Title: A Service Evaluation of Simultaneous near Patient Testing for Influenza, RSV, C. difficle and Norovirus in a UK District General Hospital

Running tittle: Evaluating near patient microbiology testing

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Structured Summary

Background: The Cepheid® GeneXpert® (GXP) can simultaneously test for norovirus (NV), *Clostridium difficile* (CD), influenza A/B (IFA/B) and respiratory syncytial virus (RSV).

Aim: To compare centralised multiplex polymerase chain reaction (PCR) testing with localised GXP testing at a district general hospital.

Methods: From December 2017 – December 2018, samples received at Whipps Cross University Hospital (WCUH) were first tested at the local laboratory before transport centrally to the Royal London Hospital (RLH). At the RLH, a non-proprietary multiplex reverse transcriptase (RT) PCR assay was performed, which also tested for GI or Respiratory pathogens not tested for by the GXP.

Findings: 1,111 stool and respiratory samples were processed at both sites. 591 were respiratory and 520 were stool samples. Compared to centralised testing, the GXP gave sensitivity, specificity, and NPV all in excess of 97%, with the exception of RSV. The RSV assay had a sensitivity of 66.7% (95%CI 24.1, 94.0) but a NPV of 99.7% (95%CI 98.6, 99.9). At the RLH, 65 (5.9%) additional respiratory or GI viruses were detected, predominantly rhinovirus 35 (3.2%) and adenovirus 11 (1.0%). Compared to centralised testing, the median time saved for local respiratory and gastro-intestinal sample testing was 19:46 (hh:mm) and 17:06 (hh:mm).

Conclusions: Local GXP testing compared to centralised multiplex PCR testing for IF, NV and CD, demonstrated sensitivities, specificities and NPV between 95% - 100%. Turnaround times were faster, enabling quicker Infection Prevention and Control decision making. In our local setting (WCUH), the GXP demonstrated the potential to reduce NV and IFA/B outbreaks.

Key Words:

Influenza; Norovirus; Cepheid; GeneXpert; Infection Control and Prevention

Introduction

With the networking of UK NHS laboratories, some hospitals have a reduced ability to provide virology results in a timely fashion. In these settings, near patient testing offers advantages both to patients and the Infection Prevention and Control (IP&C) team. Testing patients with symptoms at the first point of contact can identify infectious pathogens capable of causing outbreaks, improve patient management and mitigate the risk of infection transmission [1]. In particular, near patient testing can quickly identify sporadic cases of norovirus (NV) that may cause hospital outbreaks [2] whilst negative results can offer re-assurance that diarrhoeal outbreaks are not caused by NV.

Hospital outbreaks of NV associated gastroenteritis are well described. Between July 2013 and June 2016, it is estimated that 290,000 bed days were lost displacing 57,800 patients at a cost of almost £300 million to the UK health economy [3]. Whipps Cross University Hospital (WCUH) is a 730 bed district general hospital in East London and part of Barts Health (BH) NHS Trust. It serves an elderly, ethnically diverse population of approximately 275,500. Built in 1903, it has 54 isolation rooms and is mainly comprised of 'Nightingale' wards which facilitate the spread of infection. In 2010, from the 8th January 2010 to the 20th February 2010, because of patient safety concerns, the hospital closed to all adult emergency admissions. During this busy 6 week winter period there were 92 new cases of NV, 31 primary and 61 secondary, and the hospital lost operational control and approximately £6 million of patient related revenue.

With near patient testing, to achieve optimal sensitivity, specificity, positive, and negative predictive values (PPV and NPV respectively), the tested pathogen must be easily detectable within diagnostic samples and the infection must have a high prevalence in the target population. The cost of implementing near patient testing can be justified when the disease has a high cost burden and creates great pressure on health care services. In addition to NV, respiratory infections caused by influenza A/B (IFA/B) and respiratory syncytial virus (RSV) all fit these criteria.

In 2013, within the four hospital sites comprising BH NHS Trust, all microbiology and virology sample analysis were centralised at the Royal London Hospital (RLH) site. Samples were transported by courier van for the eight mile journey between WCUH and the RLH. Despite a regular dedicated courier service, there remained delays in receiving and processing patient samples. Consequently, positive and negative influenza (IF) and NV results were not acted upon in a timely manner, and there were delays in moving patients in and out of limited isolation facilities.

The Cepheid® GeneXpert® (GXP) is a random access rapid, PCR platform, capable of testing for NV, *Clostridium difficile* (CD), IFA/B and RSV. It has a small footprint and can easily be incorporated into a small local blood science laboratory. From December 2017 – December 2018, the aim of this study was to compare centralised multiplex PCR testing at the RLH to localised testing at WCUH for NV, CD, IFA/B and RSV using the GXP. We also determined the proportion of less pathogenic GI and respiratory viruses, not detected by GXP testing, and transport and processing turnaround times (TAT) at both sites.

Methods

Sample Capture

Testing was requested on the trust Cerner Millennium® (Cerner Ltd London, England) electronic patient record (EPR) system and samples sent to the WCUH pathology reception as per local trust standard operating procedure. Trained Medical Laboratory Assistant staff intercepted nasal, throat, and naso-pharyngeal viral transport media (VTM) swabs for IFA/B and RSV from ambulatory care, the emergency department and ward based patients. Stool and vomit samples were also intercepted from the same requesters and tested for NV and CD. The original samples were tested using the GXP and an aliquot made if it was not possible to test that sample immediately. Samples were then returned to the transport system so not to delay arrival at the RLH for multiplex PCR testing, our gold standard comparator.

Cepheid® GeneXpert® PCR Method

The GXP cartridge system use reverse transcription polymerase chain reaction (RT-PCR) for NV, IFA/B, RSV and standard PCR for CD. Inhibition of PCR was detected by transcription of a specimen control which reduced false negative reporting.

Gastro-intestinal Sample Processing

The GXP four module system and the proprietary analysis kits were used throughout this study; Xpert® C.difficile BT (GXCDIFFBT-CE-10), Xpert® Norovirus (GXNOV-CE-10) and Xpress Xpert® Flu/RSV (XPRSFLU/RSV-CE-10, Cepheid UK Ltd Woburn Green, England). The Cepheid SOP was adhered to. The only deviation was to use different specimen collection equipment. This meant that dry rayon tipped swabs (Medical Wire, Bath England, MW118S) were used for processing faeces and vomit. Raw unformed stool specimens and vomit were collected in a sterile universal container, free from any additives. Specimens were stored at 2-8°C when necessary for up to 48h. Stool samples were processed using a seasonal priority algorithm; between October and March they were processed for NV and then, if negative, CD. From April to September the converse occurred, stool samples were analysed for CD and then, if negative, NV. The only deviation from this was when particular patient samples were flagged as part of a possible outbreak of NV. This algorithm helped maximise module availability and speed of sample processing. We operated a five day service, Monday to Friday from 9am – 5.30pm.

Respiratory sample processing

Xpress Xpert® Flu/RSV cartridges (Cat. No.XPRSFLU/RSV-CE-10) were used. The only deviations from the Cepheid's SOP were to use different specimen collection equipment and additional sample types. The only sample type validated for the Xpress Flu/RSV is a naso-pharyngeal swab. However we processed nose, throat, naso-pharyngeal viral transport media swabs (Sigma Virocult® (Medical Wire, Bath, England, Cat. No.MW951S) instead of the Cepheid collection kit. We also accepted naso-pharyngeal aspirates and bronchoalveolar lavage samples. Specimens were transported at 2-8°C and stored at room temperature at 15-30°C for up to 24 hours and refrigerated at 2-8°C up to seven days before testing.

Multiplex PCR testing for GI and Respiratory viral pathogens at the RLH

The laboratory at RLH used QIAGEN® (Manchester, England) equipment for the non-proprietary respiratory and gastro-intestinal PCR assays. Samples were extracted using QIAGEN® QIAsymphony® DSP DNA Mini Kit (Cat. No.937236) for the QiASymphony® SP/AS instrument (Cat. No.9020246).

QuantiTect® Virus Kit (Cat. No.211031) along with Sigma® assay specific primers and probes ribed previously were used to create the master mix, this was then mixed with patient samples in individual reaction tubes using the QIAGEN® QIAgility® instrument. The sample reaction tubes were then loaded into a QIAGEN Rotor-Gene®. Analysis of melt curves took place via the Rotor-Gene® software and samples were deemed positive where there was a sufficient increase in fluorescence over a number of cycles. This service was offered 7 days a week, Monday – Friday, 09.00am – 17.00pm and Saturday and Sunday, 09.00am – 13.00pm.

Reporting Method and data analysis (GXP at WCUH)

There was no operator interpretation, the instrument had a specific programme for each cartridge and would signal whether the target pathogen was detected or not. The IP&C staff was informed of positive and negative results which were also recorded in the EPR.

For stool and respiratory samples, sensitivity, specificity, PPV and NPV were calculated to assess the performance of the Cepheid® instrument at WCUH compared to the gold standard of multiplex PCR testing at the RLH. Less significant viral respiratory or GI pathogens missed due to selective testing were also recorded. When results were discordant, samples were sent for third party testing. Third party assays used were the BD MAX^{TM} CDifficile (BD Wokingham, England), the *High*-Plex® Upper Respiratory Pathogens MT-PCR (AusDiagnostics UK Ltd) and a non-proprietary influenza triplex PCR (Public Health England VRD, Colindale, London, England). The Laboratory Information System (LIS) was interrogated to determine the date and time of sample request, date and time sample received, processing time and date and time a final report was released from both laboratories. Collated project data was matched to the RLH analysis via the laboratory assigned number. Samples were matched using a database giving the results from both labs and whether the results agreed or disagreed. TAT were calculated for both sets of data by subtracting the sampling time and date from the receipt time and date, giving the time taken for the sample to reach the laboratory. In the same way the sample receipt in the laboratory time and date was subtracted from the final report time and date, to give the time from receipt to the issue of the final report. These were expressed as median times with interquartile ranges (IQRs).

Patient pathways

If a patient tested NV positive (either test) they were immediately isolated or, depending upon bed availability, cohorted. If a patient tested IFA/B positive (either test) a clinical decision was made on hospital admission based upon age, co-morbidities and severity assessment. If admitted to hospital, patients were isolated or cohorted and treated with an anti-viral agent. If the decision was not to admit, the patient may have been prescribed an anti-viral agent, based on clinical assessment.

Results

From December 2017 to December 2018, 1,111 stool and respiratory samples were processed at both RLH and WCUH. 591 (53.2%) were respiratory samples and 520 (46.8%) were stool samples. Due to the seasonal testing algorithm, not all 520 stool samples were tested for both CD toxin genes and NV. 455 were tested for CD and 490 tested for NV. The number of samples received and the positivity rate demonstrated seasonality, with most samples and positives received in the winter months. From January to February (2017/8 winter months), more IF was detected than NV, although in December 2018, substantially more NV was detected than influenza (Figure 1). There was also variation in the types of wards or areas where patients with IFA/B (105) and NV (47) were detected. For IFA/B, patients were in the following areas at time of diagnosis; A&E 10 (9.5%), acute assessment or clinical decision unit 36 (34.3%), ambulatory care 6 (5.7%), medical wards 37 (35.2%), HDU/ITU 6 (5.7%), surgical wards 5 (4.8%) and Paediatric wards 5 (4.8%). For NV, most patients were on medical wards 37 (78.7%), AAU 6 (12.8%) and on Paediatric wards 4 (8.5%). None were diagnosed in A&E, Ambulatory care, HDU/ITU or on surgical wards.

Of the respiratory samples tested locally at WCUH, 109 (18.4%) tested positive for IFA, IFB or RSV and, of the stool samples, 47 (9.6%) and 28 (6.2%) were positive for NV and CD respectively. Compared to testing at the RLH, there were 45 false positive results and 6 false negative results (Table I). Using RLH testing as our gold standard, the sensitivity, specificity, PPV and NPV for the five major pathogens, with the exception of RSV, demonstrated excellent sensitivity, >95%, and all assays had an NPV of >99%. In nearly all cases, a negative result ruled out suspected infection (Table II).

During this service evaluation, the GXP assays gave 51 (4.6%) of 1,111 non-concordant results. When results were discordant, some of these were sent for third party testing where 18 (35.3%) RLH results were confirmed, 10 (19.6%) GXP results were confirmed and 23 (45.1%) were not retested due to insufficient remaining specimen (Table III).

Because local testing at WCUH was limited to 5 major pathogens, 65 (5.9%) other less pathogenic viruses were detected at the RLH site. Most commonly these included rhinovirus, adenovirus, and metapneumovirus. The aetiology and proportion of other viral pathogens isolated are summarised in table IV.

The median time taken for a stool or respiratory sample to reach the WCUH laboratory was 3:55 (hh:mm) (IQR 1:02-18:39) and 2:26 (IQR 0:46-12:42) respectively, compared to 17:51 (IQR 7:59-22:31) and 18.50 (IQR 10:09-24:19) at the RLH Virology Department. The median time to process a stool or respiratory sample at the WCUH laboratory was 2:53 (IQR 2:25-3.30) and 1:08 (IQR 0:52-1:42) respectively, compared to 6:06 (IQR 4.44-21:48) and 4:30 (IQR 3:52-5:46) at RLH. Therefore, when the combined transit and processing times are compared, the median difference was approximately a 19 hour delay for a respiratory sample and a 17 hour delay for a gastrointestinal sample.

Discussion

GXP testing for NV, CD and IFA/B proved similar to multiplex PCR testing at the RLH. Sensitivity and specificity, compared to RLH testing, were all >95% with only the RSV assay significantly less sensitive. GXP missed 7 IFBs (table 3) so may be less sensitive than centralised testing for IFB detection. A small number of false positive samples for IFA/B and RSV (compared to centralised testing) probably meant that some patients were unnecessarily isolated and treated with antivirals, although another explanation is that the GXP was more sensitive for IFA and RSV testing. For the five major pathogens NV, CD, IFA/B and RSV, the NPVs were all >99%, indicative of a high level of certainty in ruling out suspected infection. This was a valuable aid in keeping open wards with suspected diarrhoeal or IF outbreaks which, ordinarily, may have been closed. A significant number of less pathogenic viruses were detected (e.g. rhinovirus) but none of these were deemed to be of significant infection control importance in immunocompetent patients. Taking this into account, we concluded that the viral targets covered by the GXP were sufficient, without the need for full panel PCR testing at the RLH, unless patients were aged <16 years or immunocompromised.

TAT for receipt and reporting of samples at WCUH was considerably shorter compared to centralised testing at the RLH. This meant that the IP&C team could immediately act on results; keeping wards open following negative results but also isolating infectious patients with positive results in a more timely fashion.

There are three other comparable instruments to the GXP; bioMérieux® Biofire® FilmArray®, QIA GEN® QiaStat® and GenMark® ePlex®. All of these are random access bench top analysers which offer simultaneous NV and IFA/B testing. The complexity of operation is low for all platforms, although cost per test for the GXP is cheaper, making it, at present, our platform of choice. The GXP was easily incorporated into a blood science laboratory and can be interfaced with the WinPath®

(CliniSys Group Ltd) LIS (widely used in the UK), which can then transfer results to the Cerner Millennium® EPR. It is possible to interface the other instruments to WinPath®; however there are no sites in the UK where this is operational at this time (information from CliniSys Group Ltd).

The GXP also demonstrated versatility in processing different types of samples although we did not analyse results according to sample types. A variety of respiratory samples were processed including Sigma Virocult® VTM swabs and naso-pharyngeal aspirates. As there were sometimes delay in collecting stool samples, we would like to have processed rectal swabs, particularly in an outbreak situation, but this was not possible and should be the subject of a further study.

Our service evaluation was unique in that we found no other near patient platforms that tested simultaneously for IFA/B and NV. There were significant differences in the types of wards or areas patients were at the time of diagnosis. For IFA/B, most patients were diagnosed in the A&E, acute assessment and clinical decision unit whereas for NV the majority of patients were diagnosed on medical wards. Therefore this platform, when testing for IFA/B and NV simultaneously, is not suitable for use as an A&E point of care instrument.

There have been two studies of NV using the GXP, both in laboratory settings, one using the CDC NV reference method [4], and one using an in-house NV PCR assay [5]. This group was the only one to investigate TAT. Regarding IF, there have been three GXP IFA/B evaluations, one compared the Hologic® Prodesse® ProFlu +® and cell culture [6]. The second evaluated the impact of rapid PCR on patient length of stay (LOS) and, finally, a point of care evaluation which also used Hologic® Prodesse® ProFlu +® as their comparator [8]. These publications varied in sample size, comparative methods and settings, with only one study comparing localised with centralised testing [4]. Overall, like our study, the sensitivity of the GXP for IFA/B and NV was between 93-100% and the NPV between 99-100%. The speed of the GXP proved to be advantageous in making quicker patient care decisions and offering IP&C advice when compared to batch non-random access PCR platforms. One study proved that there was a significant reduction in patient LOS and microbiology test requests for patients with a positive GXP result [7].

Our study demonstrated that the high sensitivities and NPVs for IFA, IFB and NV were, again, reproducible. The value of simultaneous testing for IF and NV was also demonstrated with high numbers of IFA and IFB detected in the 2017/18 winter season compared to far higher numbers of NV detected in the 2018/19 winter season. The impact on patient flow and initial management, which we also experienced, has been demonstrated in other studies [9-10]. Although we have emphasised the utility of rapid IF and NV results, the RSV results also had an impact on patient flow in paediatric wards. As previously described, availability of a positive RSV result in a symptomatic child can allow for cohorting of patients that is reflected in better utilisation of isolation facilities [11].

There were some limitations to this study. Automated results were taken at face value. We did not take into consideration Ct values in discordant samples that may have been false positives, nor did we have verification data available to provide evidence that the automated result was valid for our population. We did not collect data on patient clinical speciality (including Paediatrics) and compare and contrast results. We were unable to accurately calculate bed days lost, although, anecdotally, at least 300 bed days were saved over a 12 month period. Based on non-tariff prices, the GXP costs £30 for Xpert® Flu/RSV compared to £21 for multiplex in-house respiratory panel. A cost effectiveness argument for localised testing can therefore be made on the basis of a reduction in bed days lost and reduced cleaning costs, despite our lack of precise data.

In the UK, following the Carter report [12], there has been an emphasis on centralisation and formation of Pathology networks. The efficiency of networks relies upon good transport and IT links which can be difficult to organise, particularly for hospitals in different NHS Trusts. A competing model is the Lord Darzi vision [13] which can be summarised as 'localise where possible, centralise where necessary'. Our service evaluation demonstrates the utility of a localised random access real time PCR platform incorporated into a blood science laboratory. The advantages were faster turnaround times and comparable performance when testing for IFA/B, CD and NV. Clearly, this is a local testing innovation which aligns with Darzi's vision.

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Conflict of Interests Statement

None declared.

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