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Comparison of gargle samples and throat swab samples for the detection of respiratory pathogens

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ABSTRACT

Respiratory illness causes significant morbidity especially in children, the elderly and the immunocompromised. The sample type taken and the quality of that sample are of great significance in providing an accurate diagnosis. Gargle samples are easy to take and sample the same area as a throat swab (THS). In this study, we assessed the utility of gargle samples for the molecular detection of common respiratory infections. Paired gargle and THS samples collected on the same day from the same patient were compared. We also included in our analysis paired THS and gargle samples that were collected within three days of each other as these samples are likely to have been taken during the same illness. Overall the data suggests that gargle samples are a more sensitive sample type than THS samples as overall the diagnostic yield was higher in the gargle samples and the Ct value of the gargle samples was stronger for the majority of samples in comparison to THS samples. Similar data was seen in the paired samples collected within one to three days of each other, as although the diagnostic yield between the sample types was similar (similar discrepant results), the majority of gargles had stronger Ct values than THS samples. This paper highlights the usefulness of gargle samples as non-invasive sensitive respiratory sample in comparison to THS samples. We recommend that other testing sites should consider using gargle samples for respiratory diagnosis as it will bring benefits in terms of sensitivity and sampling ease of use.

1. Introduction

Respiratory illness causes significant morbidity especially in children, the elderly and the immunocompromised. It is of great importance to identify the causative pathogen to aid infection control and the appropriate patient management. Respiratory pathogen testing has been revolutionised with the advent of molecular techniques and in particular multiplex real-time PCR (Ginocchio and McAdam, 2011) which is more sensitive and rapid than traditional methods and allows for the detection of a variety of pathogens simultaneously.

Various upper respiratory tract (URT) samples and lower respiratory tract (LRT) samples can be used for molecular respiratory pathogen testing. In general URT sample types such as throat swabs (THS) and nasal swabs (NS) are used as these are easy to take and acceptable to the patient. Nasopharyngeal aspirate (NPA) samples can also be used in children but are not acceptable in adults (Heikkinen et al., 2002; Abu-Diab et al., 2008; Chan et al., 2008; Debyle et al., 2012; de la Tabla et al., 2010; Gruteke et al., 2004; Lambert et al., 2008; Meerhoff et al., 2010; Sung et al., 2008). Lower respiratory tract samples such as sputum, and bronchoalveolar lavage (BAL) for detection of LRT infection are useful (Falsey et al., 2012; Branche et al., 2014; Jeong et al., 2014) but can be difficult to collect and may require pre-treatment before nucleic acid extraction.

Gargle samples (also known as mouth/throat wash) are an additional sample type that could be useful for the detection of respiratory pathogens. Gargle samples are easy to take and, by their very nature, sample the same area as a THS. However, there is little data assessing gargles as a sample type for respiratory diagnosis.

The WoSSVC accept all types of respiratory samples for respiratory testing including gargle samples. Occasionally we receive both a THS and a gargle for a particular patient. In this study, we took advantage of such duplication to assess the utility of gargle samples for the detection of common respiratory infections by comparing them to THS's.

2. Materials and methods

2.1. Study design

Between October 2014 and June 2015 the WoSSVC received 16503 samples for respiratory PCR testing from a number of different health boards across the West of Scotland (Greater Glasgow and Clyde, Lanarkshire, Forth Valley and Ayrshire and Arran). This included 6459

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Table 1
Patient details.

		Samples collected	
		Same Day	Within 1–3 days
Male		27	16
Female		52	12
Age	0–14	1	1
	15–24	8	1
	25–44	20	6
	45–64	28	12
	65+	22	8
Outpatient		25	7
Inpatient		54	21

THS samples and 5341 gargle samples. From these we identified 158 samples (79 THS and 79 gargles) that were collected from 79 patients on the same day. A further 58 samples (29 THS and 29 gargles) were identified that were collected from 28 patients within three days of each other. Any samples collected more than three days apart were excluded from the analysis due to concerns that any variation in Ct or positivity could be a result of the stage of infection rather than the sample type. Patient details are given in Table 1.

2.2. Laboratory methods

This was a retrospective study of THS and gargles received into the laboratory, the laboratory recommends to users that lysis buffer tubes (containing 1 ml lysis buffer) provided by the WoSSVC are used for transport of THS, and that gargles are taken as follows:- 5–10 ml sterile water or saline is gargled for 10 s and sent in a sterile universal container. Total nucleic acid was extracted from respiratory specimens using QIAamp Viral RNA kit (Qiagen, Crawley, United Kingdom) 263 µl of sample extracted and eluted into 110 µl or the bioMerieux easyMag (BioMerieux, Basingstoke, United Kingdom) 200 µl of sample eluted into 110 µl, according to the manufacturer's instructions. Samples were extracted and tested as they were received into the laboratory.

Sample were extracted and tested for respiratory pathogens by in-house molecular methods as described in Gunson and Carman, 2011. For each set of paired samples we compared the Ct values for all pathogens detected.

3. Results

3.1. Samples collected on the same day

In the study period 158 samples (79 paired gargle/THS samples) were collected from 79 patients on the same day. A total of 83 positive result sets were obtained from the 79 paired samples as four patients had two pathogens detected in the sample pair.

3.2. Results obtained from positive paired samples

Where both sample types were positive, a range of pathogens where detected. Influenza A was the most commonly detected pathogen and was detected in 18 paired samples. This was followed by influenza B (n = 11) and RSV (n = 6), other pathogens were detected in smaller numbers (Fig. 1).

The Ct values obtained for each positive gargle/THS pair is plotted as a difference plot in Fig. 2. As described in Fig. 2 these results indicate that the sensitivity of the THS sample type is less than that of the paired gargle sample, i.e. gargle samples give a lower ("stronger") Ct value than that of the THS sample in the majority of paired samples.

Examining this data by the pathogen detected showed a similar pattern to that outlined above. Difference plots for influenza A, influenza B and RSV are shown in Fig. 3a–c. As the figure illustrates the

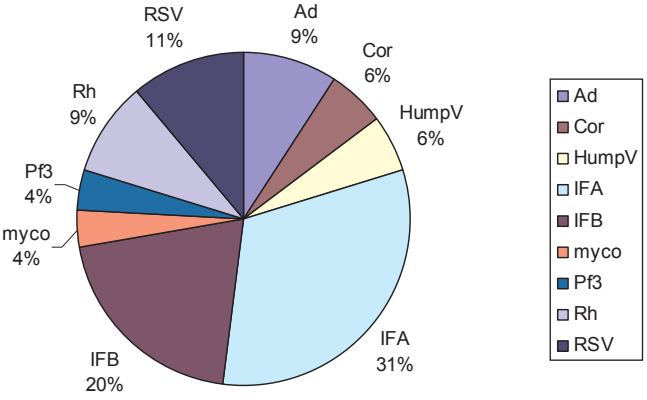

Fig. 1. Pathogens detected in same day testing.

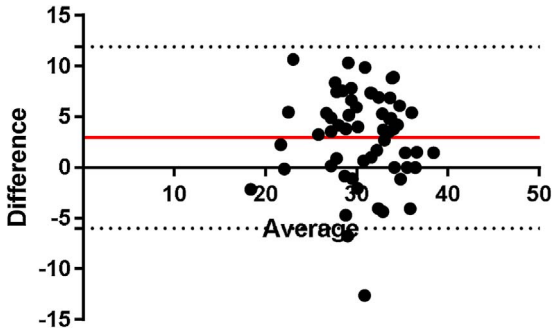

Fig. 2. Difference plot of Ct values of THS versus gargle samples in sample pairs collected same day.
Fig. 2 illustrates that the gargles samples are comparable to paired THS samples (the reference sample type) as a sample type for respiratory screening. This plot indicates that there is a bias (indicated by the line of mean difference) as the ct value of the THS is higher than that of that of the gargle samples.

results indicates that the sensitivity of the THS sample type is less than that of the paired gargle sample for these specific pathogens. Please note that we did not examine the other viral pathogens detected further due to the low numbers in the study cohort.

Of the discrepant results there were eight paired samples where the THS was positive and the paired gargle sample was negative, whereas there were 18 paired samples that were positive in the gargle and negative in the THS (Table 2). Of the eight THS positive/gargle negative pairs, four THS samples had a Ct value of greater than 35. The remaining four positive THS had Ct values between 28 and 35. Of the 18 gargle positive/THS negative pairs, five samples had a Ct value in the gargle greater than 35. The remaining 13 gargle positive/THS negative samples had Ct values higher between 29 and 35. A 2 × 2 contingency table (Table 2) was made and an Exact McNemar's test determined that there was no significant difference between the results (P = 0.755). These results suggest that the gargles are a comparable sample type to THS, the reference sample type. However, even though not statistically significant there is a bias of the gargle samples being more sensitive than THS sample as illustrated in Figs. 2 and 3.

3.3. Results obtained from positive paired samples collected within three days of each other

In the study period there were 29 paired THS/gargle samples that were collected within three days of each other. There were 20 paired samples where both samples were positive for at least one pathogen; nine paired samples had discrepant results. Of the 20 paired samples that gave positive results in both samples types a range of pathogens were detected. The results are plotted as difference plots in Fig. 4a and b.

Fig. 4a and b illustrate that there is a bias (indicated by the line of