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Review Article

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A narrative review of nine commercial point of care influenza tests: an overview of methods, benefits, and drawbacks to rapid influenza diagnostic testing

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Abstract

Context: Rapid influenza diagnostic tests (RIDTs) are becoming increasingly accurate, available, and reliable as the first line of testing when suspecting influenza infections, although the global burden of influenza infections remains high. Rapid diagnosis of influenza infections has been shown to reduce improper or delayed treatment and to increase access to diagnostic measures in public health, primary care, and hospital-based settings.
Objectives: As the use of RIDTs continues to expand in all healthcare settings, there is a multitude of molecular techniques being employed by these various testing platforms. With this in mind, we compare the sensitivity, specificity, and time to diagnosis for nine highly utilized commercial RIDTs.
Methods: Nine commercially available RIDTs were identified from the US Centers for Disease Control and Prevention (CDC) website, which were also referenced on PubMed by name within the title or abstract of peer-reviewed publications examining the sensitivity and specificity of each test against a minimum of three influenza A virus (IAV) strains as well as seasonal influenza B virus (IBV). Data from the peer-reviewed publications and manufacturers' websites were combined to discuss the sensitivity, specify, and time to diagnosis associated with each RIDT.
Results: The sensitivity and specificity across the examined RIDTs were greater than 85.0% for both IAV and IBV across all platforms, with the reverse transcriptase–polymerase chain reaction (RT-PCR) assays maintaining sensitivity and specificity greater than 95.0% for all viruses tested. However, the RT-PCR platforms were the longest in time to diagnosis when compared to the other molecular methods utilized in the examined RIDTs.
Conclusions: Herein, we discussed the benefits and limitations of nine commercially available RIDTs and the molecular techniques upon which they are based, showing the relative accuracy and speed of each test for IAV and IBV detection as reported by the peer-reviewed literature and commercial manufacturers.

Keywords: flu testing; influenza; influenza diagnostics; molecular diagnostics; point of care testing; rapid test; RIDT.

Annually, 3 million to 5 million people globally are infected with influenza, resulting in 290,000 to 650,000 deaths [1]. Common clinical signs and symptoms of influenza infections are fever, pharyngitis, cough, arthralgia, malaise, and rhinorrhea [2, 3]. These symptoms are often also seen in many other viral upper respiratory infections (URIs), such as those caused from SARS-CoV-2 (COVID-19), respiratory syncytial virus (RSV), parainfluenza, adenovirus, and rhinovirus infections [4]. These respiratory viral infections commonly produce subtle and occasionally indistinguishable differences in the symptomatology seen in a clinical setting [2, 3]. However, differentiating between influenza infections from the other commonly seen

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respiratory pathogens has proven significant for the appropriate clinical management of these patients [2, 3, 5]. The current diagnostic gold standard for influenza A virus (IAV) and influenza B virus (IBV) identification is through reverse transcriptase–polymerase chain reaction (RT-PCR) along with the reference standard of viral culture methods to confirm the presence of the influenza virus [3, 6]. Both of these are regarded as the most sensitive and specific testing methodologies for viral detection [3, 6]. However, these diagnostic standards often take valuable time and resources that are not always afforded in all settings, prompting the increased development of rapid diagnostic testing. Through the increased development and utilization of rapid influenza diagnostic tests (RIDTs), it has been reported that rapid diagnosis of influenza infections leads to quicker administration of appropriate antiviral therapy, improved antibiotic stewardship, decreased hospital stays, decreased mortality rates associated with IAV and IBV, as well as decreases in the average cost per test for patients when compared to PCR or viral cultures [7–15].

Sparked by the 2009 H1N1 influenza pandemic, innovation in influenza diagnostics resulted in cheaper and faster delivery of results, ultimately improving access to testing [16]. While these factors have helped reduce socioeconomic burdens impacting accurate diagnostics of influenza infections, faster results from the RIDTs have also been coupled with variable sensitivities and specificities of these tests when compared to the RT-PCR diagnostic workflows [17]. These RIDTs have been developed utilizing a broad scope of molecular detection methodologies, including antibody-mediated immunoassays, RT-PCR, and isothermal nucleic acid amplification tests (Isothermal NAATs) to target various components of the influenza virus for accurate detection [18–21]. These currently utilized and US FDA-approved rapid viral diagnostics technologies are all rooted in either antibody-mediated antigen detection or nucleic acid detection [10, 16]. While the immunological based methods utilize the presence of an antigen or antibody for the detection of proteins specific to influenza viruses, the nucleic acid detection methods target the genetic makeup of the virus, amplifying specific regions of RNA [19]. In utilizing these rapid detection methods, some of the current RIDTs are only able to test for influenza without distinguishing between the clinically relevant types A or B due to their targeting of highly conserved regions of the influenza genome [21–23].

The global pandemic caused by COVID-19 highlighted the need for quickly deployable and accurate viral testing that could be conducted with minimal resources. Although this need was vastly exposed through the COVID-19 pandemic, it was merely an amplification of the existing need for accurate and accessible viral testing for all communities. With accurate viral diagnostics sitting as the cornerstone of public health responses to outbreaks and treatment efforts, rapid diagnostic testing provides an opportunity for primary care providers and public health agencies to increase access to virus detection outside of the hospital and large health systems settings [9, 10]. Herein, we discuss the basis of the molecular biology techniques employed in nine commonly utilized commercial RIDTs, while examining sensitivity, specify, and some of the other benefits and pitfalls of these tests. To our knowledge, no current review exists combining the reported real-world data on point-of-care influenza tests from peer-reviewed studies juxtaposed to the reported sensitivity and specificity of the manufacturers. Our purpose of this narrative review is to provide a comprehensive resource to clinicians, researchers, and public health officials examining the various methodologies utilized in RIDTs while evaluating the current literature reporting on the utility of these commercial assays in the detection of IAV and IBV.

Methods

Study design

Nine commercially available RIDTs were identified through the US Centers for Disease Control and Prevention (CDC) website, which showed a list of FDA-approved RIDTs that were also referenced by name in PubMed-indexed publications examining sensitivity and specificity of a minimum of three IAV strains [24]. Publications examining the sensitivity of IAV/IBV detection in clinical and public health settings were included in this review, while those evaluating the co-detection of COVID-19 and IAV/IBV were excluded from the review. Each referenced RIDT's reported time to diagnosis was obtained from the manufacturer's protocol published on each of their respective commercial website. The IAV/IBV sensitivity and specificity is reported as a combination of data points from the manufactures' websites and PubMed-indexed studies that reported sensitivity and specificity of any of the tests when calling out the RIDT by name in the title or the abstract. Only peer-reviewed data published within the last 10 years that described studies containing a minimum of 30 human clinical samples were included in the evaluation of the sensitivity and specificity of the RIDTs.

RT-PCR and RT-qPCR: RT-PCR is the current gold-standard methodology for molecular detection of RNA viruses, such as influenza and many other common respiratory viruses [3]. In RT-PCR, reverse transcriptase converts the single-stranded, genomic viral RNA into cDNA prior to the standard PCR amplification of a targeted gene product to confirm the presence or absence of viral RNA in the sample [25]. Optimized primer and probe mixtures utilized in the PCR methodology are virus RNA-specific, targeting conserved regions of the viral genome [26–28]. Although the traditional RT-PCR methodology has demonstrated high sensitivity and specificity for influenza detection, this technology requires multiple reagents and laboratory equipment

such as a thermocycler, a biosafety cabinet, and consumable plastics [25]. Therefore, these workflows require that these tests be completed at central pathology and/or public health laboratories and may be seen as cost-prohibitive for individual medical practices to run independently. Currently, there are multiple commercially available RT-PCR–based diagnostic platforms, including a multitude of multiplex primer reactions that are sent off to a laboratory for processing, as described previously. Additionally, some of the commercially available point-of care influenza diagnostics examined in this review also employ RT-PCR–based technology, implementing condensed versions of the equipment and reactions utilized in the large-scale pathology and public health labs for IAV/IBV detection.

In addition to the RT-PCR detection modality, a later iteration of the technology has grown in popularity among diagnostic laboratories, real-time or quantitative RT-PCR (RT-qPCR). RT-qPCR follows the same pattern as described previously, with the only major difference being the inclusion of a read step after each individual cycle to monitor in real time the amplification occurring as the PCR progresses. This technology produces a readout of cycle threshold (Ct) values denoting the cycle in which a given sample begins to show target amplification. RT-qPCR technologies have been employed in diagnostics to provide increased quantitative information from each sample. The World Health Organization (WHO) and US CDC have issued guidelines for these tests, indicating that any diagnostic RT-qPCR panel should only be considered positive for the targeted sample if the Ct values fall at 40 cycles or below [1, 6]. Aside from the increased data provided by these tests, there are currently no approved differences in diagnostic or therapeutic utility of RT-PCR vs RT-qPCR, and both remain highly utilized in viral diagnostic technology dependent on the equipment available in each laboratory.

The Xpert Xpress Influenza A/B (Cepheid, Sunnyvale, CA) and the Cobas Influenza A/B Nucleic Acid Test (Cobas, Roche, Basel, Switzerland) provide real-time RT-PCR–based diagnostic solutions for point-of-care testing [22]. The diagnostic platforms from Cepheid and Cobas are CLIA-waived, cassette-based RT-PCR testing that can be completed in the office, allowing for similar accuracy to traditional RT-PCR/RT-qPCR workflows without the need for certified laboratory personnel or multiple different machines for sample preparation and processing [25, 29].

Isothermal nucleic acid amplification assays: Isothermal nucleic acid amplification is a relatively new molecular biology technique that is similar to the previously mentioned RT-PCR in that it amplifies targeted DNA or RNA nucleic acid sequences, yet unique because it does not require temperature fluctuations for the amplification process to complete, thus ruling out the need for a thermocycler [30]. Additionally, these reactions are contained to a single tube, reducing the overall resource burden for completing testing [30, 31]. In this review, we discuss two different isothermal techniques utilized in RIDTs, nicking enzyme amplification reactions (NEAR) and loop-mediated isothermal nucleic acid amplification (LAMP) [30]. The Abbott IDNow Influenza A&B test (Abbott Laboratories, Chicago, IL) is based on the NEAR technology, while many laboratory-derived rapid respiratory virus panels are in development utilizing LAMP [22, 30].

Both NEAR and LAMP assays identify and amplify RNA fragments utilizing multiple primers that continue to bind and replicate different locations on the targeted nucleic acid sequence until the targeted sequence is replicated in its entirety. Once the full target sequence has been created, the primers continue to amplify this sequence in a similar fashion to the PCR reactions described above [25, 30]. The use of multiple primers creating multiple fragments allows for an increased production of targeted nucleic acid sequences in the form of amplicons, or synthesized DNA, when compared to standard PCR reactions working with a single primer set per target sequence. This rapid amplification of the target sequence allows for either chromatography visualization of the amplicons or fluorescent labels to measure the intensity of amplification for determining the relative concentration of the amplicons produced [30].

Specifically, the Abbott IDNow Influenza A&B test (Abbott Laboratories, Chicago, IL) targets RNA segments PB2 and PA for both A and B, respectively, with fluorescently labeled probes [22, 32]. When amplification occurs and the complete target sequence is produced, the probes will bind to the amplicon and fluoresce when attached to their corresponding nucleotide sequence. This fluorescence is picked up by the IDNow instrument, qualifying a positive IAV or IBV detection depending on which fluorophore is emitted [32].

Lateral flow immunoassay: RIDTs detect specific influenza viral antigens, usually nucleocapsid proteins (NPs), from swabs or aspirates by utilizing lateral flow immunoassays (LFA) such as immunochromatographic (ICT), fluorescent (FIA), and chemiluminescent methods. These methods utilize the enzyme-linked immunosorbent assay (ELISA) sandwich technique with slight variations in detecting the capture antibody-analyte complex. Patient samples are obtained via nasal, buccal, or nasopharyngeal swabs and mixed into a dilutional reagent prior to processing. The samples may then be processed to allow for denaturation of the extracellular matrix and inactivation of the virus. The analyte is then placed on a sample pad that allows for migration through the conjugate release pad down to the nitrocellulose membrane. The conjugate release pad contains antigen-specific labeled antibodies. The nitrocellulose paper contains test lines that capture targeted antibodies and an internal control line that serves as quality control to validate each sample. When the analyte-antibody complex reaches the test line, the conjugated antibody will react with the capture antibodies, producing a visibly positive result on the membrane [18]. The entirety of this process typically takes 15–20 min from the start of the test.

Currently, the majority of the RIDTs on the market are lateral flow assays [11, 16, 24]. Veritor Rapid Flu A + B (Becton, Dickinson and Company, Franklin Lakes, NJ), BinaxNOW Influenza A and B (Abbott Laboratories, Chicago, IL), X/pect Flu A&B Test (Thermo Fisher Scientific, Waltham, MA), Status Flu A&B (Lifesign Healthcare, New Delhi, India), Immunocard STAT! (Meridian Bioscience Inc., Cincinnati, OH), and OSOM Ultra Flu A&B (Sekisui Diagnostics, Burlington, MA) are the named RIDTs in this manuscript utilizing lateral flow technology to detect the presence of IAV and IBV nucleoprotein (NP) antigens [18].

Results

Sensitivities and specificities reported for these nine commercially available RIDTs were determined from the manufacturers' conducted studies, independent peer-reviewed trials, and meta-analyses published within the last 10 years (Table 1). Table 2 describes the names, specimen types, sensitivity, and specificity for these tests against both IAV and IBV detection [23, 34–46]. Whereas

Table 1: Listing of the number of peer-reviewed publications listed in the PubMed search for each test, following the inclusion and exclusion criteria laid out in the Methods section of this article.

Product name	Number of PubMed publications satisfying inclusion criteria
BinaxNOW Influenza A and B	37
Cobas Influenza A/B Nucleic Acid Test	29
Xpert Xpress Influenza A/B	19
Veritor Rapid Flu A + B	18
ID Now Influenza A&B 2	6
X/pect Flu A&B Test	2
Immunocard STAT!	2
OSOM Ultra Flu A&B	1
Status Flu A&B	0

manufacturers reported their sensitivity and specificity to be on the higher end of the ranges, the meta-analyses and other peer-reviewed trials reported many studies having lower sensitivities and specificities, producing the ranges of values for these categories denoted in Table 2 [29, 33].

When conducting a PubMed search for any publications calling out the commercial RIDTs by name in either the abstract or the title, the Veritor, Xpert Xpress, BinaxNOW, and Cobas NAAT tests had significantly more results than the remaining five tests. Abbott's BinaxNOW Influenza A and B has the most named studies in the peer-reviewed literature, with 37 results appearing on PubMed following our search criteria, while Status Flu A&B had the fewest, with no peer-reviewed references under our inclusion criteria (Table 1).

Table 3 demonstrates the products along with their type of testing method and the time to results reported by the manufacturers [32, 33, 41–46]. The table shows that one of the newer RIDTs, known as ID NOW Influenza A & B, can obtain results in the shortest period of time, 5–13 min, whereas the RT-PCR gold-standard method produces results in roughly 30 min [3]. On average, the most commonly utilized methods in the clinics today are LFAs that take 10–15 min on average to produce results (Table 3) [2, 5–7, 24].

Discussion

Herein we compiled a thorough list of the commonly utilized, commercially available RIDTs from the US CDC's listing of FDA-approved influenza diagnostic tests to provide an overview of the molecular methods, sensitivities, specificities, and time to results. As seen by the high variability in test sensitivity in Table 2, the many studies incorporated in this review have shown that the reported sensitivities and specificities by manufacturers' protocols are not always comparable to what is observed in a variety of clinical and public health settings. For every assay examined in Table 2, the upper end of the sensitivity and specificity ranges reported comes from the manufacturers' own reported data on the tests, with all nine reporting IAV and IBV sensitivity and specificity greater than 90.0% when compared to RT-PCR or viral culture [32, 33, 41–46]. However, as seen in Table 2, the range of reported sensitivities stretches far below the manufacturers-reported 90.0% for every test in both IAV and IBV detection. This implies that the real-world data obtained from the peer-reviewed studies included in this review demonstrates that the clinical performance of these assays does not always mirror the initially reported data by the manufacturers. The large disconnect between the data reported from the manufacturers on the clinical performance of their assay and the data that are reported in the peer-reviewed literature is not to be overlooked. This significant overestimation of performance by the manufacturers could result an in a high rate of false negatives that directly impact patient and community health.

However, there is a critical need for expanded utilization of RIDTs in both the public health and primary care fields, with accurate viral diagnostics becoming the cornerstone for infection prevention and quick and appropriate therapeutic initiation [10, 15]. Although sensitivity and specificity are shown to greatly fluctuate with many of the RIDTs, the utility of rapid testing with the potential for false readouts still outweighs foregoing testing or waiting days for results to come in, especially when dealing with a viral illness in which time is critical for appropriate antiviral initiation [15]. It is our opinion that these technologies, while having technical limitations, provide a strong benefit to increasing accurate viral diagnostic testing capabilities. The thermostability and the low cost associated with many of these tests makes them ideal candidates for field implementation or utility in primary care offices, yet neither of these settings may have access to the advanced pathology testing laboratories that typically house the large-scale PCR equipment utilized in viral diagnostics.

Of the influenza testing methods discussed, conventional RT-PCR remains the gold standard due to its excellent sensitivity and specificity [3]. Although this method of accurate detection of influenza is clinically important, there are many difficulties to overcome in implementing traditional RT-PCR workflows into medically rural and underserved communities where primary care and public health efforts are often at work attempting to provide

Table 2: A comparison of sensitivity and specificity for the detection of IAV and IBV strains as reported by the manufacturers and the current peer-reviewed literature.

Product name		Specimen type	Sensitivity IAV	Specificity IAV	Sensitivity IBV	Specificity IBV
Veritor Rapid Flu A + B	Becton Dickinson and Co.	NP A/W	64.0–94.0%	97.5–99.2%	64.0–94.2%	97.5–99.8%
BinaxNOW Influenza A and B	Alere; Abbott	NPS & N A/W	4.4–100.0%	91.7–100.0%	80.8–93.0%	97–99.5%
Remel X/pect Flu A&B Test	Thermo Fisher Scientific	NW, NS, TS,	36.3–100.0%	98.4–100.0%	83.3–100.0%	100.0%
Status Flu A&B	Lifesign	NPS/NS, NP A/W	85.1–91.4%	95.7–100.0%	85.7–87.6%	95.9–99.5%
ID Now Influenza A&B 2	Abbott	NPS, VTM	92.8–96.3%	97.4–99.2%	97.2–100%	97.1–98.8%
Immunocard STAT!	Meridian Bioscience, Inc	NS/NPS, NP A/W	66.7–98.4%	92.2–99.4%	86.4–94.4%	99.0–100.0%
OSOM Ultra Flu A&B	Sekisui Diagnostics	NPS/NS, NP A/W	85.1–91.4%	95.7–100%	85.7–87.6%	95.9–99.5%
Xpert Xpress Influenza A/B	Cepheid	NS, NPS	94.4–100.0%	97.6–100.0%	91.7–100.0%	98.4–99.3%
Cobas Influenza A/B Nucleic Acid Test	Cobas; Roche	NPS	91.0–100.0%	96–100.0%	94.4–100.0%	97.6–100.0%

Under the "Specimen type" column, different sample types were analyzed in the detection of virus and denoted with the following designations: aspirate (A), nasopharyngeal swab (NPS), nasal swab (NS), nasal wash (NW), throat swab (TS), viral transport medium (VTM), wash (W). All tests were compared to either conventional RT-PCR of viral culture methods for the confirmation of viral presence in a given sample. Veritor Rapid Flu A + B (Becton Dickinson and Co.), Immunocard STAT! (Meridian Bioscience Inc.), and OSOM Ultra Flu A&B (Sekisui Diagnostics), Xpert Xpress Influenza A/B (Cepheid), and Cobas Influenza A/B Nucleic Acid Test (Cobas; Roche) reported their findings as positive percent agreement (PPA) and negative percent agreement (NPA) within their protocol manuscripts. To maintain consistency, we will be documenting the PPA and NPA as sensitivity and specificity for these companies. IAV, influenza A virus; IBV, influenza B virus; RT-PCR, reverse transcriptase–polymerase chain reaction.

Table 3: A depiction of the molecular techniques utilized in each of the nine commercially available RIDTs alongside the reported time required to reach a result when detecting IAV and IBV as designated by the manufacturer's approved testing protocol.

Product name	Type of test	Time to result
ID Now Influenza A&B 2	Isothermal nucleic acid amplification (NEAR)	5–13 min
Veritor Rapid Flu A + B	Lateral flow immunoassay	10 min
BinaxNOW Influenza A and B	Lateral flow immunoassay	15 min
X/pect Flu A&B Test	Lateral flow immunoassay	15 min
Status Flu A&B	Lateral flow immunoassay	11–16 min
Immunocard STAT!	Lateral flow immunoassay	15 min
OSOM Ultra Flu A&B	Lateral flow immunoassay	10–15 min
Xpert Xpress Influenza A/B	Real time RT-PCR	20–30 min
Cobas Influenza A/B Nucleic Acid Test	Real time RT-PCR	20 min

"Time to result" is defined as time from sample placement into the test until positive or negative results can be determined. If a range is given, positive results have the potential to appear as early as the front end of the range, whereas the back end is the time required to observe the test prior to the diagnosis of a negative result. IAV, influenza A virus; IBV, influenza B virus; NEAR, nicking enzyme amplification reactions; RIDT, rapid influenza diagnostic test; RT-PCR, reverse transcriptase–polymerase chain reaction.

accurate viral diagnostic strategies. Due to the cumbersome equipment and supplies associated with RT-PCR, the financial burden on both the clinical practice and patient can quickly mount when having to either establish a CLIA testing lab for in-house sample processing or specimen transport to an external testing lab. This has led to a hybrid type of method in which many primary care clinics perform their testing in-house with the LFAs or other RIDTs. However, due to the known variability in sensitivity of the current commercially available RIDTs, negative test results obtained via RIDTs for a patient with a clinical presentation highly suspicious for influenza are often sent for confirmatory testing via conventional RT-PCR or viral culture [2, 6]. While this is a more economical solution than having

to house the equipment for RT-PCR, the time to receive a confirmatory test is still slower due to transit time and the time to perform the test, ultimately delaying the appropriate treatment and care for the patient [7, 9]. However, if the test is positive, studies have shown that due to the high specificity of LFAs, the likelihood of being infected with the virus is extremely high [37–39]. Additionally, LFAs are roughly $15-$20 per test, which makes it financially reasonable to perform a quick test [37]. Through investigation via the study's PubMed inclusion criteria, individual studies document that LFA sensitivities for IAV are variable, ranging from 4.4 to 100% (Table 2) [32, 33, 41–46]. Although this is a wide range, a meta-analysis from 2017 evaluating 162 publications showed that traditional RIDTs reported a pooled sensitivity and specificity of 54.4% (95% CI, 48.9–59.8%) and 99.4% (95% CI, 98.9–99.8%), respectively for IAV, with 53.2% (95% CI, 41.7–64.4%) and 99.8% (95% CI, 99.7–99.9%), respectively, for IBV [37]. Among the RIDTs currently on the market, most are equipped with a digital reader that allows for more accurate readings when the test lines may be too faint to provide a reliable human interpretation of results [33, 35, 37]. Although the specificities are high, the wide range of sensitivities can lead to many errors in the clinical setting, such as the inappropriate use of antibiotics, the underprescribing of antivirals, the increased cost for confirmatory testing, and increased hospitalizations. In order to combat the high rates of misdiagnosed influenza cases, the FDA placed a minimum sensitivity range of 80.0% compared to RT-PCR on RIDTs in 2017 [47].

Another methodology gaining popularity in the RIDT market are nucleic acid amplification tests (NAATs), such as the Abbott ID Now platform that reported sensitivities and specificities greater than 90.0% in the reported data (Table 2). The utility of this technology is growing in its application given the accuracy of results similar to that of RT-PCR while reducing the time and resources needed to achieve viral detection [29, 33, 48]. As a NAAT-based assay, ID NOW IAV and IBV can run a test in as few as 5 min with a negative read at 13 min [32]. The quick turnaround time from this rapid-detection technology in clinical settings results in one-third the original time could drastically decrease the exposure time to other patients or staff, providing a theoretical reduction in community propagation secondary to exposure. However, the increased detection capabilities associated with NAATs also demonstrate an increased sensitivity to contamination. It has been documented that NAATs carry a high risk of false positives due to cross-contamination in the machine or preparation space with persistent genetic material or amplicons from previous samples [49]. It is essential that the spaces utilized for conducting these tests are thoroughly cleaned between samples in an attempt to minimize this risk of false positives for point-of-care testing.

In addition to the logistical and financial considerations discussed previously, our review of the literature has highlighted other factors worth noting that may impact the reported sensitivity and specificity of the RIDTs; antigenic variation, sample population demographics, and clinical sample type are all factors to consider in point-of-care influenza testing. Clinicians, researchers, and public health officials should all consider these factors when determining if an RIDT would appropriately fulfill their needs.

Antigenic variation implications on RIDTs

The interspecies transmission of the influenza virus results in its constant state of evolution annually [3]. This variability results in RNA segment changes by either point mutations leading to antigenic drift or gene reassortment leading to antigenic shift primarily within the hemagglutinin (HA) and neuraminidase (NA) glycoproteins [3]. Due to these mutations, a plethora of strains have emerged leading to varying degrees of infectivity, morbidity, and mortality around the world [3]. These changes lead to outbreaks that may result in epidemics or pandemics, such as the 2009 H1N1 influenza strain [3]. These changes in HA and NA can also lead to changes in the sensitivity and specificity of the tested strains for immunoassays [48].

However, it is worth noting that not all diagnostic assays discussed herein target the HA and NA proteins due to this risk of antigenic variation. The NP is a member of the core viral ribonucleoprotein complex (vRNP), which is relatively conserved due to its critical function in viral replication [3, 50, 51]. Due to this relative conservation of the antigenic structure and high abundance of NP in infected cells, this protein is becoming increasingly targeted in lateral flow assays and therapeutic efforts to bypass the highly variable HA and NA glycoproteins [51]. Through the targeting of NP, LFAs are attempting to account for shift and drift, but in doing so are unable to differentiate between different strains of IAV and IBV. While the inability to differentiate between strains currently holds no diagnostic or therapeutic repercussions, this limitation should be considered as future assay development occurs that may or may not need the ability to identify the antigenic variation of influenza strains.

Sensitivity and specificity changes based of patient demographics and sample type

Patient demographics and sample type have both been shown to change the sensitivities and specificities.

Merckx et al. [37] synthesized 162 studies in a meta-analysis that reported a subgroup analysis of children consistently having higher sensitivities and specificities than adults. There was an 12.1% (95% CI, 3.1–22.1%) difference in the pooled sensitivity of IAV with a 31.8% (95% CI, 6.1–52.6%) difference in the IBV for RIDTs [37]. One hypothesis for this event is that children have higher secretions of antigens nasally than adults, although to our knowledge, this has not been tested nor confirmed. Another factor is that while LFAs provide quick and affordable results, the sensitivity and specificity of these assays have reported a wide range in the current literature depending on the input sample type utilized. In a meta-analysis published in 2020 synthesizing 13 published studies, the sensitivity of LFAs ranged from 62.0 to 93.0%, while the specificity was 88.0–100% based on the type of swab performed [11, 39, 51]. Nasopharyngeal aspirates were found to have the highest sensitivity (93%, $p<0.001$), while nasopharyngeal and oropharyngeal swabs have the highest specificity (100%, $p=0.001$ and $p=0.1058$ respectively) [52].

Clinically, both of these factors should be considered when determining whether to utilize one testing method over another. For example, in pediatric facilities, it may be a more cost-effective method to utilize LFAs due to the higher sensitivity and specificity in their targeted demographic, whereas other outpatient facilities should be prepared to send more cultures to laboratories for RT-PCR. Finally, picking a test based on its sampling method should also be examined to ensure the maximum results.

Availability of RIDTs and their impact on public health efforts

There are more than 30 commercially approved RIDTs currently on the market today; however, our review only focused on nine commonly utilized RIDTs. As seen in Table 1, due to the large number of tests currently available, the total number of studies testing the sensitivities and specificities of each test varies, limiting researchers and clinicians to how these tests actually hold up in practical settings. This is a gap in the current literature, and we believe that more testing should be conducted to determine the appropriate efficacy of each test.

Limitations

One limitation to this review is the possibility of missing eligible studies outside of our search. In particular, no studies were found under our search criteria for Status Flu A&B. The only data regarding the sensitivity and specificity of this product is through the manufacture's study. All other products showed decreases in the sensitivities and specificities, so one should assume that the reported numbers for this product are not accurate. Another limitation to this review is that we were not able to obtain the pricing for the products in the study. The manufacturers' websites state that sales managers or product managers should be contacted for purchase. The majority of the product companies are third-party sellers that have contracts with corporations that have varying prices. Future directed studies should be geared toward determining the average cost per test for each product and its overall benefit.

Further studies should be directed at testing the newer NAATs with confirmation to RT-PCR to determine their appropriate sensitivity and specificity ranges. Additionally, future studies should be conducted to evaluate additional parameters of these assays and factors that may impact them, such as positive and negative predictive values for each assay type, the impact of influenza incidence in a community and how it may skew these results, and any other factor that could impact the interaction of influenza with patients or diagnostic modalities. We acknowledge that this is not a comprehensive review of the topic, but it is merely a starting point for a review of these technologies and their performance. Given our review of the literature and differences from manufacturers' reports and real-world applications, further testing and research should be performed to make accurate predictions of this test's capabilities.

Conclusions

As seen in the current global pandemic caused by COVID-19, the need for accurate and accessible viral diagnostic tests is great [53]. Herein, we have conducted a review of nine of the commonly utilized commercial RIDTs. The molecular basis of each test, including the assay target and the time to diagnosis, was shown as reported by the manufacturer (Table 3). Additionally, the sensitivity and specificity of each assay has been reported in Table 2.

Through this review of the literature, we recorded the fastest diagnostic modality of the RIDTs to be the NEAR technology utilized by the Abbott ID Now Influenza A&B test, recording positive results in as little as 5 min, with the longest time being the RT-PCR–based Cepheid Xpert Xpress Influenza A/B taking up to 30 min to finalize a negative result (Table 3). Of the nine RIDTs examined in this review, six of the nine reports finalized a result within

15 min or less (Table 3). The lateral flow assays and NEAR tests report faster results when compared to the RT-PCR based tests; however, the inverse seems to be the case with regard to reported sensitivity and specificity for detection of IAV and IBV. The RT-PCR–based assays outperformed the LFA and NEAR RIDTs, averaging sensitivity and specificity above 90.0% for all reported data (Table 2).

At the time of this submission, we did not identify any recent review articles compiling this information, including the manufacturer's reported data on each of the RIDTs. It is our hope that this narrative review on the current RIDT technology can be utilized by clinicians and public health professionals as a resource for determining the appropriateness of a given assay for their patients. While we acknowledge that more real-world research is needed around these assays and their diagnostic accuracy for various IAV and IBV strains, this compilation of the current research surrounding these nine commercially available RIDTs should serve as a robust source of information on the technology employed by each test as well as the current reported statistics on their performance.

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