A reliable and easy to transport quality control method for chlamydia and gonorrhoea molecular point of care testing

S. G. Badman1, L. M. Causer1, R. Guy1, H. Wand1, B. Donovan1,2, S. N. Tabrizi1,4,5, D. Speers6,7, M. D. Shephard8, A. Vallely9 and D. Whiley9,10, on behalf of the TTANGO Investigators11

1Kirby Institute, UNSW Sydney, Sydney, NSW, 2Sydney Sexual Health Centre, Sydney, NSW, 3Department of Microbiology and Infectious Diseases, The Royal Women’s Hospital, Melbourne, Vic, 4Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Vic, 5Murdoch Children’s Research Institute, Parkville, Vic, 6Pathwest Laboratory Medicine WA, Nedlands, WA, 7School of Medicine and Pharmacology, University of Western Australia, Crawley, WA, 8International Centre for Point-Of-Care Testing, Flinders University, SA, 9UQ Centre for Clinical Research, The University of Queensland, Brisbane, Qld, and 10Pathology Queensland Central Laboratory, Brisbane, Qld, Australia; *members are listed in the Acknowledgements

Summary
Quality control (QC) is an essential component of point-of-care testing programs. In the context of a randomised-controlled trial (TTANGO) using GeneXpert (Xpert) Chlamydia trachomatis and Neisseria gonorrhoeae (CT/NG) point-of-care testing in remote areas of Australia, we aimed to develop and utilise a stable positive control material.

Bacterial cultures of CT and NG were resuspended together to provide cycle threshold (Ct) values of approximately 25 cycles for both CT and NG when tested on the Xpert CT/NG assay. These positive control suspensions were dried in aliquots, heat inactivated, and then provided to 12 participating health services as research-only QC samples in kit form. At each service, a QC sample was resuspended and tested each month on the Xpert. QC results, including Xpert Ct values, were analysed from each site over 30 months and we calculated costs per QC sample.

Overall, at 12 health services there were 89 QC samples tested (average of 8 tests per site per year). Mean Ct values for the 89 controls samples were 25.25 cycles (SD = 1.15) for CT, 24.04 cycles (SD = 1.400) for one NG target and 23.35 cycles (SD = 1.55) for the other NG target. No significant differences in Ct value for CT or NG controls were observed over a trial period of 30 months.

Positive QC samples for research use in a trial of a molecular point-of-care assay were inexpensive to produce and stable when stored at 2–8°C. For routine use, additional requirements such as meeting National Association of Testing Authority (NATA) regulations and Therapeutic Goods Administration (TGA) approval will need to be achieved.

Key words: GeneXpert; chlamydia; gonorrhoea; DNA; quality; control; assurance; point-of-care.

INTRODUCTION
Nucleic acid amplification testing (NAAT) is now a common laboratory method used to diagnose a range of bacterial and viral infections. For many pathogens NAATs have the advantage of shortening the window period to diagnosis, and for others they are the only way of determining if the infection is active versus a past exposure. However, use of NAAT-based point-of-care (POC) tests by primary care services has been mainly limited to tuberculosis (TB), with the scope beginning to expand to other infections. Availability of molecular POC devices provides an opportunity for accurate test results to be generated by clinical staff, rather than laboratory, and more timely treatment which has advantages for clinics that are significant distances from laboratories or where there are high rates of patients lost to follow up.

With increasing use of POC assays has come the need to ensure that quality management frameworks, including quality control (QC) and external quality assurance (EQA) testing, are available. Quality management frameworks are important to monitor the reliability of results, alert operators if a change in test performance has occurred, and reduce the risk of misdiagnosis. In Australia, the National Pathology Accreditation Advisory Council (NPAC) guidelines recommend QC be run regularly to ensure that all testing is performed using instruments, reagents and consumables which are working correctly and according to specifications. They also recommend that POC testing devices should achieve an acceptable standard of performance in external proficiency testing programs whereby the provision external quality assurance (EQA) panels are provided for testing. The EQA panels typically comprise a series of positive and negative samples in a panel for testing at the location of the device. Operators are usually blind to the results, and the results are returned to the provider for review and assessment of test accuracy when compared to other laboratories who participate in the same peer program.

The Test-Treat And Go (TTANGO) cluster randomised trial was implemented from June 2013 to December 2015 in 12 remote Australian Aboriginal and Torres Strait Islander...
primary health services to determine the acceptability and cost-effectiveness of POC testing for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) and the operational performance of the POC test in the real-world. The trial was implemented in remote Aboriginal communities where there is a high sexually transmitted infection (STI) prevalence, and coinfection rate in young people
 delays in treatment, with up to 25% of patients not receiving treatment and an average time to treatment of 3 weeks with laboratory-based testing. Full details of the TTANGO trial protocol are provided elsewhere. The POC device chosen for the TTANGO trial was the GeneXpert CT/NG (Xpert) (Cepheid, USA) due to ease of use, dual detection of CT and NG by NAAT and demonstrated accuracy. Xpert CT/NG is also approved for testing female endocervical swabs, patient-collected vaginal swabs and for female and male urethral specimens.

A quality framework was established in the study, including QC and EQA. For EQA, we purchased CT/NG external EQA panels manufactured by the National Reference Laboratory (Melbourne, Australia) for the trial. In regards to QC, the Xpert has three in-built QC measures for each test conducted. These are used to assess the adequate performance of critical processes in each test reaction, and include: a sample adequacy control (SAC); testing for the presence of human DNA, a sample processing control (SPC); testing for polymerase chain reaction (PCR) inhibition or extraction failure by the use of internal control DNA and a Probe Check Control (PCC), which verifies reagent rehydration, the PCR tube filling in the cartridge, probe integrity, and dye stability. All must process correctly for the CT/NG test to be valid. The probes in the Xpert detect one sequence for CT (CT1) and two different sequences for NG (NG2 and NG4). Both NG targets must be detected for the Xpert to return a positive NG result. Independent CT and NG controls, however, are not provided with the Xpert CT/NG kit to assess the performance of these test reactions.

In this study, we sought to identify an independent positive control for the Xpert CT/NG test that combined both CT and NG DNA (for simultaneous confirmation of both CT and NG test performance) within the one reaction for the purpose of QC testing and clinical staff training. The control sample also needed to be inexpensive and ideally be produced in a dried tube format (DTF) such that it could be mailed to health services in the conventional postal system at an affordable cost and be easily adapted for staff training purposes. To our knowledge, the only commercially available QC option available at the start of the trial was the ZeptoMetrix NATrol (USA) CT/NG material which required CT and NG positive controls to be purchased separately at a combined cost of AUD$126 and in a liquid format. Based on the total number of samples needed for the trial, including those required to support staff training, the cost for this commercial option was considered to be outside of our available budget and in a format not suitable for our needs. Therefore, we developed in-house CT and NG positive DTF control samples and determined the cost of production and stability over the course of the trial (Table 1).

METHODS

Ethics

Ethical approval for this trial was provided by UNSW Sydney, and the Children’s Health Services, Queensland Human Research Ethics Committee. The trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000808741). Written consent to publish de-identified QC test data was obtained from participating health services prior to the commencement of this trial.

Development of the CT/NG in-house quality control samples

The positive control samples were prepared using bacterial cultures of CT and NG and comprised a local wild-type clinical CT strain grown in HEP-2 cells. As the Xpert SAC requires the presence of human DNA to provide a valid result, it was advantageous that the CT was cultured from a human-derived cell line. The NG culture was a local wild-type clinical strain and grown on selective agar. CT and NG cultures were initially tested individually with the Xpert to provide cycle threshold (Ct) value estimates for each. Based on these Ct values the CT and NG cultures were then combined and diluted such that a 10 µL aliquot (when made up to 1 mL with sterile water as outlined below) would provide Ct values of approximately 25.0 cycles for both CT and NG when tested using the Xpert assay. In doing so it should be noted that the controls were provided as research-use-only for the TTANGO Trial.

Pretesting of in-house quality control samples

A 10 µL aliquot of the dilution was added to 10 separate 2 mL sample tubes. All tubes (with caps removed) were placed in a heater block at 95°C for 10 min to dry and render the material non-infectious. After cooling, the tubes were capped. To prepare the controls for testing, each 2 mL dry tube sample was reconstituted using 1 mL of sterile water, agitated briefly by hand, allowed to settle and then transferred into the Xpert CT/NG cartridge. The Xpert CT/NG test was then performed on each of the 10 samples as per manufacturer’s instructions and results kept for stability record purposes.

QC and training sample kits

A total of 144 QC samples were provided to health services in a kit form, which comprised a re-sealable plastic bag containing 12 in-house CT/NG QC samples and 12 tubes containing 3 mL of sterile water for reconstitution. Using the same CT/NG dilution, each site also received a separate training kit with five positive CT/NG DTF samples designated for training and a matching number of tubes of sterile water for reconstitution, plus five positive air dried CT/NG swabs for use with Cepheid swab collection tubes. On delivery, it was recommended that both the controls and training samples be refrigerated immediately at 2–8°C until ready for use.

Delivery to sites

QC and training kits were transported by air freight to health services along with Xpert cartridges in insulated foam containers. Given the significant distance to these remote service locations, up to 3740 kilometres, and the fact that ambient daytime temperatures at airline transit locations and the final service destination can exceed 40°C during the summer months, a temperature logger to monitor transport temperature history was included with each shipment.

POC test operator training

Clinical staff underwent comprehensive Xpert competency based training as a part of this trial, including a specific component on the reconstitution and testing of QC samples. A specific standard operating procedure (SOP) for this process and a visual wall aid were developed; the latter included photographic thumbnails, which guided test operators through the process of preparing and testing the controls. A hard-copy training manual, which covered the Xpert testing system, was available at each site and newly trained operators were encouraged to use the QC section as a primary reference source. Xpert test results, including those performed using the controls, were monitored using real-time, remote login software. A paper-based record of each monthly QC test event was also maintained by operators at each service.

This software allowed trial coordinators to remotely monitor testing and test performance directly from the Xpert laptop computer at each health service and to provide telephone support to services as needed. The monitoring system was also used to identify when device maintenance was required and to determine when calibration of the Xpert machine should take place.
Data collection and statistical analyses

Electronic data from each Xpert QC test result was transferred into a Microsoft Excel spreadsheet by a trial coordinator; this data included the date of test, time of test and all individual Xpert output results including Ct values for CT1, NG2 and NG4 detections, SAC, SPC and PCC internal control check details. Probe check results, which determine the successful completion of each test phase, were also collected.

We used descriptive analyses to calculate the mean of all Ct values for QC samples tested across all sites, and for each test type (CT and NG). We then stratified the mean Ct values by four geographical regions (1, 2, 3 and 4) and the elapsed time between the QC sample being manufactured and used for QC testing (<6 months, 6–12 months, 12–18 months, 18–24 months and >24 months). This was done to take account of any potential variation between regions such as the distance from the point of QC manufacture and the conditions under which controls and training samples had to travel to sites. In some cases, this meant having to use a mixture of air and road transport given the remoteness of some health services. Linear mixed models were then applied to determine if there was any change in the Ct values over time, accounting for potential correlation due to repeated measurements. Data were explored to determine any interaction between time elapsed and region and its impact on the Ct value of each QC test conducted. Estimated fixed effects and their 95% confidence intervals (CIs) were reported. Standard deviations and their 95% CIs of the random components were also obtained. The impact of time between the date of QC sample manufacture and date of each QC test was then assessed using statistical software (Stata 14.0; StataCorp, USA).

From the perspective of a research trial, the cost of production per QC sample was also calculated; these costs included the purchase of consumables used to prepare the QC samples (2 mL aliquot tube and barrier pipettes) and laboratory staff time to prepare the samples (to aliquot samples from the prepared CT/NG dilution, place tubes in a heater block for drying and remove them to a storage box for cooling and labelling). Preparation of sterile water was explored to determine any interaction between time elapsed and region and its impact on the Ct value of each QC test conducted. Estimated fixed effects and their 95% confidence intervals (CIs) were reported. Standard deviations and their 95% CIs of the random components were also obtained. The impact of time between the date of QC sample manufacture and date of each QC test was then assessed using statistical software (Stata 14.0; StataCorp, USA).

RESULTS

The pre-testing of the 10 QC samples showed consistent amplification for all targets (CT1, NG2, NG4, SAC and SPC) as well as valid probe check results. At baseline, the mean Ct value was 25.39 cycles (SD = 0.83, CI 23.7–27.0) for CT1, 24.24 cycles (SD = 1.23, CI 21.8–26.6) for NG2 and 23.66 cycles (SD = 1.45, CI 20.8–26.5) for NG4. The mean SAC and SPC control Ct values were 27.6 cycles (SD = 1.07, CI 25.5–29.7) and 33.2 cycles (SD = 0.98, CI 31.2–35.1), respectively.

Based on this consistency a total of 144 positive CT/NG DTF control samples for QC were manufactured and distributed to the 12 health service sites participating in the trial. All electronic test results, including those for QC, were systematically reviewed from all sites by a trial coordinator. Only one invalid QC test was noted in the data, and the SAC indicated the sample volume was below the required amount to process the test correctly. A repeat of the QC test on the same day, and using a new sample, indicated a valid result.

In all, 89 QC results were available from 12 sites for analysis of their Ct values. Mean Ct values were 25.25 cycles (SD = 1.62, 95% CI 24.7–25.8) for CT1, 24.04 cycles (SD = 1.40) for NG2 and 23.35 cycles (SD = 1.55) for NG4.

All 89 QC samples tested provided amplification in the SAC reaction whereas two SPC reactions did not amplify. The mean SAC Ct value was 27.96 cycles (SD = 1.62, 95% CI 27.6–28.3) for the static Xpert SPC internal control. and any observed Ct value variation was otherwise consistent with that observed for the static Xpert SPC internal control.

The pre-testing of the 10 QC samples showed consistent amplification for all targets (CT1, NG2, NG4, SAC and SPC) as well as valid probe check results. At baseline, the mean Ct value was 25.39 cycles (SD = 0.83, CI 23.7–27.0) for CT1, 24.24 cycles (SD = 1.23, CI 21.8–26.6) for NG2 and 23.66 cycles (SD = 1.45, CI 20.8–26.5) for NG4. The mean SAC and SPC control Ct values were 27.6 cycles (SD = 1.07, CI 25.5–29.7) and 33.2 cycles (SD = 0.98, CI 31.2–35.1), respectively.

It should be noted that a failed SPC reaction does not invalidate a test where the CT and NG reactions have provided positive results. This is because the SPC reaction has been developed as a weaker PCR reaction to ensure that it will not interfere with amplification of the test targets. The mean Ct values for CT and NG did not appear to vary considerably by geographical region or time elapsed (Fig. 1A,B). The analysis also noted that one health service in region 4 did not commence POC testing until Dec 2013 which meant there were no QC test results to report within the first 6 months of the trial. Using linear regression, Ct values for both CT and NG showed no trend during the 30-month testing period (p = 0.747). Results did not change when the models were fitted using the interactions between time elapsed and the regional geographical locations where QC testing took place (p = 0.589) (Table 1).

The temperature logger data collected during the transportation of QC kits to health service sites were reviewed by a trial coordinator for each service after delivery, and all kits remained below 28°C in transit to services.

From the perspective of a research trial and based on the in-house development and production of 144 positive QC samples; we were able to prepare these controls at an estimated cost of AU$4.25 per sample.

| Table 1 | Trends in Ct value for CT and NG QC samples (n = 89) at 12 remote health services, June 2013 – December 2015 |
|---|---|---|---|
| **Chlamydia trachomatis** | **Model – 1 (no interaction)** | **Model – 2 (with interaction)** |
| Estimated fixed effects (95% CI) | p value | Estimated fixed effects (95% CI) | p value |
| Time points (overall trend days) | -0.0002 (-0.001, 0.0008) | 0.747 | -0.0003 (-0.001, 0.0007) | 0.589 |
| Estimates of random component (sites) | 1.15 (1.00, 1.33) | 1.14 (0.98, 1.32) |
| Standard error | Interactions | 0.0001 (-0.000, 0.0003) | 0.211 |
| Neisseria gonorrhoeae | 2-way interaction (days x site) | 0.169 | 0.0008 (-0.002, 0.003) | 0.166 |
| Time points (overall trend days) | 1.38 (1.19, 1.60) | 1.37 (1.19, 1.59) |
| Estimates of random component (sites) | 2-way interaction (days x site) | 0.000 (-0.0002, 0.0003) |

Please cite this article in press as: Badman SG, et al., A reliable and easy to transport quality control method for chlamydia and gonorrhoea molecular point of care testing. Pathology (2017), https://doi.org/10.1016/j.pathol.2017.09.012
The samples were stable when stored under refrigerated conditions over a 30-month period. The samples were regularly used by most clinical sites and produced valid monthly QC results across a variety of different remote regions and POC sites.

The cost of developing an in-house sample for dual QC testing of CT and NG for this trial was minimal (less than AU$5.00 per sample). This was possible using a dry tube format (DTF) and offered considerable financial savings when compared to commercially available alternatives. The samples were stable when stored under refrigerated conditions over a 30-month period. The samples were regularly used by most clinical sites and produced valid monthly QC results across a variety of different remote regions and POC sites.

To our knowledge, this is the first report of a non-commercial method to manufacture CT/NG QC and training samples for use with the Xpert CT/NG molecular POC assay. The production of the samples was feasible and relatively inexpensive for research use when compared to commercially available alternatives. The samples were stable when stored under refrigerated conditions over a 30-month period. The samples were regularly used by most clinical sites and produced valid monthly QC results across a variety of different remote regions and POC sites.

The cost of developing an in-house sample for dual QC testing of CT and NG for this trial was minimal (less than AU$5.00 per sample). This was possible using a dry tube format (DTF) and offered considerable financial savings when compared to the liquid QC option recommended by the manufacturer of Xpert which is AU$63.00 for each CT positive QC sample and AU$63.00 for each NG positive sample. This amounts to a total of AU$126.00 which does not account for freight importation if required or other costs such as customs duty and taxes. The use of these controls would also mean having to prepare and test each type of QC in a separate Xpert cartridge given it appears they are not supplied in a dual CT/NG positive only format.

The in-house production method proved very useful for staff training. Having positive training samples available on site at the commencement of trial operations allowed POC coordinators to immediately demonstrate (1) sample preparation, (2) entry of test details into the Xpert laptop computer, and (3) visual demonstration of positive test results before the Xpert device was implemented in remote health service settings. Interviews with clinical staff, including aboriginal health workers and registered nurses, indicated a high level of operator satisfaction when using Xpert under remote conditions, including the processes of preparing QC samples and performing QC tests.

A limitation of this QC strategy is that it does not truly mimic the urogenital and extragenital urine and swab samples that are routinely being tested on the instrument; it is effectively a suspension of CT and NG DNA and therefore does not adequately test the extraction protocols used by the instrument that may potentially be affected by sample viscosity or matrix issues. Nevertheless, we believe this limitation is outweighed by the simplicity of the QC sample production and use and the fact that it otherwise provides valid performance criteria for the remaining amplification and detection processes.

Use of this in-house developed QC material outside of the research environment, either commercially or in a routine programmatic implementation, would require the material to be developed and manufactured under local regulatory requirements (e.g., as per National Association of Testing Authority and Therapeutic Goods Administration specifications, Australia). These authorities may require further information on the provenance of the controls, including DNA sequencing to define the CT and NG strain-types used in the controls, through to an overall assessment and subsequent registration of the QC process. Meeting these requirements would likely incur a number of additional costs and therefore increase the final price of production.

For QC samples to achieve the greatest benefit, they need to be regularly used. In the trial, only 62% of QC samples distributed to health services (89/144) were tested and their results available for this analysis. Although some follow-up occurred to remind services about the QC testing, there was no formal feedback loop. Heavy service workloads and frequent staff turnover at some remote health service sites, and hence a loss of training continuity, may have also contributed to this interrupted participation in QC testing. It is also possible that staff at some health services did not enter the correct QC sample identifier before testing the control which resulted in it being missed on review. The TTANGO trial has now been expanded into a program, called TTANGO2, involving 33 remote and regional health services across Australia. In this model, the QC and EQA program is being managed by the Flinders International POC Centre which will provide additional oversight, governance, staff training and feedback to health services to enhance and maintain this system and ensure compliance with national standards.

**CONCLUSION**

In summary, these results show CT/NG positive DTF samples can be reliably produced in-house and used for both QC purposes and training in remote Australian settings where CT/NG POC testing is being conducted. In the context of this research trial, the estimated low cost to produce each in-house control also provides a viable alternative where commercially prepared controls are unavailable, unsuitable or too
The authors state that there are no conflicts of interest to disclose.

Address for correspondence: Steve Badman, The Kirby Institute, Level 6, Wallace Wurth Building, UNSW Sydney, NSW 2052, Australia. E-mail: sbadman@kirby.unsw.edu.au

Conflicts of interest and sources of funding: The TTANGO Trial was funded by a National Health and Medical Research Council (NHMRC) project grant #109902 and an NHMRC partnership project grant #GNT1092503. The authors state that there are no conflicts of interest to disclose.

References