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Letter to the Editor

In-house validation of the cobas Liat influenza A/B and RSV assay for use with gargles, sputa and endotracheal secretions

Sir,

Seasonal influenza and respiratory syncytial virus (RSV) are common causes of respiratory infection. As such, these viruses can place a significant burden on healthcare systems during the winter season. Point-of-care tests (POCT) provide rapid respiratory testing at the patient's bedside, allowing the implementation of effective patient management and infection control procedures. Providing timely diagnosis of influenza and RSV, in turn, improves the management of respiratory infections in healthcare settings, and may provide monetary savings to the National Health Service [1].

Nasopharyngeal swabs are a common specimen type for use with the majority of respiratory POCT; however, this sample type is fairly difficult to obtain and the procedure is uncomfortable for patients [2]. In our hospital, gargle samples are the specimen of choice for the investigation of upper respiratory tract infections in the majority of patients, due to their ease of collection, lack of requirement for users to possess viral transport medium, and acceptability to patients. Furthermore, polymerase chain reaction (PCR) detection of respiratory pathogens in gargle samples has been shown to be equivalent, if not superior, to throat swabs [3]. Sputum and endotracheal secretions (ETS) are also useful specimen types for the investigation of lower respiratory tract infections, in addition to cases where patients are unable to produce a gargle sample (e.g. if they are intubated).

The cobas Liat system (Roche, Basel, Switzerland) is an automated multiplex real-time reverse transcription PCR (RT-PCR) POCT for the qualitative detection of influenza A, influenza B and RSV, with results available in just 20 min. This platform is validated for use with nasopharyngeal swabs alone. Here, we describe the in-house validation of the cobas influenza A/B and RSV assay for use with gargles, sputa and ETS samples.

In total, 35 samples (20 gargles, 11 sputa and four ETS) were tested in this validation (Table I). Respiratory samples had previously been tested using the in-house real-time RT-PCR multiplex respiratory screen [4], and had since been stored at -80°C. Positive samples, with adequate remaining volume, were selected at random to cover a range of Ct values between Ct 15 and Ct 35, with the aim to reflect the typical range of Ct values observed throughout the season. We were unable to source any influenza A (H1N1)-positive gargle samples; therefore, we prepared simulated gargles for this validation. To prepare simulated gargles, tissue cultured influenza A (H1N1) was diluted in a pooled gargle, negative for the three viruses of interest, and dilutions of this sample were tested on our in-house RT-PCR to determine Ct values. Four simulated influenza A (H1N1)-positive gargles (ranging from Ct 20.00 to Ct 32.03) were included in this validation. Three gargle samples tested herein were dual positives: two gargle samples were positive for both influenza A and B, and one gargle sample was positive for both influenza A and RSV.

The Ct values for influenza-A-positive samples ranged from 20.00 to 34.36 (mean 27.90), Ct values for influenza-B-positive samples ranged from 15.26 to 34.89 (mean 25.50), and Ct values for RSV-positive samples ranged from 16.96 to 30.25 (mean 25.04). Prior to testing on both the in-house RT-PCR and the cobas Liat, sputa and ETS samples were mixed with an equal volume of mucolyse and incubated at room temperature for 15 min. Gargles were tested directly.

One sputum sample, which was influenza-A-positive by in-house RT-PCR, was reported as invalid by the cobas Liat, taking the total number of samples tested successfully to 34. All remaining samples tested positive for at least one virus. In total, 35 of 37 pathogens were detected correctly by the cobas Liat compared with the gold standard, resulting in a sensitivity of 94.59% (95% confidence interval 81.81–99.34%). In samples with single infections, all viruses were detected correctly by the POCT (100% sensitivity); this included all sputum ($N = 10$) and ETS ($N = 4$) samples, and 17 gargle samples.

There were three dual-infected gargles in this study (Table I). The cobas Liat identified both viruses correctly in one sample, but only identified the predominant virus in the remaining two samples. These two samples were re-run on the in-house RT-PCR and, again, influenza A and B were detected in both samples. The Ct values of viruses were similar (Ct 29.10 and 30.18) in the sample in which the cobas Liat correctly identified a dual infection, whereas the Ct differences in the other two samples were approximately 11 and 18; hence, it is likely that these pathogens were missed in the POCT due to competition. It should be noted that influenza A and B are in two separate multiplex assays in the in-house test, and hence competition would not occur.

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Table I
In-house real-time reverse transcription polymerase chain reaction (RT-PCR) and cobas Liat results for respiratory specimens included in this study

	In-house respiratory real-time RT-PCR				Cobas Liat	
Sample	Pathogen 1	Ct value	Pathogen 2	Ct value	Pathogen 1	Pathogen 2
G1	Flu A	32.03	–	–	Flu A	–
G2	Flu A	26.31	–	–	Flu A	–
G3	Flu A	23.67	–	–	Flu A	–
G4	Flu A	20.00	–	–	Flu A	–
G5	Flu A	26.32	–	–	Flu A	–
G6	Flu A	34.36	–	–	Flu A	–
G7	Flu B	25.64	–	–	Flu B	–
G8	Flu B	27.53	–	–	Flu B	–
G9	Flu B	23.40	–	–	Flu B	–
G10	Flu B	32.49	–	–	Flu B	–
G11	Flu B	20.81	–	–	Flu B	–
G12	Flu B	30.04	–	–	Flu B	–
G13	RSV	30.25	–	–	RSV	–
G14	RSV	24.45	–	–	RSV	–
G15	RSV	27.01	–	–	RSV	–
G16	RSV	16.96	–	–	RSV	–
G17	RSV	21.19	–	–	RSV	–
G18	**Flu A**	**23.75**	**Flu B**	**34.89**	**Flu A**	–
G19	**Flu A**	**33.10**	**Flu B**	**15.26**	**Flu B**	–
G20	Flu A	30.18	RSV	29.10	Flu A	RSV
S1	Flu A	24.26	–	–	Flu A	–
S2	Flu A	32.08	–	–	Flu A	–
S3	**Flu A**	**30.65**	–	–	**INVALID**	–
S4	Flu A	30.37	–	–	Flu A	–
S5	Flu B	27.31	–	–	Flu B	–
S6	Flu B	33.62	–	–	Flu B	–
S7	Flu B	19.52	–	–	Flu B	–
S8	Flu B	22.66	–	–	Flu B	–
S9	RSV	30.19	–	–	RSV	–
S10	RSV	26.59	–	–	RSV	–
S11	RSV	24.02	–	–	RSV	–
E1	Flu A	27.34	–	–	Flu A	–
E2	Flu A	24.01	–	–	Flu A	–
E3	Flu B	18.37	–	–	Flu B	–
E4	RSV	20.62	–	–	RSV	–

G, gargle; S, sputum; E, endotracheal secretions; RSV, respiratory syncytial virus.
Samples in bold represent discordant results.

Overall, the results suggest that, in addition to the validated specimen type (nasopharyngeal swabs), gargle, sputa and ETS specimens are suitable sample types for testing on the cobas Liat, although it should be noted that the number of samples tested in this study was small. This assay had excellent sensitivity for all three specimen types in samples positive for a single virus. Previous studies have also demonstrated that the cobas influenza A/B and RSV assay performs very well, and has a similar sensitivity to in-house RT-PCR methods and other commercial assays [5–8]. However, this study also found that dual-positive samples may not be detected if one virus is more prevalent in the specimen than the other.

Conflict of interest statement
None declared.

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