -2 Assay*	Hologic	PCR	3	FDA-EUA
18.	*Smart Dectect SARS -CoV -2rRT -PCR Kit*	InBios International	PCR	4-6	FDA-EUA
19.	*CoV -19 IDx assay*	Ipsum Diagnostics	PCR	24	FDA-EUA
20.	*Covid -19 RT -PCR test*	LabCorp	PCR	24	FDA-EUA
21.	*ARISES SARS -CoV -2 Assay*	Luminex Molecular Diagnostics	PCR	2	FDA-EUA
22.	*NxTAG CoV Extended Panel Assay*	Luminex Molecular Diagnostics	PCR	4	FDA-EUA
23.	*Accula SARS -CoV -2*	Mesa Biotech	PCR-PoC	<1	FDA-EUA

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	test				
24.	*SARS -CoV -2 Assay ,288/96 Molecular System*	NeuMoDx	PCR	1-2	FDA-EUA
25.	*New Coronavirus RT -PCR test*	Perkin Elmer	PCR	4-6	FDA-EUA
26.	*COVID -19 genesing Real -Time PCR assay*	Primerdesign	PCR	2	FDA-EUA
27.	*QIAstat -Dx Respiratory SARS -CoV-2 Panel*	Qiagen(acq.by Thermo Fisher)	PCR	96-120	FDA-EUA
28.	*Quest SARS -CoV-2rRT -PCR*	Ouest	PCR	1	FDA-EUA
29.	*Lyrra SARS -CoV -2 Assay*	Quidel	PCR	4-6	FDA-EUA
30.	*Cobas SARS -CoV-2 Test*	Roche	PCR	3-8	FDA-EUA
31.	*SARS -CoV -2 RTqPCR Detection Kit*	ScienceCell Research Labs	PCR	4-6	FDA-EUA
32.	*TaqPath COVID -19 Combo Kit*	Thermo Fisher	PCR	4	FDA-EUA
33.	*NY SARS -CoV -2 Real -time RT-PCR*	Wadsworth Center ,NY State Dept of Public Health	PCR	24-72	FDA-EUA
34.	*SARS-CoV-2 +Influenza A & B RT -qPCR Kit*	3D Medicines	PCR	4-6	CE Mark
35.	*REALQUALITY RQ-2019-nCoV*	AB ANALITICA	PCR	4-6	CE Mark
36.	*Bosphore 2019 -nCoV Detection Kit*	Anatolia Geneworks	PCR	2	CE Mark
37.	*SARS -CoV-2, influenza ,RSV panel*	AusDiagnostics	PCR	4-6	CE Mark
38.	*AccuPower COVID 19 Real -Time RT -PCR Kit*	Bioneer	PCR	8	CE Mark
39.	*Q-Sens 2019-nCoV Detection Kit*	Cancer Rop	PCR	2	CE Mark
40.	*VIASURE SARS-CoV-2 Real -TimePCR*	CerTest Bioter ,BD	PCR	3	CE Mark
41.	*VitaPCR SARS -CoV2 Assay*	Credo Diagnostics Biomedical	PCR-PoC	<1	CE Mark
42.	*QuantiVirus SARS -CoV-2 test*	DiaCarta	PCR	2	CE Mark
43.	*EasyScreen SARS -CoV -2 Detection Kit*	Genetic Signature	PCR	4-5	CE Mark
44.	*Detection Kit for SARS -CoV-2*	Genetic Health	PCR	4	CE Mark
45.	*qCOVID-19 ,CLART COVID-19*	Genomica /PharmMar Group	PCR	5	CE Mark
46.	*2019Real-time PCR Kit*	Kogene Biotech	PCR	4-6	CE Mark
47.	*GeneFinder COVID -19 RealAMp Kit*	OsangHealth	PCR	4-6	CE Mark
48.	*Allplex 2019 -nCoV*	Seegene	PCR	4	CE Mark

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Assay				
49. *DiaPlex Q 2019-nCoV Detection Kit*	SoIGent	PCR	2	CE Mark
50. *SARS -CoV -2 Clinical Sequence assay*	Vision Medicals	NGS	>12	CE Mark
51. *Multiple Real -Time PCR Kit*	Beijjing Applied Biological Technologies (XABT	PCR	4-6	CE Mark
52. *SARS-CoV-2RT -PCR test*	Children Hospital of Philadelphia (CHOP)	PCR	4-6	LDT(EUA)
53. *MGH COVID -19qPCR assay*	Massachuesetts General Hospital	PCR	4-6	LDT(EUA)
54. *SARS -CoV -2 Assay*	Northwestern Medicine	PCR	4-6	LDT(EUA)
55. *Viracor SARS -CoV -2 assay*	Viracor Eurofins Clinical Diagnostics	PCR	4-6	LDT(EUA)
56. *Applied Biosystems TaqPath COVID-19 Combo Kit*	Rutgers Clinical Genomics Laboratory	PCR	4-6	LDT(EUA)
57. *SDI SARS-CoV-2 Assay*	Special Diagnostic laboratories	PCR	4-6	LDT(EUA)
58. *UNC Health SARS-CoV-2 real-time RT-PCR test*	University of North Carolina	PCR	4-6	LDT(EUA)
59. *Stanford SARS-CoV-2 assay*	Standard Health care	PCR	4-6	LDT(EUA)
60. *Orig3n 2019 Novel Coronavirus (COVID-19) Test*	Orig3n, Inc.	PCR	4-6	LDT(EUA)
61. *SARS -CoV-2 PCR test*	Yale new Haven Hospital	PCR	4-6	LDT(EUA)
62. *CDI Enhanced COVID-19 Test*	Hackensack University Medical Centre	PCR	4-6	LDT(EUA)
63. *CirrusDx SARS-CoV-2 Assay*	CirrusDx Laboratories	PCR	4-6	LDT(EUA)
64. *Childrens – Altona-SARS-CoV-2 Assay*	Infectious Diseases Diagnostics	PCR	4-6	LDT(EUA)
65. *SARS-CoV-2 Test*	Exact Sciences Laboratories	PCR	4-6	LDT(EUA)
66. *SARS-CoV-2 Test*	Integrity Laboratories	PCR	4-6	LDT(EUA)
67. *COVID-19 RT-PC Test*	Medicine Lab of Baptist Hospital	PCR	4-6	LDT(EUA)
68. *Explify Respiratory*	IDbyDNA	NGS	24-48	LDT
69. *COVID -19 Home Test Kits*	Carbon Health	PCR	72-144	Discontinued

 doi:10.20944/preprints202008.0353.v1

70. At- home Covid -19 test	Everlywell	PCR	48	Discontinued
71. Covid -19 Home Test Kit	Nurx Molecular Testing Labs	PCR	48	Discontinued

PCR – Polymerase Chain Reaction ; LDT – Laboratory Based Test; EUA – Emergency Use Authorization, FDA- Food and Drug Authority, PoC – Point – of – Care . NGS – Next Generation Sequencing

Available on https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations , accessed on 17 April 2020

3.0 Aptameric nanosensors as viral diagnostics

Aptamers offer a novel approach for targeted diagnosis and treatment of infections. Aptamers have short oligonucleotides that are single-stranded and consist of either RNA or DNA with the ability to detect a wide range of molecules[89-91] including cells, tissues, viruses, and bacteria. They have high specificity and affinity to their target molecules. Aptamers are generated by an artificial method known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX)[92]. To design nucleic acid sequences that are specific to target molecules, the SELEX technique employs selecting sequences and replicating them carefully over iterative cycles. Aptamers have between 20-90 nucleotides[93]. To generate an aptamer for a target molecule, all feasible aptamers sequence with specific length are selected from a combinatorial library and incubated with the molecule of interest[94-96]. During this process, the nucleic acids with high affinity to the target molecule bind to it and the sequences with low affinity are removed from the pool.

The high affinity nucleic acids and the target molecule complex are recovered and separated. This process repeated until an aptamer with a low dissociation constant and high specificity towards the molecule of interest is generated. In addition to the SELEX procedure, other techniques such as surface plasmon resonance (SPR) and capillary electrophoresis (CE) can be integrated to increase biostability and improve binding performance.

Investigating the aptamer binding features under varying conditions of temperature, pH and conductivity whilst probing the conformational dynamics of the aptamer presents a useful strategy to optimize the binding performance. Aptamers bind to their targets via unique structural transformations. This gives them some advantages over monoclonal

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antibodies in terms of binding specificity and stability. The smaller size of aptamers makes them ideal for *in vivo* use compared to antibodies. Moreover, immunofluorescence dyes and drugs could be coupled onto aptamers without functionality and properties[94, 97]. Aptamers can be employed in biosensing devices as probes and such biosensors are called aptasensors. They are superior to conventional biosensors that use antibodies as they are stable, have high affinity to targets, modifiable, and can be developed for a wide range of targets [98], using various transduction mechanisms. Aptasensors have been applied in different forms to detect chemicals, disease biomarkers and pathogens found in food. It is important to identify and diagnose diseases at an early stage to help patients receive early treatment and better healthcare delivery[93]. In light of this, several antigens and viruses have aptamers developed for them to aid in their diagnosis. Aptamers generated for this purpose either target the whole virus or surface antigens. Over the years, aptamers have been developed for viruses such as hepatitis B and C viruses, human papilloma virus, HIV, Influenza, SARS, Ebola, dengue, herpes simplex virus, west nile virus [99-109].

4.0 Aptameric nanosensors for SARS coronavirus detection

An aptamer with a dissociation constant of 4.93 nM has been generated to target the nucleocapsid protein of SARS COV[7]. From the ELISA analysis, this ssDNA aptamer specifically bound to the nucleocapsid protein. In comparison with antibody, the authors further showed that the ssDNA aptamer was able to detect the SARS-CoV nucleocapsid protein efficiently via Western blot analysis. In an earlier development by Ahn and co-workers, an RNA aptamer was developed to target the nucleocapsid protein and a 1.65 nM dissociation constant was obtained[110]. Fluorescence imaging showed a detection limit of 2 pg/ml (Figure 3.0). The signal transduction was achieved using polyclonal anti-N antibody and FITC-labelled anti-rabbit IgG secondary antibody. In another study by Jang, et al. [111], RNA aptamers was generated for the NSP10 (NTPase/Helicase) found in SARS coronavirus. They reported that isolated RNAs efficiently bound and inhibited duplex DNA unwinding activity of SARS-CoV helicase by approximately 85%. The IC_{50} value was 1.2 nM. However, they stimulated infinitesimal change on ATPase activity of the helicase protein in the presence of a cofactor (Figure 4.0).

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Similarly, Shum and colleagues reported the binding between an immobilised DNA aptamer on Ni-NTA magnetic beads and SARS-coronavirus helicase[112]. Using ATPase and fluorescence resonance energy transfer (FRET) based assay, all selected aptamers demonstrated inhibitory activity against the SARS-CoV helicase with low apparent K_m values. The SARS-CoV helicase has the ability to unwind its duplex nucleic acid (DNA and RNA) to enable the replication and proliferation of the virus[113, 114]. Through circular dichroism and gel electrophoresis techniques, the investigators identified two different aptamer classes, namely G-quadruplex and non-G-quadruplex. Interestingly, non-G-quadruplex aptamer clones efficiently and specifically inhibited the SARS-CoV helicase-unwinding activity with IC_{50} ranging from (17.5 – 120.8 nM). The opposite was observed for the G-quadruplex aptamer class.

Also, Roh and Jo sensitively and specifically detected SARS-CoV nucleocapsid protein in a one-spot experiment[115]. The investigators reached this achievement with RNA aptamer conjugated to quantum dots (QDs) using an immobilized SARS-CoV N protein - glass chip system. Florescence imaging showed a detection limit of 0.1pg/ml.

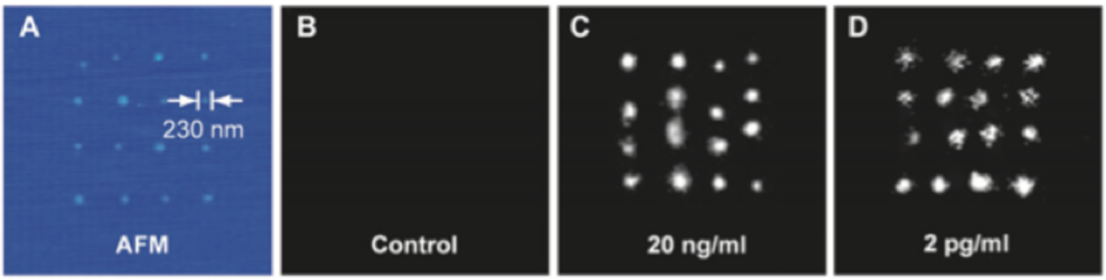

*Figure 3.0 Atomic force microscopy (**A**) and Fluorescence microscopy (**B – D**) for the detection of SARS COV N protein by a nanoarray aptamer chip. Adapted from [110].*

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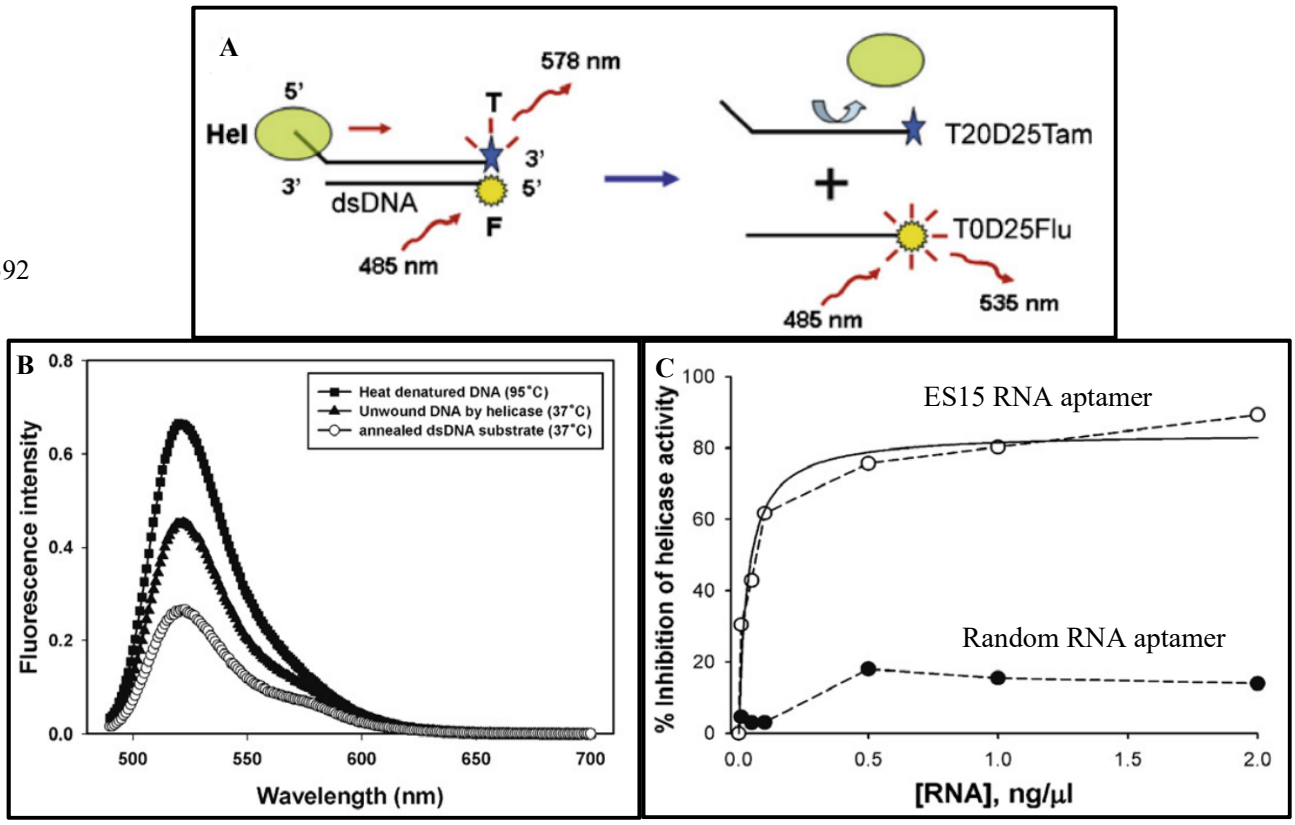

*Figure 4.0. Schematic and Principle of FRET-based fluorometric helicase assay (**A-B**) and inhibition of the SARS-CoV helicase protein activity by ES15 RNA aptamer (**C**). The inhibition activity of ES15 Aptamer is 85 % and random RNA aptamer has little effect on the helicase substrate. Adapted from* [111]

Recently, Researchers have developed new creative diagnostic kits with the use of a pioneering imaging technology called Mango for its vivid colour to sensitively detect RNA molecules, helping to improve the screening of viruses such as coronavirus while enabling fundamental discoveries into the functioning of cells[116]. The Mango system was made up of a RNA aptamer which acts like a magnet targeting the dye molecules. The dye becomes excited when bound and glows brightly. They concluded that the Mango NABSA kits can be used to detect pathogens such as the positive stranded RNA coronavirus faster and more efficiently.

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5.0 Future Perspective

Pandemics and viral diseases or outbreaks are inescapable and can lead damaging effects on every aspect of life. Due to this, there is an utmost need for effective and affordable testing or diagnostic technologies for early detection. The diagnosis of viral diseases and recent outbreak largely depend on the detection of nucleic acid, specifically RNA. A judicious selection of appropriate detection method and kits is essential. On the frontier of the current pandemic (COVID-19), FDA approved diagnostic test and technique described earlier possess unique merit and unavoidable demerit. These diagnostic methods are mainly molecular based assays and serology detection or immunoassays. The former consists of reverse – time PCR assay, full genome analysis using Next-generation sequencing (NGS) and loop-mediated isothermal amplification method (LAMP). PCR as a highly sensitive and specific standard assay is widely used for COVID-19 screening, however testing requires sophisticated equipment, facilities, clinical laboratories and well-trained personnel and finally time-consuming. NGS helps in the characterization, understanding, identification and transmission of the virus, but it is an expensive approach. The high temperature requirement of the LAMP limits its further application in coronavirus detection. Serology detection method, which is a point-of-care test helps in the detection of antibody types against SARS-CoV-2 in patient serum. Its low sensitivity and specificity inevitably hamper its usage by healthcare facilities. Additionally, only few has been developed and approved by the FDA. In this context, there is a need to develop rapid, cheaper, specific, sensitive and user-friendly diagnostic tool and point-of-care test for SARS-CoV. To date, new approaches are been developed to enhance SARS-CoV-2 detection but have not been approved by FDA yet. Practical approach is via a combination of the aforementioned methods to avoid limitations of single test and methods[70, 117, 118].

Due to their biophysical characteristics or potentials, aptamers have previously been demonstrated as a bioprobe for rapid sensing, screening and detection of infectious or pandemic pathogens and virulent diseases. These characteristics makes them ideal candidate to design and develop point-of-care test (POCT)[119, 120] for diagnosis and combating COVID-19 and other future pandemics. The Juxtaposition of molecular diagnostics and aptameric technology have been highlighted previously[121]. Briefly, In

 doi:10.20944/preprints202008.0353.v1

443 comparison to molecular – based assays or diagnostic technology, aptameric sensors have
444 advantages such as (i) easily generated for several targets (including toxins and non-
445 nucleic acid targets)[122, 123] (ii) they are less prone to contamination and sample
446 preparation is not essential[124]; (iii) they are simple, rapid, less expensive and can be used
447 in real time[125].
448 Additionally, although it is subjected to application format, aptameric assays do not
449 depend on labels, centralized or clinical laboratories and well-trained staffs[126-129]. Hence,
450 it can be developed for detection or testing for COVID-19 cases at home and this will be
451 reduced travelling to clinical/healthcare facilities which could contribute to rapid diseases
452 transmission and escalation. Similar to molecular diagnostics, aptasensors are highly
453 specific, sensitive and can be applied or incorporated into multiplex systems or
454 applications[124, 125, 128-130].
455 Through aptamer immobilization on 2Dimensional (Glass slides, silicon, chips,
456 polymethylmethacrylate) and 3 Dimensional (Microspheres made of magnets, agarose,
457 silica, monoliths and polystyrene) supports, many aptamer sensors with improved
458 characteristics or properties have been developed for dual functions, biodetection and
459 bioscreening[131-138]. The 3D immobilized aptasensors are highly efficient and possess
460 higher surface area -to-volume ratio[121]. Also, they have high loading capacity, are less
461 prone to steric hindrance and can be used efficiently in multiplex assays. Moreover, 3D
462 immobilized aptasensors have demonstrated high throughput ability and are cost –
463 effective. They can be used to detect and capture pathogens into support matrix. The
464 development of 3D aptasensors assays are quicker, hence, a criterion for rapid, specific
465 and sensitive screening of pandemic pathogens.
466 All these improved aptasensors technologies will be essential for effective mass
467 screening for pandemics, possibly further detection of asymptomatic cases and low
468 pathogen limits in suspected individuals since it takes a at least 2 - 14 days for suspected
469 patients to develop symptoms of COVID-19 infections.
470 Recently, Great strides and milestones have been reached on aptamer discovery through
471 integrated artificial intelligence-based technologies[139] and *in silico or* computational
472 methods[140-146]. These has come to solved problems of efficient and successful
473 identification of new or high-performance aptamers from combinatorial library using the

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SELEX technology. Additionally, high cost, labour and time further limits the SELEX process[147]. These computational methods have revolutionized the separation and identification efficiencies; decreased amplification bias and nonspecific adsorption; and enabled expansion of different base types. Several machine learning methods have emerged for identification of high-performance aptamers.

These include sequence clustering-based methods[148, 149] and motif finding – based methods[150-154]. Unfortunately, the former had limitations such as high amplification bias, and adsorption process is not specific. The later studies, although developed to over the challenges of the former, they faced with limitation such as high computational and analysis cost. Also, the general secondary structures of aptamers are not considered, which makes it absolutely choice dependent.

Moreover, both strategies/methods are still unable to identify highly efficient aptamers from SELEX pool. Surprisely, all these challenges have been addressed in a different study. In this recent work using machine learning based classification , Song, et al. [139] developed an Algorithm (SMART-Aptamer) to select aptamers from sequencing data SELEX pool to fill the above mentioned gaps with pioneering studies. The investigators rapidly identified three aptamers with excellent affinity, selectivity and accuracy for Epithelial stem cells, human embryonic cells and blood cells. The aptamer (SJ-3C2) demonstrated a strong binding ability with K_d value in the nanomolar range (41.43 ± 1.84 nM). Thus, using *in silico methods*, improved / enhanced aptamer sensing technologies can be developed to target different structural protein on SARS-CoV-2 for diagnosis and therapeutic purposes.

7.0 Conclusion

Diagnostics form relevant part of the toolkits in combating COVID-19 outbreaks. This enable healthcare providers or settings to distribute resources and focus on patients with severe infections. Hence, preventing the spread of the virus and reduction in mortality rate. The unique characteristics and benefits of aptamers are promising for the development of novel therapeutics and diagnostics for viral infections with particular emphasis on coronaviruses. This will aid in the development of reliable point-of-care test

 doi:10.20944/preprints202008.0353.v1

to bind specifically to SARS-CoV-2. Point-of-care test are less expensive, hand-held diagnostics that are used to test patient samples in community centres and do not require well-trained staffs, centralized facilities and clinical settings[70]. Also, these devices have short turnaround time and prevent spread of the disease as suspected individuals do not need to travel to clinical laboratories for testing. Research advances on aptameric sensors, technologies and application demonstrates that, it can be employed for the diagnosis and screening of current and future pandemics. The pandemic pathogens can be detected in a rapid, sensitive and specific manner. This will help to curtail infection aggravation, transmission and spread of the virus. Moreover, it is evident that integrated *in silico* methods and Artificial intelligence can be used to identify specific aptamer for improved aptsensing technologies against the SARS-CoV-2, consequently speeding up the design and development of point-of-care testing for combating the

Data on COVID-19 keeps changing rapidly daily. Some information in this review may change as more studies emerge and new tests and methods get approved by FDA. Some preprints were referenced.

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