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Review

Context-Aware Diagnostics Specificity (CADS) for SARS-CoV-2: A Review of Sensors and Detectors

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Abstract: Engineering a medical device as a low-cost, non-invasive diagnostic tool for surveillance of transmission and infection in humans, and animals, is not only critical in a pandemic but also a routine public health necessity. If the pharmacokinetics and pharmacodynamics of binding target proteins with specificity *in vitro* (device-based diagnostics) provide clues to therapeutic applications (*in vivo*) then we may have also laid the foundation for potential use in prevention. In this review, we establish a first-principles classification strategy for categorizing devices based on the nature of molecular interactions between targets and sensor recognition elements. In principle, it is applicable to any infectious agent or physiological dysfunction where one or more target molecules have been identified and the specificity of the interaction is documented. Using this approach, we focus on detection of SARS-CoV-2 virus. We summarize an analysis of devices that have been granted emergency use authorization (EUA from the US FDA) as well as those under development in research labs. Connected-devices may enable the underserved population to access at least some facet of public health service using smartphone-based non-invasive rapid detection of infectious agents (the approach for humans may be extended to animals and plants to embrace the OneHealth perspective).

Keywords: SARS-CoV-2; COVID-19; coronavirus, multiplexing, biosensor, mobile diagnostics

1. SARS-CoV-2

SARS-CoV-2 is a coronavirus virion (also known as a virus particle) that infects host cells through nonlytic exocytosis [1]. The capsid serves as a protein shell and is composed of structural proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, all of which

serve as an envelope encasing the single-stranded RNA genome, non-structural proteins (Nsp) and viral peptides [2]. SARS-CoV-2 contains two large open reading frames (ORF): ORF1a and ORF1b [3]. ORF1ab is the largest gene and contains overlapping ORF that encode polyproteins PP1ab and PP1a which yield NsP 1 through 16. Nsp play an important role in viral RNA replication, transcription and are critical for maintaining genome integrity [4].

The first step of SARS-CoV-2 infection is binding between S protein and host receptors, namely angiotensin converting enzyme 2 (ACE2). S protein receptor binding domain (RBD) makes first contact with ACE2 [5]. Subunit S1 is the surface-accessible portion adjacent to the RBD and is critical for recognition. Subsequent fusion of the viral envelope with the cell membrane is facilitated by exposure of the fusion loop in the S2 subunit, facilitating delivery of viral +ssRNA (positive sense) inside the host cell.

Rapid diagnosis of infection is critical to controlling disease outbreak [6]. Ideally, diagnostic tools should be non-invasive and low cost, while providing rapid results that have clinical relevance (e.g., determination of infectivity). Although we would like to have all of these features in a single device, currently a suite of tools must be used to deliver a meaningful outcome. The interactions between viral target(s) and unique receptors used in diagnostic devices vary significantly, depending on the specific target and specificity of the target. Further, the type of transduction, data acquisition approach, and data analysis (*post hoc*) are not common among different devices [7]. There are a myriad of different approaches and each diagnostic tool may generate unique output. The unequal characterization and significance of the data influences the value of these test results in the decision-making process.

A number of reviews and short communications have been published for cataloguing SARS-CoV-2 detection tools [8,9,18,10–17]. These reviews serve to index critical state-of-the-art knowledge in virus detection and are crucial for sensor development labs, but in many ways the actionable information is lacking for making the connection with diagnostic outcome(s). Beyond catalogues of what has been done, there is a critical need for classification systems which view technology development at the systems scale. Here, we review current SARS-CoV-2 detection devices based on specificity of the molecular interaction(s) between viral target and sensor recognition structure, and we discuss application of devices for diagnostic applications. These aspects are combined to create, at least in principle, a context-aware design emphasizing the specificity of detection targets. The resulting tool(s) and practical outcomes are expected to reflect the context-aware diagnostic specificity (CADS).

In section 2, we develop a simple classification system for organizing devices based on detection of either lysed virion (Type I), or intact virion particle (Type II). Using this binary classification system, we summarize the current state of the art in detection of intact SARS-CoV-2 virus, including devices that have been granted emergency use authorization (section 3). In section 4, we analyze diagnostic devices currently under development using the CADS logic. We conclude in section 5 by introducing challenges and opportunities related to CADS.

2. Classifying SARS-CoV-2 detection tools based on nature of molecular recognition specificity

The most common SARS-CoV-2 structural targets for diagnostic devices are structural proteins (S protein, N protein), or genomic RNA (see section 4). Clinically, the large amount of S protein in serum makes it an important target for serological detection [5], but testing depends on invasive sampling which may not be appropriate for some cases (e.g., frequent testing of children in schools). N protein is often targeted for detection assays and vaccines [19,20], but has shown non-specific binding to non-target DNA via electrostatic interactions [21]. Datta et al [22] recently reviewed various binding strategies and provided other examples of potential targets for detection beyond S protein, N protein and genomic RNA.

Fig 1 shows our proposed classification scheme and is based on type of molecular recognition scheme. Type I includes devices that detect targets released after lysing of the capsid envelope. For example, antigens associated with structural proteins (S, E, M, N proteins), non-structural proteins (Nsp), genomic RNA, or in rare cases viral peptides are all Type I devices. Viral accessory proteins, whose function(s) are not yet elucidated, may be a possible target for Type I devices but have not been published. Type II devices detect extra-capsid targets on intact SARS-CoV-2 virion particles. These devices target at least one of the three structural proteins (S, M, E proteins), but may include other molecules (such as glycans). The most common target is S protein, due to lack of physical access to E and M proteins without disrupting the particle. This simple classification system is intuitive, but it is important to quickly assess the nature of SARS-CoV-2 testing when considering testing outcomes (see section 4 for discussion).

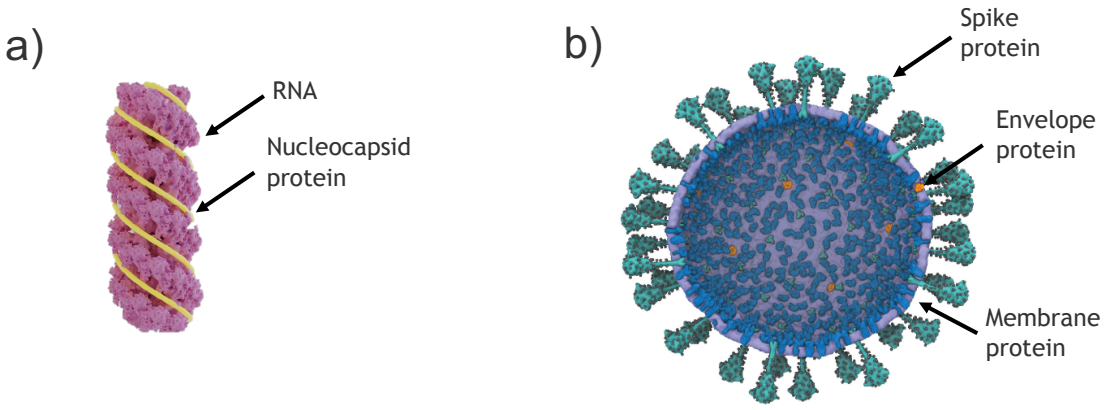

Figure 1. Classification of SARS-CoV-2 detection tools based on type of molecular interaction. **a)** Type I requires lysing of the capsid followed by subsequent detection. Cartoon shows RNA in yellow wrapped around the N protein in pink. **b)** Type II: Detecting intact SARS-CoV-2 virion particles relies on molecular targeting of exposed targets such as spike protein (S). Structures courtesy of Amaro and Mulholland [23] and image repository at www.covid.molssi.org. Non-structural proteins (Nsp) such as RNA-dependent RNA polymerase (RdRp) are not shown.

Diagnostic tools have been further organized into subcategories based on the recognition element used for detection (transduction schemes are not considered). Most recognition elements discussed here has been analyzed for potential use in SARS-CoV-2 detection and, when available, published references for use cases in COVID-19 diagnostics or research are highlighted. For recognition structures that have not yet been applied for SARS-CoV-2 detection (e.g., lectins), structural and binding features are discussed based on detection of other coronaviruses (see **Table 3** and supplemental section). The following sections introduce each subcategory, and examples of FDA approved devices (section 3) and research devices (section 4) are reviewed in subsequent sections. The subsections are summarized in **Table 1** as a reference.

Type I detection

Type I detection schemes require a lysis step (often thermal or chemical) to release viral RNA or target protein(s). Four subcategories are discussed below, and are based on viable recognition structures that have been tested. Specific examples for coronaviruses are discussed in section 4.

Type Ia detection (oligo binding in lysate): This scheme utilizes oligonucleotides as the recognition element. The most common molecular targets for polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) primers (denoted as Type Ia in **Table 3**) are the E, N, S and Nsp genes via RT-PCR [17]. Berber et al review the molecular tools for COVID-19 diagnostics and

therapeutics, including CRISPR-Cas systems, antisense oligonucleotides, antisense peptide nucleic acids, ribozymes, aptamers, and RNAi silencing approaches [24]. For detection of viral RNA, reverse transcriptase (RT) amplification is common for detection of stable reporters in most cases. Fluorescence is the transduction system of choice for nearly all of these systems, which requires a label to be inserted during synthesis. The conserved portions of the S2 subunit (responsible for fusion machinery, fusion peptide) are likely targets for aptamer binding (particular sub-segments are of particular interest). A number of reviews have been published analyzing the current state of the art for PCR primers targeting SARS-CoV-2, including establishment of reference sequences [25–30]. The gene-based detection techniques own high specificity and sensitivity, which are reliable method for authority document.

Type Ib detection (aptamer binding in lysate): Single stranded (ss) DNA aptamers (Type Ib in **Table 1**) have been developed for binding SARS-CoV-2 targets in lysate [31]. These dynamic single stranded oligonucleotides bind via tertiary structures (e.g., hairpin loop, G-quadruplex, etc.). Type Ib schemes may use numerous forms of transduction for detection of binding (fluorescence, surface plasmon resonance, electrochemical, magnetic), and in some cases more than one transduction scheme is used for a single assay.

Type Ic detection (Ab binding in lysate): Antibodies (Ab) are used as the biorecognition structure for selective target binding in lysate, commonly focusing on N and S proteins. These devices have been used for development of LFA (lateral flow assay) and ELISA (enzyme-linked immunosorbent assay) assays for SARS-CoV [31] and are currently being used for SARS-CoV-2 as well (see section 3). However, specificity of the antigen-Ab interaction may change for mutant viruses [32].

Type Id detection (lectin binding in lysate): Type Id schemes are lectin binding assays and have been used for analysis of viral lysate from herpes simplex virus [33] , Ebola [34], HIV [35] and coronaviruses such as influenza A[36] and SARS-CoV [37].

Type II detection

Biorecognition structures which are relevant for Type II devices include aptamers, peptides, lectins, antibodies, and membrane receptors. The most common exposed target on intact virion particles is S protein (RBD, subunit S1). Similar to HIV, SARS-CoV-2 S protein uses a N-glycan coat on S protein [38] to escape immune recognition. For Type II assays, it remains to be investigated if inclusion of endo-b-N-acetylglucosaminidase (ENGase) [39] is necessary to expose the binding site of S protein by partially removing the N-glycan coat. Each sub-category of Type II schemes are discussed below, specific examples are discussed in section 4.

Type IIa detection (aptamer detection of intact virus): Aptasensors may be developed for targeting any exposed structure on the virus particle (e.g., glycan coat, S protein, fusion peptides). The most common types of transduction used in aptasensing of this type are impedimetric, surface plasmon resonance (SPR), and FRET pairing [40]. Numerous DNA aptamers [41] are under development for binding S1 epitopes as shown in section 4 and the supplemental section. There are several peptide targets identified on SARS-CoV [42–46] that could be viable targets, but this has not been confirmed.

Type IIb detection (Ab detection of intact virus): Type IIb devices utilize Ab as the recognition agent. The use of neutralizing antibodies (nAbs) as immobile targets in biosensors is limited to detection of specific virus strains. Antigenic drift and protein stability are the main problems for clinical application of Type II immunosensors. Recent studies show that most monoclonal antibodies to SARS-CoV do not bind SARS-CoV-2 [47]. Nevertheless, mouse antiserum raised against SARS-CoV protein has been shown to cross-neutralize SARS-CoV-2 pseudo virus, indicating the possibility for overlapping neutralizing epitopes between SARS-CoV and SARS-CoV-2 [48]. Certainly, epitopes may be shared but that introduces doubt in terms of specificity. Accumulating evidence indicates significant disparity between dissociation constants likely due to non-conserved epitopes and post-translational modifications (N-glycosylation site at amino acid residue 370 on SARS-CoV) [49].

Generally sensing platforms are validated with one SARS-CoV-2 strain and do not account for mutations [50,51].

Type IIc detection (lectin binding of intact virus): These devices are based on interactions between lectins (the biorecognition element) [52] and saccharide targets and lectin carbohydrate recognition domains (CRD) [53]. The clearest application is targeting of the glycan shield on SARS-CoV-2, which is based on binding of N-linked glycan epitopes by CRD, a concept which has been studied for Ebola [54], SARS-CoV [55], and other coronaviruses [56,57]. Lectin arrays target specific patterns of glycan based on the pattern recognition receptor (PRR) system. PRR are the first line of innate immune response proteins that respond to pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP) in animals. These include membrane-associated PRR such as Toll-like receptors (TRL) which sense pathogen-associated and danger-associated molecular patterns extracellularly or in endosomes. Specific detection of DAMPs can lead to cell viability detection due to PRRs that bind dying cells based on changes in glycosylation patterns on the cell surface. It is unknown which features of the PAMP/DAMP system may be replicated for SARS-CoV-2 detection.

Type IId detection (membrane receptor binding of intact virus): The last subcategory of devices is based on the interaction between membrane proteins and the intact virion particle. Human angiotensin converting enzyme II (ACE2) is the candidate host membrane receptor biosensor. ACE2 mediates entry of severe acute respiratory syndrome coronaviruses (including SARS-CoV, MERS-CoV, and SARS-CoV-2) into humans as well as other animals [58–61]. The encouragement for ACE-2 in Type IId design is due to the fact that such a sensor system may act as a "platform" for a family of zoonotic viruses agnostic of the mutations a specific virus evolves. For example, sensor systems using the protein ACE2 as the binding target may identify MERS, SARS and SARS-CoV-2 viruses because the virus family still, (in an evolutionary sense) uses human ACE (hACE2) as the cellular receptor to invade human cells irrespective of the variants over decades. Though ACE2-based sensing could be prone to false-positive results when screening for a specific viral strain, these "generic" results will still be useful given the virulence and mutation potential of the family of viruses that use hACE2-mediated infection. Type IId devices may utilize various types of transduction.

Table 1. Classification scheme for SARS-CoV-2 detection schemes organized devices by nature of molecular binding event. We review seven classes of devices that are organized based on detection of either lysate soup (Type I) or intact virion particle (Type II).

	Type	Recognition type	General Strengths	General Weaknesses
Intra-capsid detection	Ia	Oligo.	Best LOD of any tool (0.1 aM is common); multiplexing, highest sensitivity and specificity of any test	Potential for false positive/false negative; requires a label for detection
	Ib	Aptamer	Long shelf life; wide range of targets; customizable features; LOD comparable to antibody	Unknown affinity/avidity for target; modeling required for determination of 2D/3D structure; unknown specificity in complex mixtures
	Ic	Antibody	Well documented results; established protocols commercially available materials; clinically relevant LOD	Short shelf life; potential for false negative; turnaround time of approximately 72 hours

	Id	Lectin	Easily accessible; well established and proven screening array technologies, vast library of glycobiology information regarding binding affinity/avidity in various biological media	Non-specific binding; poor LOD; requires a label for detection
Extra-capsid detection	IIa	Aptamer	Long shelf life; wide range of targets; customizable features; LOD comparable to antibody	Unknown affinity/avidity for target (for new aptamers); modeling required for determination of 2D/3D structure; effect of tethering to sensor surface unknown
	IIb	Antibody	Rapidly accessible to many labs for developing tools; known immobilization schemes; status quo acceptance; clinically relevant LOD	Short shelf life; potential for false negative; binding kinetics affected by surface protein mutation
	IIc	Lectin	Easily accessible; well documented binding chemistry; known affinity/avidity	Non-specific binding; poor LOD
	IId	Membrane receptor protein(s)	Binding may not be affected by mutation for some receptors (hACE-2)	Short shelf life for recombinant proteins; receptors bind most respiratory viruses (low specificity); stability of artificial (recombinant) membrane proteins unknown

Oligo.= oligonucleotides;
hACE-2= Human angiotensin converting enzyme-2.

Although not reviewed here, serological analyses are rooted in analysis of post-infection biomarkers via antibody screens using tools such as ELISA or other protein detection methods. The distinction between the two classifications here is that serological assays are neither detecting lysate nor intact virion particles. Rather, a serological test is detecting the presence of Ab (typically immunoglobulin G) that are present in fluids as a result of immune response. For example, antibody-based NanoLuc luciferase immunoprecipitation assays in HEK293 cells have been developed for serological detection of N and S protein [62]. Rosadas et al [63] note in a critical review that serological assays developed using antibodies to N protein (anti-NP) may be flawed due to an inability to determine neutralizing and potentially protective antibodies, among other problems.

In the next section, we review the approved Type I and Type II devices under the FDA emergency use authorization.

3. FDA approved devices under the Emergency Use Authorization (EUA)

Recent reviews have catalogued the list of FDA approved devices under the Emergency Use Authorization (EUA) [64], which uses a 3-tier system: EUA under the Clinical Laboratory Improvement Amendments for high complexity tests (designation H), moderate complexity tests (designation M) and patient care settings operating under a CLIA (Clinical Laboratory Improvement Amendment)

Certificate of Waiver (designation W). Static reviews of EUA devices devalue over time as the EUA and subsequent authorizations are dynamic processes that are subject to approval, re-approval, and various other factors.

In **Table 2**, we summarize the status of the EUA devices as of the date of this review, and we augment the table by providing analysis of the potential strengths and weaknesses as well as a denotation of which type of detection was used. To date, there are seven Type Ia devices (primers for lysate), six Type Ic devices (Ab for lysate antigens), and two serological tests under designation W; serology devices are shown in the supplemental section (no devices for direct detection). At the time of writing this review, each of the EUA tests other than the Lucira home testing kit have approval for use in laboratories certified under designation H, M and W; while the Lucira home test only has approval for designation W at the time of this review.

All of the Type I devices employ primers for PCR- or LAMP-based detection, commonly called "molecular tests". The other devices in **Table 2** are commonly called antigen tests, which to date have been developed for targeting antigens in lysate. One of the common disadvantages of Type Ic tests (antibody-based) is the high rate of false positives from bacterial infection or other viruses. Each of the devices in **Table 2** uses a unique control strategy to account for this problem, and the long-term success of each approach is to be determined. The most useful antigen tests are multiplexing tools that detect other bacterial and viral infections, such as the Sofia LFA. All EUA antigen tests have disclaimers stating that positive results do not rule out a bacterial infection or co-infection with other viruses, indicating that secondary validation or other multiplex approaches are highly needed.

All EUA tests to date, with the exception of Cue (Type Ia) and Ellume (Type Ic) require a prescription. This is a major problem that severely restricts access for vulnerable communities (particularly in the present pandemic, where many people are no longer working due to lockdowns and general economic downturn). Further exacerbating this problem, multiple EUA tests report a narrow window for accurate detection (15-30 min), which could be problematic if used as a home test kit or outside of a clinical setting. In addition to access and potential for operator error, every test in the EUA is a disposable device (some even require the user to dispose of the battery) which is a fatal flaw when considering the scale of the public health crisis and the landfill problems that would emerge from disposing of tens of millions of batteries. As a first generation, these devices represent a useful step toward progress, but many could be authorized as quantitative devices with minor engineering modifications. When combined with features such as low cost and rapid turnaround time, quantitative detection is critical for future detection systems which provide enhanced value (e.g., stage of infection, active shedding status, etc).

Dinnes et al [65] reviewed commercially available antigen tests, PCR tests, and one at home test kit developed in 2020. The study analyzed over 3,100 samples (approximately 55% were positive). The sensitivity and selectivity of Type Ia (PCR) was highest, followed by home test kits and then antigen tests (LFA). Only two home test kits were analyzed, and the results were highly variable, thus more analysis is needed to confirm if home test kits are more accurate than antigen tests. Antigen test kits such as the SD STANDARD Q COVID-19 SD-Biosensor kit have been independently analyzed in other studies. Cerutti et al [66] report no false positives but the test kit had a high false negative rate. Hirotsu et al [67] compared a chemiluminescence enzyme immunoassay (CLEIA) antigen test kit (LUMIPULSE) with quantitative RT-PCR for viral load with 313 samples taken by NP swab. According to this analysis, the antigen level was accurate (100%) when the sample contained >100 viral copies but was only 85% accurate when the sample contained $\leq$ 10 viral copies. This result, among others, shows that the relatively low sensitivity of antigen testing may be problematic depending on the progression of infection.

Table 2. Critical review of FDA Emergency Use Authorization (EUA) *in vitro* POC devices (category "W") approved at the time of this review. Strengths and weaknesses should be framed around our idea for rapid, quantitative data that can be de-identified, sent to a cloud database, and then made

271 available to public health databases within 24 hours. All tests other than Cue (Type Ia) and Ellume
272 (Type Ic) require a prescription. All tests are qualitative and utilize internal controls.

Name of test (Entity)	**Type**	**RTA (target)**	**Sample**	**Strengths**	**Weaknesses**
Lucira COVID-19 All-In-One Test Kit	Ia	RT-LAMP assay with colorimetric transduction based on pH-sensitive halochromic agents (N gene)	NP swab	30 min turnaround; 14 years and older; digital readout	Specialty equipment required; entire device is disposable; ability to detect in presence of other viruses unclear
BioFire Respiratory Panel 2.1-EZ	Ia	Nested multiplex PCR (S gene; M gene)	NP and nasal swab	multiplex detection (16 different viruses and 4 bacteria); 6 years and older; 45 min turnaround	requires trained specialist to conduct test
Xpert Xpress SARS-CoV-2/Flu/RSV	Ia	RT-PCR (E gene; N2 gene)	NP swab, nasal swab, nasal wash/ aspirate	Multiplex detection (CoV-2, inf. A, inf. B, RSV); 6 years and older; 30 min turnaround	no discrimination between N2 and E gene; requires trained specialist to conduct test
Mesa Biotech Accula SARS-Cov-2 Test	Ia	RT amplification with LFA (N gene)	NP and nasal swab	Partial automation, for patients 5 years and older; 30 min turnaround	No discrimination between SARS-CoV; requires specialty equipment; visual readout (no digital data)
Cobas SARS-CoV-2 & Influenza A/B Nucleic Acid	Ia	RT-PCR (ORF1a/b Nsp; N gene)	NP and nasal swab (self-collected)	Multiplex detection (CoV-2, inf. A inf. B); internal controls; partial automation; digital readout; 20 min turnaround	No differentiation between CoV; health supervisor and specialty equipment required
Abbott ID NOW COVID-19	Ia	Molecular RT, Isothermal amplification (target not identified)	NP, nasal, throat swabs	Partial automation; digital readout; 13 min turnaround	No differentiation between CoV; health supervisor, specialty equipment and trained personnel required
Cue COVID-19 Test	Ia	Isothermal RT-PCR	Nasal swab	Partial automated; RNase P control; partial automation;	Requires iPhone 8+; no differentiation CoV; direct visual readout;

(smartphone)		(N gene)		no prescription required; 25 min turnaround	specialty equipment required
Ellume COVID-19 Home Test	Ic	LFA (N protein antigen)	Nasal swab	Smartphone based test; authorized for children 2 years and older; 15 min; no prescription required	No differentiation between SARS-CoV and SARS-CoV-2; analyzer and battery must be discarded after use
CareStart COVID-19 Antigen test	Ic	Immuno-chromatographic LFA (N protein antigen)	NP swab	Partial automation; 6 years or older; 10 min turnaround	No differentiation for CoV; direct visual readout has 5 min window
LumiraDx SARS-CoV-2 Ag	Ic	Microfluidic turn-on fluorescent immunoassay (N protein antigen)	NP swab	Partial automation; 5 years and older; digital readout; 12 min turnaround	No differentiation between CoV;health supervisor required; direct visual readout; specialty equipment
Siemens BinaxNOW COVID-19 Ag Card Home Test	Ic	LFA immuno-chromatographic assay (N protein antigen)	Nasal swab	Simple one-step test; 15 years or older; 30 min turnaround	Requires iPhone IOS 11 or Android V8; Qualitative; must be performed with the supervision of a telehealth proctor
Sofia 2 Flu + SARS Antigen FIA	Ic	Multiplexing LFA, turn-on fluorescent immunoassay (N protein antigen)	NP and nasal swab	Multiplex detection (CoV-2, inf. A, inf. B); partial automation; 6 years and older; 15 min turnaround; digital readout	No differentiation between CoV; health supervisor and specialty equipment required
BD Veritor System for Rapid Detection of SARS-CoV-2	Ic	Immuno-chromatographic LFA (N protein antigen)	Nasal swab	Point of care testing with proven instrument; partial automation; digital readout; simple one-step test; 15 min turnaround	Health supervisor and specialty equipment required

RTA= Recognition-Transduction-Acquisition scheme.
NP swab = Naso-pharyngeal swab.
LFA= Lateral flow assay.
inf. A= influenza A.
inf. B= influenza B.
RSV= respiratory syncytial virus.
Se= Serological test (neither Type I nor Type II).*

4. SARS-CoV-2 detection schemes under development

Table 3 shows the tools published to date that are under development using Type I detection.

Type Ia detection (oligo binding in lysate): The primary SARS-CoV-2 targets for Type Ia assays were N gene or Nsp12 (RdRp), but detection of E gene and crRNA have also been demonstrated. For MERS-CoV, detection assays have been developed for targeting the RLKGG cleavage site and N gene. RTA schemes for Type Ia (oligo-binding) were diverse, including DNA hybridization with either SPR or colorimetric transduction. Other devices were based on RT-LAMP assays, CRISPR-Cas systems, or luciferase systems based on papain-like protease (PLpro) activity. The most common sample was NP swab, but testing to date also included plasma samples, throat swabs, and sputum. HEK293T cells and Ersatz solutions (multigene mixtures in buffer) were also tested but have low clinical relevance. The LOD for Type Ia devices published to date ranges from 100 fM (RdRp gene) to 220 fM (Nsp12), or from 1 to 2 copy/μL for PCR and CRISPR based tools.

Type Ib detection (aptamer binding in lysate): Type Ib tools (apta-detectors) have been developed for targeting either N protein or Nsp12 and tested in samples derived from human serum, sputum, urine and NP swabs (see supplemental section for sequences and K_D values for all aptamers in this review). RTA schemes include aptamer-based sandwich assays (nAu reporter), turn-on fluorescent systems with splint-based RNA detection, electro-chemiluminescent devices (labeled-DNA aptamer tetrahedrons), and naked eye quantum dot (QD) chip systems. The LOD of these emerging devices ranges from 0.1 pg/mL (QD chip in buffer) to 10ng/mL (ELISA and LFA). Response time was between 15 and 120 min, a range which is applicable to high throughput screening if clinical specificity thresholds are met. Chen et al [68] suggest that aptamers targeting N protein require two stem loops, but the study did not investigate a wide range of secondary structures so the results are inconclusive. Generalizations about secondary structure aside, there is a need to understand the effect(s) of electrostatic interactions on aptamer binding. SARS-CoV-2 is negatively charged at physiological pH [69]. Isoelectric potential of S, E, and M proteins are 6.2, 8.6, and 9.5, respectively [70]. Electrochemical biosensors are particularly sensitive to Debye shielding and zwitterionic storm at the electrode surface.

Type Ic detection (Ab binding in lysate): Type Ic tools (Ab-based detection) have been developed for N protein but a few devices have also been developed for targeting S1 subunit in lysate. Monoclonal antibodies (MAb) and polyclonal antibodies have each been used with varying degree of success, but one of the more promising approaches is the monobodies developed through phage display by Kondo [71], which may also have therapeutic potential based on S1 RBD-ACE1 binding assays. When applied in ELISA assays the performance was poor relative to other Type Ic devices, it remains to be seen if this approach has value in rapid detection. Beyond ELISA and RT-PCR, FET and fluoresce were also shown to viable, although the clinical performance remains to be proven. Compared to other Type I devices, Ab-based tools had a poor LOD and a similar response time (30 to 120 min) in nasal swab, NP swab, saliva, and urine. A label-free FET device targeting SARS-CoV (N protein) using Ab-mimic proteins functionalized on In_2O_3 nanowires [72] may have promise for application in SARS-CoV-2 but has not been shown thus far.

Type Id detection (lectin binding in lysate): To date, no Type Id tools (lectin sensors) have been demonstrated for detection of capsid lysate from SARS-CoV-2. However, there are numerous targets that can be explored in viral lysate, a review of lectins from non-mammalian sources provides details [73], including mannose-binding lectins (MBL), Ca^{2+}-dependent lectins (C-type), N-acetyl-glucosamine-binding lectins (N-type), fucose-binding lectins, and the super-family of I-type lectins (immunoglobulins excluding Ab and T cells). Use of lectins as biorecognition elements for analysis of viral lysate in other systems [33–37] provides confidence that the tool could be useful if rigorously tested and combined with other tools.

Table 3: Summary of tools developed for SARS-CoV-2 detection based on Type I detection (lysed capsid) and Type II (intact capsid) targets. See supplemental section for SARS-CoV and MERS detection.

Type	SARS-CoV-2 Target(s)	RTA	Sample(s) Tested	LOD	t_{95} [min]	Reference
Type Ia	RdRp (Nsp12)	Hybridization: LSPR combined with plasmonic photothermal effect	Multigene mixtures (ersatz)	220 fM	60	Qiu [74]
	S, N genes	Isothermal rolling circle amplification (RCA) for rapid detection of SARS-CoV-2	NP swab	1 copy/ μL	120	Chaibun [75]
	E, N genes	RT-LAMP on LFA based on CRISPR-Cas12 detection	NP swab	1 copy/ μL	40	Broughton [76]
	ORF1ab, N genes	Reverse transcription LAMP	NP swab	12 copies/ reaction	60	Zhu [77]
	4,500 crRNA	CRISPR-Cas13a (CARMEN-Cas13)	Plasma, NP, throat swabs	NR	120	Ackerman [78]
	ORF1ab, N gene	RT-PCR CRISPR-Cas12a fluorescent reporter assay	NP swab	2 copies/ μL	50	Huang [79]
Type Ib	N protein	DNA aptamer-based sandwich assay; nAu reporter for ELISA and LFA	Diluted serum	10 ng/mL	120	Chen [68]
	N protein	DNA aptamer-invertase magnetic bead assay on commercial glucometer (upon binding, antisense strand is displaced and activates invertase).	saliva	4.4 pM	NR	Singh [80]
	N protein, S protein (S1)	Aptamer-assisted proximity ligation assay with qPCR (fluorescence)	Human serum	31 pg/mL	120	Liu [81]
	N protein	Sandwiched aptamers in ELISA assay with Au label	Sputum, urine, serum	1 ng/mL	15	Zhang [82]
	RdRp (Nsp12)	DNA aptamer reporter (turn on) for sensitive splint-based RNA detection (SENSR) using T7 promoter system	NP swab	14 pg/mL	60	Woo [83]
	RdRp (Nsp12)	Electro-chemiluminescence based on labeled-DNA tetrahedrons	Human serum	0.4 pg/mL	60	Fan [84]
Type Ic	S protein (S1 RBD)	Monobodies (TRAP display with BLI) for sandwich ELISA assay and RT-PCR	Nasal swab	76.5 ng/mL	120	Kondo [71]

	N protein	Dual-labeled magnetic nanobeads for immunomagnetic signal amplification	Serum	230 pg/mL (whole serum) 100 pg/mL (dilute serum)	60	Li [85]
	S protein (S1 RBD); N protein	E-chem ELISA, PAb labeled with alkaline phosphatase on magnetic beads	Saliva, NP swab	19 ng/mL (S1); 8 ng/mL (N)	30	Fabiani [86]
	N protein	Fluorescence immuno-chromatographic assay	Urine, NP swab	NR	10	Diao [87]

RTA= Recognition-Transduction-Acquisition scheme.
t_{95}= Response time.
LFA= Lateral flow assay.
ELISA= Enzyme linked immunosorbent assay.
E-chem= Electrochemical assay (cyclic voltammetry).
FET= Field effect transistor.
NP= Nasopharyngeal swab.
SERS= Surface-enhanced Raman spectroscopy;
TRAP= Transcription-translation coupled with association of PuL;
BLI= Bio-layer interferometry;
MAb= Monoclonal antibody;
PAb= Polyclonal antibody.
NR=Not reported.

Table 4 shows the tools published to date that are under development using Type II detection (no EUA or CLIA authorization).

Type IIa detection (aptamer sensors for intact virus): Only a few Type IIa tools (apta-sensors) have been developed, and all devices targeted S protein (S1 subunit) and were tested in either buffer or diluted saliva (see supplemental section for sequences and K_D values for aptamers). RTA schemes include FET devices on silica thin films, thiolated aptamers on gold-sputtered polystyrene film electrodes with voltametric transduction, and a cascade based on invertase following aptamer displacement (amperometric glucometer for acquisition). Two of the devices employed the same aptamer (developed by Song et al [41]), which was a 51-nt with three hairpins (two hairpins joined on the dista5′ arm), and the other device used a 51-nt triple hairpin structure with a 15-nt antisense strand on the 3′ arm developed by Singh et al [80]. The LOD of these Type II aptasensors was 1 to 6 pM with response times of 30 to 60 minutes. Only two aptamers have been tested to date. Devi and Chaitanya [88] designed a number of S protein peptide aptamers *in silico* aptamers but these have not yet been tested for detection. Although more testing is required, the 26-nt DNA aptamer targeting S protein RBD by Sun et al [89] may be an interesting candidate for testing (KD=0.13 nM; no error reported). For electrochemical devices in body fluids such as saliva, regulation of ion concentration in the test medium may prove to be one of the most critical steps as it can influence signal-to-noise ratio (*see Table S1 in supplemental section*).

Type IIb detection (Ab detection of intact virus): Limited Type IIb devices have been developed to date, but a few devices targeting S protein RBD have shown results consistent with other devices. RTA schemes were either based on graphene FET or nAu-based SERS in either buffer or NP swab samples. Detection time was from 30 min to over 3 hours with LOD as low as 4 fM.

Type IIc detection (lectin binding of intact virus): To date, no Type IIc tools (lectin sensors) have been demonstrated for detection of glycan-shields on S protein but we include the category here due to

the potential for improving assays by including a control that targets the S protein glycan shield. Datta et al [22] articulate the importance of this interaction as a positive control using C-type and I-type lectins. This concept was also proposed by Rahimi in mapping SARS-CoV-2 targets to various lectins [90]. Beyond targeting sugar residues such as glycan with C-type and I-type lectins (positive control), Datta et al [22] also describe a negative control system for other microorganisms common to respiratory samples of interest. While no Type IIc sensors have been developed, yet, many studies have investigated the role of lectins in reversible binding of sugar residues on the surface of coronaviruses, including MERS [91,92], SARS-CoV [93,94], and SARS-CoV-2 [95–98]. Currently, lectins are being explored for use as antivirals targeting extra-capsid structures such as the glycan shield [99–102].

Type IId detection (membrane receptor binding of intact virus): There are few examples of the last tool category, Type IId detection, involves use of membrane receptors (e.g., ACE-2 and engineered membrane proteins) for binding intact virion particles. Chang et al [103] developed a sensor for S protein detection using human ACE-2 adsorbed on gold electrodes for SARS-CoV but to date no ACE-2 biosensors have been reported for SARS-CoV-2. Guo developed an assay for detecting SARS-CoV-2 S protein based on recombinant mammalian (Vero) cells with exposed human chimeric Ant-spike S1 antibody and an electrochemical readout. However, to date Type IId devices have not been used as a positive control in multiplex assays, an idea which may improve rapid screening tools if the materials are optimized [22].

Table 4. Summary of tools developed for SARS-CoV-2 detection based on Type II detection (intact capsid). See supplemental section for Type II biosensors developed for SARS-CoV and MERS.

Type	Target	RTA	Sample(s) Tested	LOD	t_{95} [min]	Reference
Type IIa	S protein (S1)	DNA aptamer on silicon thin film FET	PBS buffer	1 pM (1nM upper)	NR	Farrow [104]
	S protein	DNA aptamer-invertase magnetic bead assay on commercial glucometer (upon binding, antisense strand is displaced and activates invertase).	saliva	5.8 pM	30	Singh [80]
	S protein (S1)	Thiolated DNA aptamer on gold-sputtered polystyrene film electrodes	Diluted saliva (10%)	1.3 pM	60	Zakashansky [105]
Type IIb	SARS-CoV-2 S protein	Anti-S Protein on graphene FET	NP swab	16 PFU/mL (media); 242 copies/mL (clinical)	240	Seo [50]
	SARS-CoV-2 S protein	Monoclonal anti-S coated nano-Au SERS	Buffer	4.2 fM	30	Ahmadivand [106]

Type IIc	NA	NA	NA	NA	NA	NA
Type IId	SARS-CoV-2 S protein (S1 subunit)	Recombinant mammalian (Vero) cells with exposed human chimeric receptor	Buffer	1 fg/mL	180	Guo

RTA= Recognition-Transduction-Acquisition scheme;
t_{95}= Response time;
LFA= Lateral flow assay;
ELISA= enzyme linked immunosorbent assay;
E-chem= Electrochemical assay (cyclic voltammetry)
FET= Field effect transistor;
NP= Nasopharyngeal swab;
SERS= surface-enhanced Raman spectroscopy.
NA=not available;
NR=not reported.

Numerous papers have suggested various other design strategies for SARS-CoV-2 detection, including thio-NAD cycling [107], micropillar PDMS platforms with CRISPR detection [108], and nanoparticle bioassays [22], for example. However, to date the devices in **Table 3-4** are the only published biosensors at the time of this review, and the published literature is lacking in terms of rigorous clinical testing of these emerging tools. Further, the published devices do not show comprehensive and rigorous controls required to determine clinical relevance of the device.

5. Challenges and Opportunities in Type II SARS-CoV-2 diagnostics

There are numerous challenges to development of Type II diagnostic tools. Below we briefly summarize major issues related to first-principles engineering and systems-level considerations. In a follow up review, our team will discuss additional challenges and opportunities for CADS in detail.

First-principles engineering (RTA)

Fundamental research in diagnostic tools requires analysis through the lens of first principles. This fundamental approach is critical to avoid catastrophic mistakes at the clinical stage. Engineering first principles are based on the RTA triad, which includes molecular recognition, signal transduction and acquisition [7,109]. The nature of molecular interactions between viral targets and sensor nanostructures is the most granular level of analysis, and this is intimately coupled with transduction events that may include energetic changes of electrons or photons. To date, Type II devices have focused on recognition of S1 protein based on DNA aptamers, immuno-specific affinity, stratified biomolecule cascades, and whole cell biosensor systems. While each of these represent a proof-of-concept demonstration, rigorous control studies are needed to understand sample matrix effects before any success can be translated to clinical testing. High throughput technologies (e.g., surface plasmon resonance, biolayer interferometry) may offer validation of binding affinity in complex mixtures. In addition, fundamental studies to show that biomaterials are stable under testing conditions are necessary. Incorrect biomaterial arrangement and architecture are known to cause catastrophic failure if the structure becomes unstable during testing [110,111]. In addition to detailed studies of molecular recognition and material stability, there is limited data on the use of nanomaterials for enhanced transduction and material biocompatibility.

Systems-level considerations

There are many challenges to digitizing public health care at a large scale [112]. The specific design choices for selecting hardware, data curation methods, and analysis are critical to the outcome, and thus the value, of the detection tool [7,109] . Connected devices may enable the underserved population to access at least some facet of public health service using smartphone-based non-invasive rapid detection of infectious agents. Budd et al [113] reviewed current digital technologies available for surveillance, identification, tracing and evaluation of COVID-19 in 2020, focusing on legal, ethical, data privacy, organizational and workforce barriers. Although beyond the scope of this review, in a follow up review our team will discuss critical gaps in the discussion, including: i) analytical versus clinical performance, ii) data connectivity, iii) multiplexing and data fusion, iv) distinguishing infective vs. non-infective SARS-CoV-2, and v) testing access and equity.

Supplementary Materials

Supplemental material are included.

Author contributions

Conceptualization, E.S.M., S.P.A.D.; Methodology, Y.T., C.G., D.V. and E.S.M.; Formal Analysis, Y.T. and E.S.M.; Data Curation, E.S.M.; Writing – Original Draft Preparation, E.S.M. and S.P.A.D.; Writing – Y.T., C.G., E.A., D.D., T.-R. T., S.P.A.D., D.C.V., E.S.M.; Review & Editing, E.S.M., S.P.A.D., and D.V.; Visualization, E.S.M.; Supervision, E.S.M.; Funding Acquisition, C.G., D.J., S.P.A.D., D.C.V., E.S.M.

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Conflict of interest

The authors declare no conflicts of interest.

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