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WHITE PAPER

Superiority of RT-PCR Tests for the Detection of Respiratory Viruses at the Point-of-Care:

A Summary of the Literature

PL-000304 A November 2024

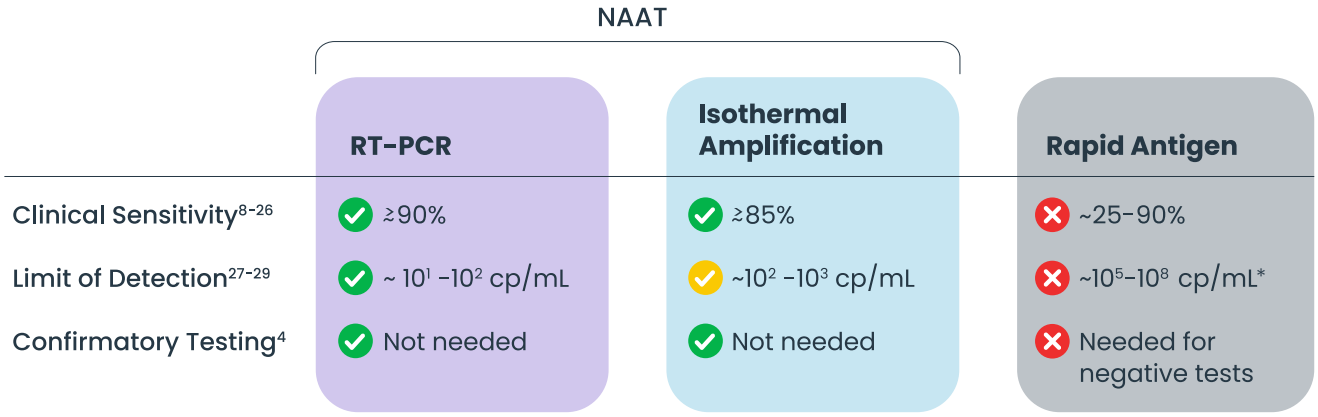
Introduction

Testing at the point-of-care (POC) for respiratory viruses such as influenza A, influenza B, and SARS-CoV-2 provides critical clinical utility - improving patient care by increasing use of appropriate antivirals, decreasing use of inappropriate antibiotics, and yielding essential diagnostic information for the overall management of patient and public health.[1-3] Historically, rapid antigen tests have been used in POC settings due to their low cost and fast turn-around times (TATs). However, the documented low sensitivity of these tests limits their diagnostic utility, and potentially increases healthcare burden by yielding false negative results and/or requiring additional confirmation testing.[4]

In contrast, reverse transcription polymerase chain reaction (RT-PCR) tests are supremely sensitive and are considered a "gold standard" for detecting respiratory viruses.[5,6] Previously, RT-PCR testing required sending specimens out for processing and testing at centralized laboratories with trained personnel, yielding long TATs (hours to days) and high costs. However, RT-PCR tests are now available at the POC, providing the best of both worlds – the superior sensitivity of molecular detection, with rapid access to results. Herein we describe the relative performance of POC diagnostic tests for detecting respiratory viruses.

Testing Options

Available tests for respiratory virus detection fall into two broad categories: immunoassay detection of viral antigens (rapid antigen tests), or molecular detection of amplified viral nucleic acids (nucleic acid amplification tests, or NAATs). In the latter category, tests can be further characterized based on their amplification modality: RT-PCR or isothermal amplification. Typically, NAATs are more sensitive than antigen tests and are more reliable for early disease detection.[7,8] Basic characteristics of each of these tests are shown in Figure 1. Product Instructions for Use (IFU) documents should be referenced for information about specific tests.



*Calculated from virions/mL, based on the conservative assumption that there is at least 1 copy/virion for all molecular markers

Figure 1: Overview of Respiratory Virus Detection Tests

Methods

For this analysis, peer-reviewed literature was searched to identify studies that reported the performance of diagnostic tests for detecting respiratory viruses. Preference was given to publications that focused on commercially available tests in POC settings. In cases where peer-reviewed performance assessments were not available for a diagnostic test of interest, the product IFU was used as the source. A summary of the published sources referenced for the clinical performance specifications reported herein is provided in Table 1.

Table 1: Description of referenced sources for clinical performance specifications, in alphabetical order by first author. Test names are as reported in each source; may not reflect exact marketed brand names.

First Author	Year	Reported Test(s)	Study Description
Bornemann	2021	Sofia SARS-CoV-2 Antigen FIA	Clinical evaluation of the Sofia SARS-CoV-2 Antigen fluorescent immunoassay (FIA) in comparison with laboratory-based NAT, across 1404 patients (symptomatic and asymptomatic), in a large tertiary care center.
Chu	2011	- BinaxNOW Influenza A&B - QuickVue Influenza A+B	Meta analysis of 17 studies assessing the performance of rapid influenza H1N1 diagnostic tests.
Dinç	2023	Panbio COVID-19/Flu A&B Rapid Panel test kit	Performance evaluation of the Panbio COVID-19/Flu A&B Rapid Panel compared with RT-PCR using 300 remnant samples.
Hansen	2021	Cobas Liat SARS-CoV-2 & Influenza A/B nucleic acid test	Prospective, multisite clinical evaluation of the Cobas Liat SARS-CoV-2 & Influenza A/B nucleic acid test compared with laboratory-based RT-PCR, using 357 specimens collected from symptomatic and asymptomatic patients across 5 U.S. sites.
Hassan	2014	- BD Veritor System for Flu A+B - Alere BinaxNOW Influenza A&B Card	Performance evaluation of the BD Veritor System for Flu A+B and the Alere BinaxNOW influenza A&B card compared with RT-PCR. using 200 frozen clinical specimens collected from January 2011 to June 2012 from pediatric patients.
Jensen	2024	- Xpert Xpress CoV-2/Flu/RSV *plus* test - STANDARD M10 Flu/RSV/SARS-CoV-2 test	Analytical and clinical evaluation of the Xpert Xpress CoV-2/Flu/RSV *plus* test and the STANDARD M10 Flu/RSV/SARS-CoV-2 test, compared with hospital-based standard-of-care testing in Denmark, using 492 remnant nasopharyngeal samples.
Kanwar	2020	- ID Now Influenza A&B 2 assay - Cobas Influenza A/B nucleic acid test - Xpert Xpress Flu	Prospective clinical evaluation of three diagnostic tests compared with the CDC Flu A/B PCR assay, across 201 symptomatic pediatric patients.
Katzman	2023	Visby Medical COVID-19 POC test	Clinical evaluation of Visby Medical COVID-19 POC test performance compared with laboratory-based RT-qPCR, using 100 residual samples collected from Mayo Clinic patients.
Kilic	2021	BD Veritor SARS-CoV-2 chromatographic immunoassay test	Clinical evaluation of the BD Veritor SARS-CoV-2 antigen assay compared with RT-PCR, across 1,384 symptomatic patients in an emergency room setting.
Lee	2021	Quidel Sofia rapid influenza FIA to detect influenza A and B	Meta analysis of 17 studies assessing the pooled performance the Sofia Rapid Influenza FIA for Flu A+B with symptomatic patients.
Mahmoud	2021	-Abbott ID NOW COVID-19 assay - Atila iAMP COVID-19 Detection Kit - AQ-TOP Plus COVID-19 Rapid Detection Kit - Genechecker UF 300 RT PCR system - Cobas Liat SARS-CoV-2 & Influenza A/B nucleic acid test - POCKIT SARS-CoV-2 (orf lab) (RT-ii PCR) assay	Clinical evaluation of six rapid SARS-CoV-2 nucleic acid detection assays compared with laboratory-based RT-qPCR, across 4981 participants (symptomatic and asymptomatic).
Park	2023	Cobas Liat SARS-CoV-2 & Influenza A/B assay	Evaluation of the Cobas Liat SARS-CoV-2 & Influenza A/B assay compared with RT-PCR using 1147 residual samples collected from patients at a tertiary care hospital.
Pollreis	2021	Abbott BinaxNOW COVID-19 Test Ag Card	Clinical evaluation of the Abbott BinaxNOW™ COVID-19 Ag Card compared with SARS-CoV-2 RT-PCR, across 214 participants (symptomatic and asymptomatic) who sought COVID-19 testing from a local public health district in Idaho, USA.
Simms	2023	Lucira Check-It COVID-19 Test	Clinical evaluation of the Lucira Check-It COVID-19 test compared with laboratory NAAT, across 405 cases (symptomatic and asymptomatic) from two hospitals in multiple care settings. Also assessed analytical sensitivity.
Smith-Jeffcoat	2024	Quidel QuickVue At-Home COVID-19 Test	Longitudinal comparison of antigen-, RT-PCR-, and viral culture-based detection methods for SARS-CoV-2 across 354 participants in 129 households.
Srivastava	2022	Abbott ID NOW COVID-19 assay	Prospective clinical evaluation of the Abbott ID NOW COVID-19 assay compared with RT-PCR across 72 symptomatic subjects.
Zahavi	2022	Lucira Check It COVID-19 Test Kit	Clinical evaluation of the Lucira Check It COVID-19 Test compared with laboratory-based RT-PCR across 190 patients (symptomatic and asymptomatic).
IFU	2023	Lucira COVID-19 & Flu Test	Published performance specifications for the Lucira COVID-19 & Flu Test based on validation clinical trial data.
IFU	2023	Visby Respiratory Health Test	Published performance specifications for the Visby Respiratory Health Test based on validation clinical trial data.

Reported Clinical Performance

NAATs vs. Rapid Antigen Tests

As described above, many studies have been conducted to assess the clinical performance of POC diagnostic tests for detecting respiratory viruses. Across the publications referenced here, **specificity** was consistently high across all reported tests – ranging from 96-100%.[8-26] However, **sensitivity** differed dramatically between rapid antigen tests and NAATs:

- **Rapid Antigen Tests:** demonstrated sensitivities for influenza A, influenza B, and SARS-CoV-2 ranging from 39-90%, 25-87%, and 49-68% respectively.[8-15]
- **NAATs (Isothermal Amplification):** demonstrated sensitivities for influenza A, influenza B, and SARS-CoV-2 ranging from 90-93%, 95-97%, and 88-95% respectively.[16-21]
- **NAATs (RT-PCR):** demonstrated sensitivities for influenza A, influenza B, and SARS-CoV-2 ranging from 98-100%, 92-100%, and 95-100% respectively.[17,18,22-26]

It is important to note that these performance specifications can vary across individual tests. For a more granular view, the reported sensitivities of selected commercially available POC tests from each category (RT-PCR, isothermal amplification, and rapid antigen) are shown in Table 2.

Table 2: Sensitivity of Select Commercially Available POC Respiratory Tests

	Test Name		Instrument Free	Sensitivity		
				Influenza A	Influenza B	SARS-CoV-2
POC RT-PCR	**Visby Medical** Respiratory Health Test*		✓	98%[23]	100%[23]	97%[23]
	Xpert Xpress CoV-2/Flu/RSV *plus* Test		✗	100%[24]	100%[24]	100%[24]
	Cobas Liat SARS-CoV-2 & Influenza A/B Assay		✗	99%[a,25]	100%[a,25]	95-100%[17,26]
POC Isothermal Amplification	**ID Now**	Influenza A&B 2 Assay	✗	93%[b,18]	97%[b,18]	93-95%[c,16,17]
		COVID-19 Assay				
	Lucira COVID-19 & Flu Test**		✓	90%[21]	95%[21]	88%[21]
Rapid Antigen	**Veritor System**	Flu A+B Assay	✗	90%[d,9]	87%[d,9]	66%[e,10]
		SARS-CoV-2 Assay				
	Sofia	Influenza A+B FIA	✗	78%[f,11]	72%[f,11]	57%[g,12]
		SARS Antigen FIA				
	BinaxNOW	Influenza A&B Card	✓	39-73%[h,9,13]	71%[h,9]	68%[i,14]
		COVID-19 Ag Card				

*Data for Influenza B is a combination of prospective fresh specimens (NP and AN), banked specimens (NP), and surrogate specimens (NP)
**Data for Influenza B is from surrogate specimens
[a] Calculated from Park et al, 2023
[b] ID Now Influenza A&B 2 Assay
[c] ID Now COVID-19 Assay
[d] Veritor System Flu A+B Assay
[e] Veritor System SARS-CoV-2 Assay
[f] Sofia Influenza A+B FIA
[g] Sofia SARS Antigen FIA
[h] BinaxNOW Influenza A&B Card
[i] BinaxNOW COVID-19 Ag Card

RT-PCR vs. Isothermal Amplification

While both NAAT types can detect respiratory viruses more sensitively than rapid antigen tests, there is evidence that RT-PCR tests have lower limits of detection (LoD) than isothermal amplification tests. Particularly, one study analytically compared two POC isothermal amplification tests (Lucira Check-It COVID-19 Test and Abbott ID NOW COVID-19 Test) to an RT-PCR test (Cepheid Xpert Xpress SARS-CoV-2 Test) for the detection of SARS-CoV-2, and found that both isothermal amplification tests were about **10-fold less sensitive than the RT-PCR test.**[19]

Workflow Considerations

In addition to clinical performance, it is critical to consider how diagnostic tests fit into the routine workflow in POC settings. Namely, speed and ease-of-implementation are essential for any test to have POC utility.

Speed

While rapid antigen tests return results quickly, there is the consideration that negative COVID-19 rapid antigen results may require confirmation: the FDA recommends retesting with an additional antigen test after 48 hours (or with two additional antigen tests done 48 hours apart, depending on whether the patient is symptomatic has been exposed to COVID-19), or to reflex to a single NAAT test, to protect against false negative results.[4] This adds time and cost to the diagnostic care process despite the quick and inexpensive initial antigen test results. RT-PCR-based POC tests may mitigate this concern, and still return results within minutes-hours versus the hours-days timeline of traditional laboratory-based RT-PCR tests.

Ease of Implementation

One advantage of many rapid antigen tests is that they operate without a separate instrument, thus virtually eliminating their footprint in POC settings where space may be limited. Table 2 includes information about which of the select commercially available tests are instrument-free, showing that the Visby Medical Respiratory Health Test is the only RT-PCR test that runs entirely without a separate instrument. Instrument-free operation is also critical for test scalability in times of spiked demand, such as during annual respiratory seasons and the COVID-19 pandemic, to prevent instrument-imposed limitations on the number of tests that can be run simultaneously.

Advantage of Multiplexed Tests

Many common respiratory infections have overlapping symptoms and carry the possibility of coinfection, making them difficult to diagnose clinically.[30-32] It is crucial to identify the right causative pathogen to ensure that appropriate treatment is applied. Because of this, tests which detect and distinguish multiple respiratory viruses simultaneously can increase speed and certainty of diagnosis while not imposing additional burden on patients or providers to collect and analyze multiple swabs. Figure 2 illustrates the degree of overlap between common symptoms of influenza A & B (Flu), and SARS-CoV-2 (COVID-19).

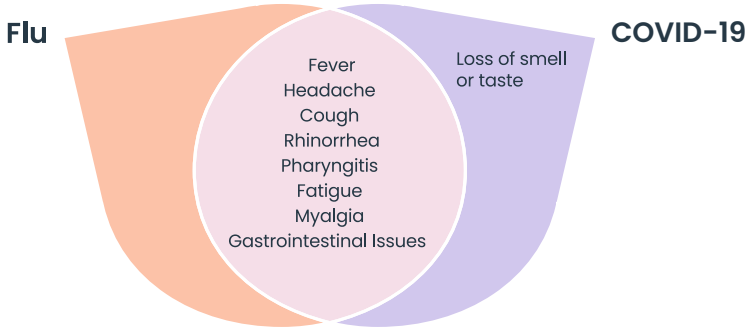


Figure 2: Symptoms of Common Respiratory Viral Infections[31,32]

Conclusion

Accurate detection of respiratory viruses at the POC is essential for fast and effective patient care and for management of public health. While rapid antigen tests have historically been used due to their quick TAT and low cost, newer RT-PCR tests are available at the POC that couple significantly better detection sensitivity with speed and ease-of-use.

Particularly, the high sensitivity of RT-PCR tests allows them to detect even small amounts of viral nucleic acid material, enabling more reliable detection of respiratory viruses like influenza A, influenza B, and SARS-CoV-2 as shown herein. Also, while traditional, laboratory-based RT-PCR tests have TATs ranging from multiple hours-days, newer POC technologies only require only minutes-hours to produce a result. Taken together, the increased sensitivity and lower TAT of newer RT-PCR tests has been shown to further increase the clinical utility of testing for respiratory viruses at the POC, even compared with rapid antigen testing (which had greater utility than no testing at all).[1–3,33] Thus leading to lower burden on patients, healthcare resources, and the public.

References

1. Stamm BD, Tamerius J, Reddy S, et al. The Influence of Rapid Influenza Diagnostic Testing on Clinician Decision-Making for Patients With Acute Respiratory Infection in Urgent Care. *Clinical Infectious Diseases*. 2023;76(11):1942-1948. doi:10.1093/cid/ciad038
2. Egilmezer E, Walker GJ, Bakthavathsalam P, et al. Systematic review of the impact of point-of-care testing for influenza on the outcomes of patients with acute respiratory tract infection. *Rev Med Virol*. Sep 2018;28(5):e1995. doi:10.1002/rmv.1995
3. Brigadoi G, Gastaldi A, Moi M, et al. Point-of-Care and Rapid Tests for the Etiological Diagnosis of Respiratory Tract Infections in Children: A Systematic Review and Meta-Analysis. *Antibiotics (Basel)*. Sep 3 2022;11(9)doi:10.3390/antibiotics11091192
4. U.S. Food & Drug Administration. At-Home COVID-19 Antigen Tests-Take Steps to Reduce Your Risk of False Negative Results: FDA Safety Communication. November 17, 2022 Update. Accessed July 2, 2024. https://www.fda.gov/medical-devices/safety-communications/home-covid-19-antigen-tests-take-steps-reduce-your-risk-false-negative-results-fda-safety
5. Centers for Disease Control and Prevention. COVID-19 Testing: What You Need to Know. Accessed April 29, 2024. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html
6. Berry GJ, Jhaveri TA, Larkin PMK, Mostafa H, Babady NE. ADLM Guidance Document on Laboratory Diagnosis of Respiratory Viruses. *J Appl Lab Med*. May 2 2024;doi:10.1093/jalm/jfae010
7. Chu VT, Schwartz NG, Donnelly MAP, et al. Comparison of Home Antigen Testing With RT-PCR and Viral Culture During the Course of SARS-CoV-2 Infection. *JAMA Internal Medicine*. 2022;182(7):701-709. doi:10.1001/jamainternmed.2022.1827
8. Smith-Jeffcoat SE, Mellis AM, Grijalva CG, et al. SARS-CoV-2 Viral Shedding and Rapid Antigen Test Performance — Respiratory Virus Transmission Network, November 2022–May 2023. *Morbidity and Mortality Weekly Report*. 2024;73:365 - 371. doi:http://dx.doi.org/10.15585/mmwr.mm7316a2
9. Hassan F, Nguyen A, Formanek A, Bell JJ, Selvarangan R. Comparison of the BD Veritor System for Flu A+B with the Alere BinaxNOW influenza A&B card for detection of influenza A and B viruses in respiratory specimens from pediatric patients. *J Clin Microbiol*. Mar 2014;52(3):906-10. doi:10.1128/jcm.02484-13
10. Kilic A, Hiestand B, Palavecino E. Evaluation of Performance of the BD Veritor SARS-CoV-2 Chromatographic Immunoassay Test in Patients with Symptoms of COVID-19. *J Clin Microbiol*. 2021. vol. 5.
11. Lee J, Song JU, Kim YH. Diagnostic Accuracy of the Quidel Sofia Rapid Influenza Fluorescent Immunoassay in Patients with Influenza-like Illness: A Systematic Review and Meta-analysis. *Tuberc Respir Dis (Seoul)*. Jul 2021;84(3):226-236. doi:10.4046/trd.2021.0033
12. Bornemann L, Kaup O, Kleideiter J, Panning M, Ruprecht B, Wehmeier M. Real-life evaluation of the Sofia SARS-CoV-2 antigen assay in a large tertiary care hospital. *J Clin Virol*. Jul 2021;140:104854. doi:10.1016/j.jcv.2021.104854
13. Chu H, Lofgren ET, Halloran ME, Kuan PF, Hudgens M, Cole SR. Performance of rapid influenza H1N1 diagnostic tests: a meta-analysis. *Influenza and Other Respiratory Viruses*. 2012/03/01 2012;6(2):80-86. doi:https://doi.org/10.1111/j.1750-2659.2011.00284.x
14. Pollreis RE, Roscoe C, Phinney RJ, et al. Evaluation of the Abbott BinaxNOW COVID-19 Test Ag Card for rapid detection of SARS-CoV-2 infection by a local public health district with a rural population. *PLoS One*. 2021;16(12):e0260862. doi:10.1371/journal.pone.0260862
15. Dinç H, Karabulut N, Alaçam S, et al. Evaluation of the Diagnostic Performance of a SARS-CoV-2 and Influenza A/B Combo Rapid Antigen Test in Respiratory Samples. *Diagnostics (Basel)*. Mar 3 2023;13(5)doi:10.3390/diagnostics13050972
16. Srivastava S, Singh P, Malhotra R, Mathur P. Comparison of Abbott ID NOW, a novel isothermal amplification based COVID-19 diagnostic method with RTPCR. *J Virol Methods*. Jun 2022;304:114521. doi:10.1016/j.jviromet.2022.114521
17. Mahmoud SA, Ganesan S, Ibrahim E, et al. Evaluation of six different rapid methods for nucleic acid detection of SARS-COV-2 virus. *J Med Virol*. Sep 2021;93(9):5538-5543. doi:10.1002/jmv.27090
18. Kanwar N, Michael J, Doran K, Montgomery E, Selvarangan R. Comparison of the ID Now Influenza A & B 2, Cobas Influenza A/B, and Xpert Xpress Flu Point-of-Care Nucleic Acid Amplification Tests for Influenza A/B Virus Detection in Children. *J Clin Microbiol*. Feb 24 2020;58(3)doi:10.1128/jcm.01611-19
19. Simms E, McCracken GR, Hatchette TF, et al. Real-world evaluation of the Lucira Check-It COVID-19 loop-mediated amplification (LAMP) test. *Microbiol Spectr*. Dec 12 2023;11(6):e0277223. doi:10.1128/spectrum.02772-23
20. Zahavi M, Rohana H, Azrad M, Shinberg B, Peretz A. Rapid SARS-CoV-2 Detection Using the Lucira™ Check It COVID-19 Test Kit. *Diagnostics (Basel)*. Aug 3 2022;12(8) doi:10.3390/diagnostics12081877
21. Pfizer Lucira COVID-19 & Flu Test [Instructions for Use] INST032 Rev. B. Accessed June 12, 2024. https://www.lucirabypfizer.com/assets/pdf/ifu-eua-long-form-lucira-by-pfizer-covid-19-flu-test-poc.pdf
22. Katzman BM, Wockenfus AM, Kelley BR, Karon BS, Donato LJ. Evaluation of the Visby medical COVID-19 point of care nucleic acid amplification test. *Clin Biochem*. Jul 2023;117:1-3. doi:10.1016/j.clinbiochem.2021.11.007
23. Visby Medical Respiratory Health Test [Instructions for Use] PS-400004 Rev D. Accessed May 17, 2024. www.visbymedical.com/static/Visby-Medical-Respiratory-Health-Instructions-For-Use-97f8e54d4710110637062O9c772ea55d.pdf
24. Jensen CB, Schneider UV, Madsen TV, et al. Evaluation of the analytical and clinical performance of two RT-PCR based point-of-care tests; Cepheid Xpert® Xpress CoV-2/Flu/RSV plus and SD BioSensor STANDARD™ M10 Flu/RSV/SARS-CoV-2. *J Clin Virol*. 2024/06/01/ 2024;172:105674. doi:https://doi.org/10.1016/j.jcv.2024.105674
25. Park K, Sung H, Kim MN. Evaluation of the cobas Liat detection test for SARS-CoV-2 and influenza viruses following the emergence of the SARS-CoV-2 Omicron variant. *Diagn Microbiol Infect Dis*. Apr 2023;105(4):115891. doi:10.1016/j.diagmicrobio.2023.115891
26. Hansen G, Marino J, Wang ZX, et al. Clinical Performance of the Point-of-Care cobas Liat for Detection of SARS-CoV-2 in 20 Minutes: a Multicenter Study. *J Clin Microbiol*. Jan 21 2021;59(2)doi:10.1128/jcm.02811-20
27. Fung B, Gopez A, Servellita V, et al. Direct Comparison of SARS-CoV-2 Analytical Limits of Detection across Seven Molecular Assays. *Journal of Clinical Microbiology*. 2020;58(9):10.1128/jcm.01535-20. doi:doi:10.1128/jcm.01535-20
28. Sohni Y. Variation in LOD Across SARS-CoV-2 Assay Systems: Need for Standardization. *Lab Med*. © American Society for Clinical Pathology 2020. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.; 2020.
29. Polvere I, Voccola S, #039, et al. Evaluation of FAST COVID-19 SARS-CoV-2 Antigen Rapid Test Kit for Detection of SARS-CoV-2 in Respiratory Samples from Mildly Symptomatic or Asymptomatic Patients. *Diagnostics*. 2022;12(3):650.
30. Pratt G, Platt M, Wong C, Rao L. Coinfection Rates of SARS-CoV-2, Influenza, and Respiratory Syncytial Virus. presented at: AACC; 2023; Anaheim, CA.
31. Neamah S. Comparison between symptoms of COVID-19 and other respiratory diseases. *EUROPEAN J MED ED TE*. 2020;13(3):em2014. doi:https://doi.org/10.30935/ejmets/8489
32. Karimi A, Rafiei Tabatabaei S, Shiva F, Hoseinialfatemi SM. COVID-19 or Influenza, or Both? A Comparison and Algorithmic Approach to Management. Review Article. *Jundishapur J Microbiol*. 2020;13(12):e112121. doi:10.5812/jjm.112121
33. Benirschke RC, McElvania E, Thomson RB, Jr., Kaul KL, Das S. Clinical Impact of Rapid Point-of-Care PCR Influenza Testing in an Urgent Care Setting: a Single-Center Study. *J Clin Microbiol*. Mar 2019;57(3)doi:10.1128/jcm.01281-18