UK NEQAS Respiratory Viruses Point of Care EQA

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Introduction

Viral respiratory tract infections are a leading cause of morbidity and mortality worldwide, contributing to significant economic burden[1]. Influenza viruses, respiratory syncytial viruses (RSV) and human SARS-CoV-2 are currently the main contributors to the viral respiratory tract infections circulating in the population[2].

Diagnosis is generally based on clinical symptoms. However, it is often not possible to determine the specific causative agent in this way due to the generalised symptoms caused[3]. Point-of-care (PoC) diagnostic tests are especially useful in the emergency or intensive care setting as they provide rapid results, aiding prompt decision-making regarding patient care.

ISO 15189 standards must be met by medical laboratories in the UK to help to ensure confidence in the quality of practice. These standards have recently been updated to incorporate requirements for PoC testing. This will improve the standardisation of such testing and require the same stringent oversight as clinical laboratories when it comes to PoC testing, therefore regulating the level of care for all patients[4].

External quality assessments (EQA) must be carried out by ISO 15189 accredited laboratories to ensure laboratory proficiency. UK NEQAS for Microbiology is an EQA provider currently trialling a respiratory virus PoC EQA panel to help care settings meet the new standards for PoC examination when testing for influenza viruses, RSV, and SARS-CoV-2. The specimens have been prepared in a liquid based format containing viral transport medium (VTM), and where possible the viruses have been inactivated to produce a suitable sample for PoC testing.

Aim of the project

- Determine if a PoC scheme is required and will be beneficial to healthcare providers testing for influenza viruses, RSV, and SARS-CoV-2 in a PoC setting.
- Determine the stability of the selected viruses over a period of 10 weeks in VTM.
- Assess the efficacy of antigen and molecular testing methods for these viruses.

Methods

Questionnaire sent to participants

A questionnaire consisting of five questions was sent out to participants enrolled in the ‘SARS-CoV-2 Point of Care’, ‘Molecular Detection of SARS-CoV-2’ EQA schemes, which tests for SARS-CoV-2; in addition to those testing for Influenza viruses, RSV, and enteroviruses enrolled in the ‘Molecular detection of Respiratory Viruses’ EQA scheme

The questions included were:

1) What respiratory viruses does your laboratory test for?

2) What sample volume is required to run all respiratory virus assays in your laboratory?

3) Does your laboratory type Influenza virus (Influenza A and Influenza B)?

4) Does your laboratory sub-type Influenza A virus?

5) Does your laboratory type respiratory syncytial virus (RSV) (RSV A and RSV B)?

Stability testing study

- Specimens positive for influenza viruses, RSV, and SARS-CoV-2 were made in VTM at various concentrations.
- Specimens were incubated at room temperature (RT) and +37°C.
- Specimens were tested at one week intervals for ten weeks.
- Antigen testing as well as molecular testing was carried out on each sample.
- The intensity of the bands on the lateral flow tests were graded based on their strength.
- The influenza A strain was the Slovenia strain. The influenza B was the Phuket strain. The RSV A and RSV B strain were taken from clinical isolates, circulating within the last five years. The SARS-CoV-2 strain was omicron BA.2.

Questionnaire feedback

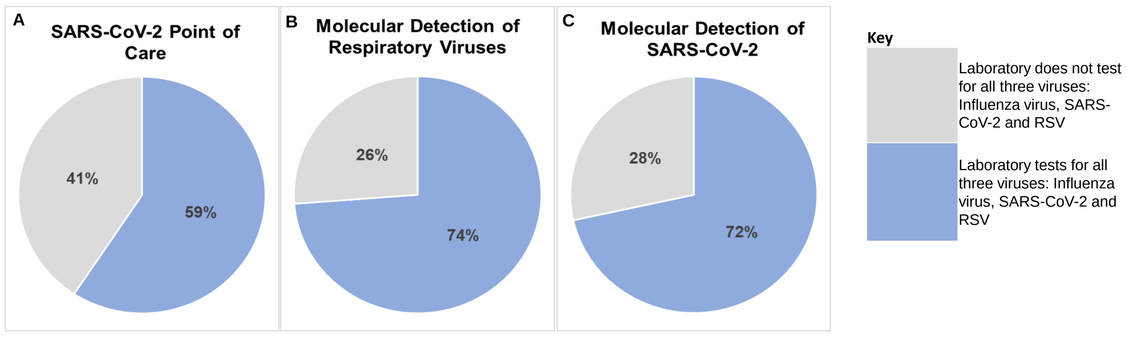

Figure 1: The percentage of participants for each of the UK NEQAS for Microbiology schemes that test for SARS-CoV-2, influenza virus and RSV in their laboratory. A: n= 27, B: n= 187, C: n= 253

- Responses from the questionnaire in the three EQA schemes shows that majority of laboratories test for all three of the respiratory tract infection viruses.
- This shows that there is a clinical need for a POC test that incorporates SARS-CoV-2, Influenza (A and B), and RSV (A and B) testing in the same panel.

Results

Antigen testing results

Virus	Week 1		Week 9		Week 10	
	RT	37°C	RT	37°C	RT	37°C
Influenza A	3+	3+	2+	Negative	2+	Negative
Influenza B	3+	3+	2+	Negative	2+	Negative
RSV A	2+	2+	1+	Negative	1+	Negative
RSV B	3+	3+	2+	Negative	2+	Negative

Figure 2: Results from the lateral flow tests for each of the specimens from week 1, 9, and 10, for influenza A, B, RSV A and RSV B at RT and 37°C. A 3+ score = A very strong band, 2+ score = a strong band, 1+ score = weak band, +/- band = a very weak band, negative = no visible band.

- The lateral flow tests were able to correctly identify the viruses for the duration of the stability testing.
- Specimens stored at ambient RT demonstrated greater organism stability after ten weeks compared to those kept at 37°C.
- Results for the SARS-CoV-2 has been omitted as this work was previously presented by Heather Crowton *et al*. at the 2022 IBMS congress.

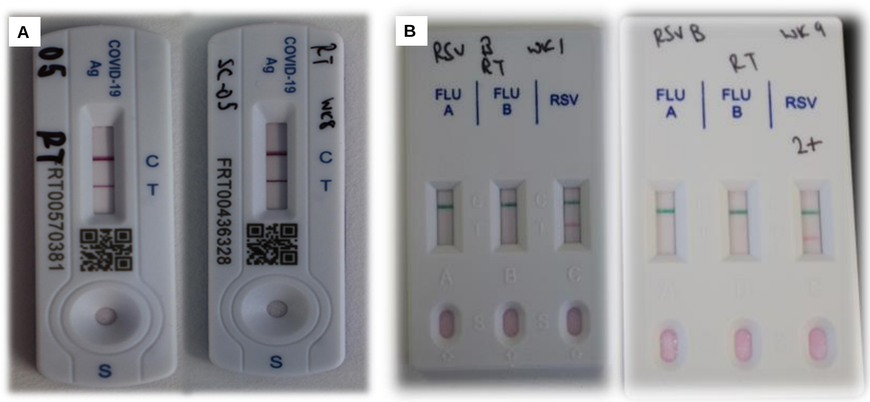

Figure 3: A) Lateral flow results for SARS-CoV-2 at week 1 and week 10. B) Lateral flow results for RSV B at week 1 and week 10.

- The SARS-CoV-2 test produced the same intensity (3+) of results throughout the duration of stability testing (figure 3.A).
- The RSV B test for the RSV B positive sample decreased in intensity from week one (3+) compared to week nine (2+) (figure 3.B).

Molecular testing results

Virus	Week 1	Week 10
Influenza A	17.23	23.0
Influenza B	19.86	20.75
RSV A	22.94	22.01
RSV B	22.15	21.01

Figure 4: Ct results for molecular analysis of influenza (A and B), RSV A and RSV B specimens carried out at week 1 and week 10.

- The nucleic acid concentration remained the same over the course of the stability testing, except for influenza A.
- For influenza A, there is Ct increase of 5.77 from week one compared to week ten, suggesting degradation occurred in the nucleic acid by week ten.

Discussion

- Specimens were made in VTM to best suit the needs of a PoC setting.
- An increased degradation was observed during antigen testing at 37°C, this is likely due to the increased enzymic activity at higher temperatures.
- The stability study confirmed viability and efficacy of all the selected viruses was maintained to an acceptable and detectable level throughout the duration of the stability study for both the antigen and molecular testing.
- This suggests that the RV PoC EQA can be aimed at laboratories that carry out antigen testing to identify respiratory viruses, as well as laboratories that carry out molecular testing for respiratory viruses.

Conclusion

- There is a clinical need for a PoC EQA for the detection of respiratory viruses.
- Due to the changes of working practices for PoC testing, as well as the recent changes to ISO 15189 concerning PoC testing, the Respiratory Virus PoC EQA should incorporate antigen and molecular testing.
- The stability testing study was able to confirm viability of specimens for the duration of the study.

The UK NEQAS Respiratory Viruses Point of Care EQA will be introduced in April 2024.

Please look out for further details of this new EQA on the UK NEQAS for Microbiology website.

Acknowledgements

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References

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