Verification of Cepheid Xpert Xpress Flu/RSV assay for use with gargle samples, sputa and endotracheal secretions

Sir,

Point-of-care respiratory virus testing is growing increasingly important in many healthcare settings, including primary care and hospital admissions, and also in vulnerable patient groups such as those requiring intensive care (ITU) or on haematology wards [1]. Rapid detection allows appropriate patient cohorting for infection control and improved patient management. This can have a significant impact on healthcare costs [1].

The Cepheid GeneXpert Xpress Flu/RSV assay is an automated in-vitro rapid diagnostic test for qualitative detection of influenza A, influenza B and respiratory syncytial virus (RSV), with results being obtained in just 33 min. At present, the Cepheid assay is CE marked for nasopharyngeal swab samples alone. At NHS Greater Glasgow and Clyde, we process many different sample types to look for causes of respiratory illness. For example, in addition to the commonly used throat swabs and nasopharyngeal aspirates, we often use gargle samples as they are sensitive sample types and acceptable to patients. Gargle samples are also a common sample type taken from haematology patients who can be prone to bleeding following throat swabbing. We also test lower respiratory tract samples such as sputum and endotracheal secretions (ETS) from patients with suspected lower respiratory tract infection (e.g. patients in ITU) [3].

In this letter, we describe the in-house validation of the Cepheid assay for use with gargle, sputa and ETS samples using a panel of samples known to be positive for influenza A, influenza B and RSV by our in-house reverse transcription polymerase chain reaction (PCR) multiplex respiratory screen [2]. In addition, the endpoint sensitivity of both methods was compared by assessing a 10-fold dilution series of each target.

In total, a panel of 33 gargle, sputa and ETS samples known to be positive for influenza A (N=16), influenza B (N=10) or RSV (N=7) were assessed as described in Table I. Gargle samples were intercepted on arrival at the laboratory and tested in parallel (no freeze–thaw of samples or long-term storage). Sputum and ETS samples were known positive samples selected from stored samples (-80°C) that had been screened recently through our in-house assay. The sputa and ETS samples were diluted in 500 μL of mucolyse and incubated at room temperature for 15 min. Samples were then processed in accordance with the kit instructions.

The Cepheid assay correctly detected 31 of 33 of the known, in-house positive samples as positive. Two gargle samples were not detected as positive by the Cepheid assay: Samples 1 (in-house RSV positive) and 10 (in-house influenza B positive) (Table I). These samples were repeated by in-house methods, and Sample 1 repeated as weakly positive (RSV Ct 36.67) and Sample 10 repeated as negative for influenza B. For two sputum samples, there was a notable difference of 4–5.5 log between the in-house and Cepheid methods (Table I). Sputum samples were reprocessed for the Cepheid testing; therefore, there may be some variation due to the variability of the starting material. This has been seen on retesting sputum samples using our in-house PCR method.

Table I
Gargle sample panel results

Sample	Sample type	Pathogen	In-house Ct	Cepheid Ct
1	Gargle	RSV	34.52 (R 36.67)	N[a]
2	Gargle	RSV	34.97	33.4
3	Gargle	RSV	21.07	22.7
4	Gargle	H1N1	34.57	34.3
5	Gargle	H1N1	34.53	30.6
6	Gargle	H3	24.15	23.9
7	Gargle	H1N1	18.4	13.8
8	Gargle	FLUB	18.68	20.3
9	Gargle	H1N1	17.9	15.7
10	Gargle	FLUB	34.47 (R N)	N
11	Gargle	H1N1	33.9	34.9
12	Gargle	H1N1	34.02	34.3
13	Gargle	FLUB	34.15	36.5
14	Gargle	FLUB	34.09	37.7
15	Gargle	FLUB	33.28	35.5
16	Gargle	RSV	32.77	37.9
17	Sputum	H3	29.1	24.5
18	Sputum	H3	24.93	20.3
19	Sputum	H3	25.08	28.2
20	Sputum	H3	32.65	23.6
21	Sputum	RSV	13.92	17.2
22	ETS	RSV	32.21	29.4
23	ETS	RSV	31.51	32.3
24	Sputum	H3	28.74	16.2
25	Sputum	H3	33.85	34.9
26	Sputum	H3	28.91	16.4
27	ETS	H3	25.08	15.9
28	ETS	H3	32.65	39.0
29	ETS	FLUB	20.16	23.1
30	ETS	FLUB	16.63	20.6
31	Sputum	FLUB	26.45	29.45
32	Sputum	FLUB	18.23	17.1
33	Sputum	FLUB	22.85	24.1

ETS, endotracheal secretions; RSV, respiratory syncytial virus; Ct, cycle threshold; N, negative; R, repeat.
[a] Weak trace observed below positive threshold.

For the assessment of endpoint sensitivity, dilution series of each target were first extracted and screened using in-house methods, and the endpoint dilutions were subsequently defined as the last two positive dilutions. These dilutions were tested in duplicate using the Cepheid assay. Influenza A and influenza B obtained the same endpoint of detection for both the Cepheid and in-house methods. For RSV, the Cepheid assay was slightly less sensitive at the endpoint of detection, failing to detect the last dilution detected by the in-house method.

Overall, the results suggest that gargle, sputa and ETS samples are suitable sample types for screening by the Cepheid assay with comparable sensitivity to in-house respiratory screens. This will allow for rapid infection control and treatment where appropriate for different patient

groups in many healthcare settings, such as point of care on the ward. The ability to process a wider range of respiratory samples will also aid use of the Cepheid assay in the laboratory setting.

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Conflict of interest statement
None declared.

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