

Evaluation of the fully automated Luminex ARIES real-time PCR instrument for rapid detection of Influenza A/B and Respiratory Syncytial Virus in urgent samples

Background

Influenza and RSV are significant winter pressures in the Sheffield region, with large numbers of patients and their associated samples being routinely tested by real time PCR. Sample numbers peak in the winter months. The availability of a rapid turnaround system to compliment the routine 24hr service is crucial to patient management and infection control. The ARIES instrument provides an easy to use; rapid turnaround means to test these samples, with sample to answer in approximately 2 hours.

The Northern General Hospital Virology Laboratory in Sheffield, England has evaluated the Luminex ARIES instrument in comparison with currently used Taqman based assay for the detection of Influenza A, Influenza B, RSV A and RSV B in routine clinical samples during the period of January-March 2016.

Aims:

- To assess the performance of the Luminex ARIES Influenza A/B/RSV assay.
- To compare this performance to that of existing methodology for Influenza/RSV detection.

Materials & Methods

Using both prospective and retrospective testing, 114 mixed respiratory samples were tested using the Luminex ARIES Flu A/B & RSV assay alongside the routine Taqman PCR currently in use for respiratory virus testing at this laboratory. Prospective specimens were taken from the wards for which it is anticipated the ARIES assay would be run on an urgent basis- adult admissions units, infectious diseases unit and the Sheffield children's hospital. Retrospective testing was also carried out on known positive and negative specimens in order to guarantee sufficient sample numbers over the study period.

A mixture of respiratory specimens were tested, consisting of 60 Nasopharyngeal Aspirate (NPA) samples, 49 Throat Swabs, 3 Bronchoalveolar Lavage (BAL), 1 Nasal Swab and 1 Endotracheal secretion sample.

For the Taqman PCR, all specimens were extracted

using the Roche MagNA Pure 96 DNA and Viral NA Small Volume extraction kit on the Roche MagNA Pure 96. PCR for respiratory viruses was performed using the Applied Biosystems ABI7500. Total testing time for this method was calculated as 180 minutes (40 minutes hands-on), excluding analysis and reporting.

The Luminex ARIES is an automated sample-to-answer platform, needing no prior extraction or sample preparation steps. Specimens were tested by pipetting 200ul of sample into the ARIES Flu A/B & RSV assay cassette containing lyophilized primers and extraction reagents, which is then ordered on the ARIES using an integrated barcode scanner. Total testing time was calculated as 120 minutes (6 minutes hands-on), excluding analysis and reporting.



Figure 1 (Above): The Luminex ARIES Influenza A/B/RSV assay cassette, with 3D barcode. Figure 2 (Right): The Luminex ARIES instrument.



Results

114 samples were tested by Taqman PCR and ARIES FluA/B & RSV. 106 specimens were positive, with 8 negative samples tested. A summary of the results achieved is shown in table 1. The study saw agreement across both methods in 112/115 (97.4%) of samples.

Three discrepant results were observed, two of which were RSV detected by Taqman PCR, but negative using the Luminex assay. Both of these samples were repeated and still found to be negative on the ARIES. The initial Taqman assay was observed for incorrect interpretation, however this was not the case. Future work using a user developed protocol on the ARIES is in progress, and aims to provide a third method by which these samples can be tested.

The final discrepant result (the asterisk in Table 1),

	FLUA LUMINEX +	FLUB LUMINEX +	RSVA LUMINEX +	RSVB LUMINEX +	LUMINEX NEGATIVE
FLUA TAQMAN PCR +	29	0	0	0	0
FLUB TAQMAN PCR +	0	27	0	0	0
RSVA TAQMAN PCR +	0	0	25	0	2
RSVB TAQMAN PCR +	0	0	0	24	0
TAQMAN NEGATIVE	1*	0	0	0	8

Table 1: Results summary; Taqman assay versus ARIES Flu A/B & RSV

was a sample in which Influenza A was detected in the ARIES Flu A/B & RSV assay but not in the Taqman method.

It was noted upon review of the Taqman PCR results that a small amplification curve was present underneath the threshold. This sample was subsequently re-extracted and re-tested, and reported again as influenza negative by Taqman PCR.

The sensitivity, specificity, positive and negative predictive values were calculated, with results as shown in table 2, below. Due to limitations of time and resources, only 8

Sensitivity	98.11%
Specificity	100.00%
Positive predictive value	100.00%

negative samples were tested during this study. It can be seen in table 1 that these specimens displayed complete agreement between both methods of testing

Table 2: ARIES Flu A/B & RSV performance characteristics

A small study was undertaken to determine in limit of detection (LOD) for both assays. This used a known positive specimen, of which serial dilutions were made and tested using both methods. The endpoints for each marker within each assay are display in table 3, below.

	FluA	FluB	RSVA	RSVB
Luminex	1:10000	1:1000	1:1 000 000	1:100 000
Taqman	1:10000	1:1000	1:1 000 000	1:10000

Table 3- Limit of Detection Study results for Taqman and : ARIES Flu A/B & RSV performance characteristics

Both assays performed comparably across all targets, with the ARIES Flu A/B & RSV performing better in the RSVB assay.

Conclusions

The Luminex ARIES sample-to-answer platform is a rapid, easy to use means of testing urgent respiratory samples. The high level of concordance with existing Taqman methodology is encouraging, and further work using laboratory developed primers on the ARIES will give more data on the performance of the platform as a whole. Sensitivity, specificity and PPV are acceptable, with further work required to establish NPV.

The ARIES can test 12 samples simultaneously, using two magazines of 6 specimens. Each magazine runs independently, with samples being added whenever a magazine becomes available, allowing for urgent samples to be prioritized easily. This is currently difficult when working in batches for extraction and PCR. The small amount of operator time is beneficial, both in prioritizing samples and freeing up staff time during busy winter periods.

Overall, with assay performance comparable to Taqman PCR, ease of use and rapid results, the ARIES Flu A/B & RSV is a useful tool in diagnosing infections with Influenza and RSV, with associated benefits in patient management.