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Rapid and Accurate PCR Diagnosis of Influenza and RSV with the cobas® Liat® System in Point of Care Settings

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1. Abstract

Background: Point-of-care testing for influenza and respiratory syncytial virus (RSV) needs to be both rapid and accurate in order to guide effective patient management and infection control. While rapid antigen-based tests are sometimes used, they often demonstrate low sensitivity. The **cobas**® Influenza A/B and RSV assay is an easy-to-use automated PCR test performed on the **cobas**® Liat® System for detection of influenza A, influenza B, and RSV from nasopharyngeal (NP) swabs in approximately 20 minutes. We evaluated the performance of the test in CLIA-waived point of care settings.

Methods: NP swabs were collected and tested from patients with influenza-like illness presenting to primary care clinics and emergency departments during the 2013-2014 and 2014-2015 flu seasons. Testing was done by users with no formal laboratory training. **cobas**® Influenza A/B and RSV assay results were compared to a conventional laboratory PCR test. Rapid antigen test results, if used by sites as part of routine clinical care, were also analyzed.

Results: NP swabs were prospectively collected from 1361 patients at 8 primary care clinics and 4 emergency departments across the US. 24% of patients were ≤5 years of age. Testing was conducted by 38 CLIA-waived intended use operators, primarily nurses and medical assistants, who also tested an additional 300 retrospectively collected NP swabs. Compared to the lab-based PCR test, the **cobas**® Influenza A/B and RSV assay showed sensitivities of 99.6%, 99.3%, and 96.8% for influenza A, influenza B, and RSV, respectively. Specificity was 97.5%, 99.7%, and 98.8% for influenza A, influenza B, and RSV, respectively. In comparison, rapid antigen testing only demonstrated sensitivities of 79.7%, 80.0%, and 87.1% for influenza A, influenza B, and RSV, respectively.

Conclusion: The **cobas**® Influenza A/B and RSV assay was accurate, rapid, and easy to use in point of care settings. No reagent preparation, sample manipulation, or formal laboratory training is required, making it a highly suitable point-of-care PCR test. Laboratory quality PCR results at the point-of-care can improve treatment decisions, patient management, and surveillance efforts for influenza and RSV.

2. Background

- Influenza and respiratory syncytial viruses are the leading cause of respiratory infections.
- Signs and symptoms of Influenza and RSV infections overlap extensively with other infectious diseases.
- Early detection and discrimination of influenza and RSV is important for optimizing patient management, enabling infection control measures, and preventing unnecessary use of antibiotics, hospital procedures, and laboratory tests[1]. Viral culture has traditionally been the "gold standard" but is time consuming and less sensitive.
- Rapid antigen tests are commonly used in outpatient clinics, physicians' offices, and hospitals despite the fact that they have insufficient sensitivity[2].
- Point of care PCR tests can provide lab quality results with the ease of use of rapid antigen tests.

3. Methods

Objective: The **cobas**® Influenza A/B and RSV assay is a point of care PCR test on the **cobas**® Liat® System that detects Influenza A, Influenza B, and RSV in nasopharyngeal swabs in approximately 20 minutes. The objective of this study was to evaluate the clinical sensitivity and specificity of the **cobas**® Influenza A/B and RSV assay for use on the **cobas**® Liat® system in point-of-care CLIA-waived settings.

Clinical Study Sites and Operators

- A multicenter clinical trial was conducted to evaluate the performance of the **cobas**® Influenza A/B and RSV assay during the 2013-2014 and 2014-2015 respiratory virus seasons at 8 primary care clinics and 4 emergency departments distributed across the United States.
- 38 test operators, primarily nurses and medical assistants were provided with **cobas**® Influenza A/B and RSV Assay Package Insert, Quick Reference Instructions, the **cobas**® Liat® System Quick Start Guide and User Manual. No additional instructions on the operation of the **cobas**® Liat® system were provided. All testing was done in CLIA-waived settings.

Study Subjects, Specimen Collection and Testing

- Consenting subjects who presented with 2 or more flu-like symptoms were enrolled in the study.
- Subjects taking anti-viral medication within 7 days of the visit or who had received a nasal flu vaccine within the last 6 weeks were excluded.
- Nasopharyngeal swabs were collected from subjects and tested with the **cobas**® Influenza A/B and RSV assay followed by a laboratory PCR test. Results of rapid antigen tests were also collected if done as part of a site's routine clinical workflow.
- Prospectively collected specimens were supplemented with retrospective specimens tested with **cobas**® Influenza A/B and RSV assay at CLIA Waived intended use sites.
- PCR and bi-directional sequencing was used to investigate discordant results.

Figure 1: cobas® Influenza A/B and RSV Assay Workflow

Statistical analysis

Sensitivity and specificity of the **cobas**® Influenza A/B and RSV Assay was determined by comparing results with a conventional laboratory PCR test. Results were also compared to rapid antigen tests results, when available at sites.

4. Results

Demographics

Table 1. Point-of-care testing study site characteristics

Site Location	Site Classification	Number of Samples Tested	Number of CLIA-Waived Test Operators
Virginia	Clinic	322	2 N, 3 MA, 1 RA
Connecticut	Clinic	18	1 N, 1 MA
South Florida Site 1	Clinic	149	1 N, 1 MA
South Florida Site 2	Clinic	90	2 MA
Nebraska	Clinic	130	5 MA
Texas	Clinic	20	3 N
Indiana	Clinic	56	1 N, 2 MA
California	Clinic	138	2 MA
Ohio	Emergency Department	148	1 N, 1 MA
Massachusetts	Emergency Department	243	1 N 2 RA
New York	Emergency Department	295	6 RA
Central Florida	Emergency Department	47	1 NS, 1 RA

N-Nurse, MA-Medical Assistant, RA-Research Assistant/Study Coordinator, NS-Nursing Student

The Virginia, California, and Nebraska sites tested both prospective and retrospectively included samples. All other sites tested prospective samples only.

Table 2. Subject demographics for prospectively collected NP swabs

	Number Of Prospective Samples	Percentage
Age		
<5 years	320	23.5
6-21 years	408	30.0
22-59 years	505	37.1
>60 years	128	9.4
Total	**1361**	
Sex		
Male	663	48.7
Female	698	51.3

Clinical performance of cobas® Influenza A/B and RSV assay against FDA-cleared laboratory PCR test

- Testing was done on 1656 total NP swabs (1361 prospective and 295 retrospective samples)
- The **cobas**® Influenza A/B and RSV Assay demonstrated a sensitivity of 99.6%, 99.3%, and 96.8% for influenza A, influenza B, and RSV, respectively; and specificity of 97.5%, 99.7%, and 98.8% for influenza A, influenza B, and RSV, respectively (**Table 3**).
- For all 3 viruses, positive sequencing and **cobas**® Influenza A/B and RSV Assay results show that the **cobas**® Influenza A/B and RSV Assay assay could detect the virus RNA in many positive specimens that were not detected by the laboratory PCR test.

Table 3. Performance of the cobas® Liat® assay compared to conventional laboratory PCR

Influenza A		Lab PCR		Total
		Positive	Negative	
Liat®	Positive	267	34[a]	301
	Negative	1[b]	1352	1353
	Total	268	1386	1654[c]

	%	95% CI
Sensitivity	99.6%	(97.9%–99.9%)
Specificity	97.5%	(96.6%–98.2%)

[a]Of 34 Liat® positive, lab PCR negative specimens, 15 were positive and 19 were negative by sequencing.
[b]1 Liat® negative, lab PCR positive specimen was negative by sequencing.
[c]2 specimens were indeterminate by lab PCR due to late Ct and/or poor PCR amplification curve.

Influenza B		Lab PCR		Total
		Positive	Negative	
Liat®	Positive	144	4[a]	148
	Negative	1[b]	1507	1508
	Total	145	1511	1656

	%	95% CI
Sensitivity	99.3%	(96.2%–99.9%)
Specificity	99.7%	(99.3%–99.9%)

[a]Of 4 Liat® positive, lab PCR negative specimens, 3 were positive and 1 was negative by sequencing.
[b]1 Liat® negative, lab PCR positive specimens was negative by sequencing.

RSV		Lab PCR		Total
		Positive	Negative	
Liat®	Positive	184	18[a]	202
	Negative	6[b]	1438	1444
	Total	190	1456	1646[c]

	%	95% CI
Sensitivity	96.8%	(93.3%–98.5%)
Specificity	98.8%	(98.1%–99.2%)

[a]Of 18 Liat® positive, lab PCR negative specimens, 7 were positive and 11 were negative by sequencing.
[b]Of 6 Liat® negative, lab PCR positive specimens, 1 was positive and 5 were negative by sequencing.
[c]10 specimens were indeterminate for RSV by lab PCR due to late Ct and/or poor PCR amplification curve.

Clinical performance of cobas® Influenza A/B and RSV assay against Rapid Antigen Detection Tests (RADT)

- **Table 4** shows the performance of the **cobas**® Liat® assay and the RADTs compared to the conventional laboratory PCR results.
- The **cobas**® Liat® assay had significantly higher percent positive agreement to lab PCR than RADTs, while having similar percent negative agreement.

Table 4. Performance of the cobas® Liat® and Rapid Antigen Detection Tests (RADT) compared to conventional laboratory PCR

	Influenza A		Influenza B		RSV	
	Positive Agreement with Lab PCR	Negative Agreement with Lab PCR	Positive Agreement with Lab PCR	Negative Agreement with Lab PCR	Positive Agreement with Lab PCR	Negative Agreement with Lab PCR
Liat®	99.6%	97.5%	99.3%	99.7%	96.8%	98.8%
RADT	79.7%	96.6%	80.0%	99.5%	87.1%	98.7%

5. Conclusions

- The performance of the **cobas**® Influenza A/B & RSV Assay in the hands of non-laboratory trained staff is comparable to, if not better than, conventional laboratory PCR. Additionally, the **cobas**® Liat® assay shows higher clinical sensitivity when compared against other rapid influenza and RSV tests widely used today, while preserving similar ease-of-use and time-to-result.
- The high accuracy, rapid time to result and remarkable ease-of-use make the **cobas**® Influenza A/B and RSV assay a highly suitable point-of-care solution in CLIA-waived settings. Manual intervention is only required to initiate the test and observe the final result, ensuring only ~1 minute of hands-on time and minimal involvement of the staff.
- Laboratory quality PCR results at the point-of-care can improve treatment decisions, patient management, and surveillance efforts for influenza and RSV.

References

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