Journal of Clinical Microbiology

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***J. Clin. Microbiol.*** **2013, 51(12):4018. DOI: 10.1128/JCM.01715-13.**
**Published Ahead of Print 25 September 2013.**

JCM
Journals.ASM.org

# Performance Monitoring of *Mycobacterium tuberculosis* Dried Culture Spots for Use with the GeneXpert System within a National Program in South Africa

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**The use of dried culture spots (DCSs) has been reported in the verification of GeneXpert instruments as being "fit for purpose" for the South African National implementation program. We investigated and compared the performance of the DCSs for verification across different bulk batches, testing the settings and cadre of staff, and the Xpert MTB/RIF assay version. Four bulk batches (V005 to V008) were used to prepare (i) 619 DCS panels for laboratory testing on G3 or G4 cartridges by a technologist, (ii) 13 DCS panels (batch V005) used for clinic verification on G3 cartridges by a nurse or lay counselor, and (iii) 20 DCS panels (batch V005) used for the verification of 10 GeneXpert 16 module instruments in mobile vehicles on the G3 cartridge performed by a scientist. The stabilities of the DCSs over 6 months at 4°C, room temperature, and 37°C were investigated. The mean cycle threshold ($C_T$) and standard deviation (SD) for probe A were calculated. The proportions of variability in the $C_T$ values across bulk batches, assay versions, and settings and cadre of staff were determined using regression analysis. Overall, the DCSs demonstrated SDs of 3.3 ($n = 660$) for the G3 cartridges and 3.8 ($n = 1{,}888$) for the G4 cartridges, with an overall error rate of 1.5% and false rifampin resistance rate of 0.1%. The proportions of variability ($R^2$) in the $C_T$ values explained by batch were 14%, by setting and cadre of staff, 5.6%, and by assay version, 4.2%. The most stable temperature in a period of up to 6 months was 37°C (SD, 2.7). The DCS is a robust product suitable for storage, transport, and use at room temperature for the verification of the GeneXpert instrument, and the testing can be performed by non-laboratory-trained personnel in nonlaboratory settings.**

Following the endorsement of the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) by the World Health Organization (1), the South African National Department of Health (NDoH) and the National Health Laboratory Service (NHLS) undertook national implementation of the GeneXpert MTB/RIF assay in March 2011. The implementation involved rapid successive placement of GeneXpert instruments in a phased approach into smear microscopy centers across high-burden tuberculosis (TB) districts, encompassing all 9 provinces in South Africa. By 31 March 2013, approximately 2,315,380 Xpert MTB/RIF cartridges had been sold globally, over half of which had been procured for use in South Africa alone (2). In parallel to this implementation, a GeneXpert instrument verification program consisting of inactivated *Mycobacterium tuberculosis* organisms spotted onto filter cards, termed dried culture spots (DCSs), was developed and successfully used to verify and ensure that newly placed instruments during phase I of the implementation ($n = 26$ sites) were "fit for purpose" before clinical specimen testing (3). A website (www.tbgxmonitor.com) was developed to automatically perform statistical analyses and to generate verification reports in real time.

With the ongoing South African national GeneXpert implementation program (4), continuous monitoring and field testing of the DCS program need to be investigated to ensure that the material is not only suitable for the verification of instruments in laboratory settings by skilled personnel but that it is also appropriate for instrument verification in remote nonlaboratory settings, such as clinics, by non-laboratory-trained personnel.

We report here the GeneXpert assay verification results for DCSs in various settings, namely: (i) laboratory instrument verification from the NHLS national implementation program, (ii) clinic instrument verification by non-laboratory-trained personnel, and (iii) instrument verification of GeneXpert assays situated in mobile vehicles for an intensified case finding event for World TB Day 2012 at KDC gold mine in Carltonville, South Africa (see http://www.nhls.ac.za/?page=world_tb_day_2012&id=77). The performance of the DCSs under the most common transport and storage conditions was also evaluated to demonstrate the stability of the material.

## MATERIALS AND METHODS

**Preparation of DCSs and testing in different settings.** The manufacture of the DCSs has been reported previously (3); briefly, it involves growing the culture strain *M. tuberculosis* ATCC 25618 (H37Rv) in a single-cell suspension (5) in bulk, followed by inactivation. Although not reported, prior to DCS panel preparation, all bulk manufactured stock was quantified using flow cytometry, which included quality control parameters for single-cell counting, such as measurement of the percentage of doublets. The material was then spotted with a blue dye (Sigma-Aldrich) onto perforated Munktell specimen collection cards (Lasec, South Africa) as previously described, dried, packed, and sent to the sites (3). Four bulk batches (V005 to V008) of this inactivated single-cell stock were used to prepare DCS panels for the program.

Received 1 July 2013 Returned for modification 28 August 2013
Accepted 16 September 2013
Published ahead of print 25 September 2013
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doi:10.1128/JCM.01715-13

**TABLE 1** Performance of DCSs by bulk stock, assay version, and cadre of staff or testing setting[a]

| Performance variable and result | Performance by bulk batch and cartridge type | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | V005 | | | | V006 | | V007 | | V008 |
| | G3 | | | G4 | G3 | G4 | G3 | G4 | G4 |
| Setting (operator) | Clinic (nurse) | Mobile (scientist) | Laboratory (technologist) | Laboratory (technologist) | Laboratory (technologist) | | Laboratory (technologist) | | Laboratory (technologist) |
| Incubation time for DCSs | 15 min | 1 h | 15 min | 15 min | 15 min | 15 min | 15 min | 15 min | 15 min |
| Method of DCS resuspension | Hand shaking | Hand shaking | Vortex | Vortex | Vortex | Vortex | Vortex | Vortex | Vortex |
| Results (no.) | 48 | 79 | 520 | 444 | 24 | 548 | 4 | 504 | 432 |
| Errors (no. [%]) | 1 (2.1) | 3 (3.8) | 8 (1.5) | 6 (1.4) | None | 9 (1.6) | None | 7 (1.4) | 7 (1.6) |
| "No result" (no. [%]) | | | 1 (0.4) | | | | | 2 (0.4) | |
| Invalid results (no. [%]) | | | | | | | | | 2 (0.5) |
| Rifampin sensitivity results (no. [%]) | | | | | | | | | |
| RIF sensitive | 47 (100) | 76 (100) | 509 (99.6) | 437 (99.8) | 24 (100) | 537 (99.6) | 4 (100) | 491 (99.2) | 423 (100) |
| RIF indeterminate | | | | | | 1 (0.2) | | 3 (0.6) | |
| RIF resistant | | | 2 (0.4) | | | 1 (0.2) | | | |
| *M. tuberculosis* not detected | | | | 1 (0.2) | | | | 1 (0.2) | |
| $C_T$ for probe A (mean [SD]) | 24.1 (3.3) | 16.2 (1.9) | 21.6 (3.3) | 17.7 (3.5) | 22.8 (3.4) | 17.3 (3.0) | 17.8 (2.1) | 21.5 (3.0) | 21.4 (3.5) |
| %CV of the $C_T$ for probe A | 13.8 | 11.8 | 15.3 | 17.7 | 14.8 | 17.5 | 11.6 | 13.8 | 16.2 |

[a] The overall SD and error rate for G3 ($n = 660$) were 3.3 and 1.8%, respectively, and for G4 ($n = 1{,}888$) were 3.8 and 1.5%.

At the testing sites, a single-use DCS was tested for each GeneXpert module by pushing the perforated spot into a sterile 50-ml Nunc tube using an additional pipette. A volume of 2.8 ml of sample reagent buffer (SR; Cepheid, Sunnyvale, CA) was added to the spot to resuspend the bacteria. This was incubated for 15 min, unless otherwise stated. During the incubation period, the DCS was mixed by either hand shaking (field test setting) or vortex (laboratory setting). Following incubation, all the SR mixture (>2 ml) was added to the cartridge and tested as per the manufacturer's instructions (6). In January 2012, a new cartridge version, G4, was released for use in the national program. The most prominent modifications to the G3 cartridge included sequence changes to probe B, a new quencher, and minor PCR cycle time reductions.

At the completion of testing, all comma-separated value (CSV) files were uploaded onto the Web-based platform (www.tbgxmonitor.com) by staff performing the verification, and all data were downloaded from the website into MS Excel format. These included the semiquantitative ranges and the cycle threshold ($C_T$) values, which is the point at which fluorescence from the hybridized probes increases with product amplification and is used as an indication of the bacterial load (7). A $C_T$ of <16 indicates a high bacterial load, 16 to 22 indicates a medium load, 22 to 28 indicates a low load, and >28 indicates a very low load.

**DCS batch usage. (i) NHLS laboratories.** Four bulk batches, V005 to V008, of the *M. tuberculosis*-positive rifampin (RIF)-sensitive stock cultures were used to prepare DCSs for the verification of GeneXpert modules in NHLS laboratory sites performing the Xpert MTB/RIF assay. A total of 619 DCS panels were sent out to verify 2,476 GeneXpert modules, some of which had already been switched to the newer G4 cartridge. All verification was performed by the technologists who routinely performed GeneXpert testing.

**(ii) Primary health care clinics.** Batch V005 was used to prepare DCSs for 13 nongovernmental organization (NGO)-funded primary health care clinics using the GeneXpert assay for research purposes. All these clinics are primarily HIV counseling and testing (HCT) sites that provide antiretroviral and TB treatment to patients. Thirteen DCS panels were sent out as follows: 7 sites in region F, Johannesburg, 4 sites in Motlasana District, North West Province, one site at Witkoppen Clinic, and one at Themba Lethu Clinic, both in Johannesburg. Verification at the clinical sites was performed on G3 cartridges by the nurse or lay counselor doing the GeneXpert testing for the study.

**(iii) Mobile vehicles.** Twenty DCS panels were prepared from batch V005 for verification of 10 GeneXpert 16 module instruments situated in mobile vehicles at the KDC gold mine in Carltonville, South Africa, for an NDoH health and wellness campaign held on National TB Day 2012. Due to electrical power failures on the day of testing, DCSs were incubated in SR buffer for up to 1 h. All verification was performed on G3 cartridges by a scientist. Time constraints on the day caused by the power failures led to only 79 modules being randomly chosen for verification (in an even distribution across the four frames of the instrument).

**DCS performance testing: stability over time and temperature.** Twenty-seven panels (3 DCSs/card) were prepared from batch V005 for DCS performance testing. DCS panels were packaged in zip-locked plastic packets with a desiccant and stored in either a 4°C refrigerator (range, 4° to 8°C), at room temperature (RT; approximately 25°C), or at 37°C (IncoTherm digital incubator; Labotec, South Africa), with stability evaluations performed at 9 different time points. One entire card (containing 3 DCSs) per temperature was then tested in the Xpert MTB/RIF assay at each time point: 1, 2, 3, 4, 8, 12, 16, 20, and 24 weeks (6 months).

**Statistical analysis.** The mean cycle threshold ($C_T$) values, standard deviations (SD), and coefficients of variation (%CV) for probe A (the first probe to bind) (3) were calculated for all DCS Xpert verification and stability results. All errors/invalids/no results findings were described but excluded from the quantitative analysis. The proportions of variability in $C_T$ values across (i) bulk batches (same assay version and operator or setting), (ii) operators and settings (same batch and assay version), and (iii) assay version (same batch and operator or setting) were determined using regression analysis and reported as the $R^2$ values (%) using Stata 12 software.

## RESULTS

**Performance of DCSs across bulk batches.** The bulk batches V005, V006, V007, and V008, which were tested in similar laboratory settings on the same assay version (G4) by laboratory technologists, excluding errors, gave an overall mean $C_T$ value of 19.4 ($n = 1{,}888$) and overall variability of 3.8 SD (Table 1). Batches V007 and V008 had greater overall mean $C_T$ values (fewer bacteria on average). The overall error rate across all bulk batches (V005 to V008) was 1.5% (29/1,928), with an invalid rate of 0.1% (2/1,928).

Only one false-resistant result was reported for batch V006 (delayed hybridization on probe B), but the module passed verification on a repeat DCS. If batch V005 was used as the reference in regression analysis, the proportion of variability in $C_T$ values explained by batch was approximately 14% ($R^2$).

**Performance of DCS by operator/setting.** A total of 647 DCSs (48 at the clinics, 79 at mobile vehicles, and 520 at the laboratory) from the same bulk batch (V005) were performed on the same cartridge assay version G3 but by a different cadre of staff (nurse or lay counselor, scientist, or laboratory technologist) in different test settings (Table 1). Both clinic and laboratory testing had similar variability (measured by the SD), while DCSs tested in the mobile vehicles had a lower SD of 1.9. The latter testing site was the only one where the incubation time in SR buffer increased beyond 15 min to approximately 1 h. In addition, the $C_T$ values from the clinic (where no vortex was used) was greater than that from the laboratory. The error rate was highest for DCSs tested in the mobile vehicles, with only one of these being operator dependent (volume related). The proportion of variability in $C_T$ values explained by setting (and, therefore, the cadre of staff) was 5.6% overall ($R^2$).

**Performance of DCSs across different cartridge assay versions.** In order to compare any differences in the assay versions, DCSs tested on the G3 and G4 cartridges from the same bulk batch V005 and in the same laboratory testing setting were compared. Both assay versions showed a similar SD for probe A and similar error rates (Table 1). G3 cartridges generated a higher false rifampin resistance result of 0.4% (1 dropout on probes D and E and 1 delayed hybridization on probe B), but one false *M. tuberculosis*-negative result was reported for the G4 cartridge, probably due to low bacterial load on the DCS. The proportion of variability in the $C_T$ values (mean $C_T$ values, 21.2 for G3 cartridges and 19.4 for G4 cartridges) explained by assay version was 4.2%.

Irrespective of the bulk batch or test setting, DCSs gave an overall SD of 3.3 ($n = 660$) on G3 cartridges and 3.8 ($n = 1{,}888$) on the G4 cartridges. DCSs tested on the older G3 cartridge had an overall higher mean $C_T$ of 21.2 (medium semiquantitative category) versus 19.4 for G4 cartridges (also medium semiquantitative category) but lower overall %CV across the data set (17.5% versus 19.5%). The percent error rate on any G3 cartridge was 1.8% (12/675), whereas G4 cartridges had slightly fewer errors at a rate of 1.5% (29/1,928).

**Long-term performance of DCS material: stability testing.** A total of 81 DCSs were evaluated to determine the performance of the DCSs under various storage and temperature conditions (Table 2). Across all time points, three errors (signal loss failures) and 3 "no results" (2 due to on-site temporary power failures and 1 syringe motion error) were reported. Of the remaining results ($n = 75$) reported in Table 2, 2 RIF-indeterminate values were reported from two samples yielding very low semiquantitative results. The SD for probe A remained low for all temperatures tested, with the lowest SD (2.7) occurring for DCSs stored at 37°C. Overall, most of the DCS results were in the low semiquantitative category (mean $C_T$ range, 22.6 to 24.7).

**TABLE 2** Overall performance data for DCSs after 6 months at various temperatures in batch V005

| Performance data | Results at temp of[a]: 4°C | RT | 37°C |
|---|---|---|---|
| No. (%) of errors, invalids, or no results | 1 (3.7) error | 2 (7.4) errors, 2 (7.4) no results | 1 (3.7) error |
| False RIF resistance calls | 1 RIF indeterminate | 1 RIF indeterminate | None |
| No. of analyzable results | 25 | 22 | 26 |
| Semiquantitative results (%) | | | |
| Very low | 3.9 | 12.5 | 7.7 |
| Low | 61.5 | 62.5 | 76.9 |
| Medium | 34.6 | 25 | 15.4 |
| High | 0 | 0 | 0 |
| $C_T$ value for probe A (mean [SD]) | 22.6 (3.2) | 24.5 (4.1) | 24.7 (2.7) |
| %CV of the $C_T$ for probe A | 13.9 | 16.6 | 10.9 |

[a] $n = 27$ for each temperature.

## DISCUSSION

DCS verification results from the National GeneXpert rollout showed comparable variation (660 G3 cartridges [SD, 3.3] and 1,888 G4 cartridges [SD, 3.8]) to previously reported findings on DCSs (268 G3 cartridges [SD, ≤3.8]) (3). The stability of intact mycobacterial cells on filter paper for up to 6 months at all temperatures (4°, RT, and 37°C) was well within the expected time limits for shipping to testing in a national program. Of the temperatures tested, DCSs were most stable at 37°C, which may be due to an increased number of bacteria being more easily able to resuspend from the paper. One potential limit would be that if GeneXpert users are found to prefer using a liquid external quality assessment (EQA) format instead of using DCSs, stability testing will need to be done on the bulk liquid batches.

The variability in the DCS product was minimal for the differences in assay version (4.2%) and operators or settings (5.6%) but higher between bulk batches (14%). This is to be expected due to the manufacturing process, which ensures single-cell format and spotting procedures. This did not, however, affect the overall mean semiquantitative results reported; all bulk batches, assay versions, and operator results were determined to be in the medium category.

The overall error rate across all batches, regardless of assay version, was 1.5%. This is currently below the average failure rate for the national program (~3%) (8), indicating that it is the minimum error rate to be expected in a national program.

The false rifampin resistance result generated from the DCS material was 0.1% (3/2,548), highlighting the overall good performance of the Xpert MTB/RIF assay. It is worth noting that two of these were due to the reduced probe B hybridization that is typical of the G3 cartridge. A total of four *M. tuberculosis*-positive/RIF-indeterminate values (stocks V006 and V007) and two *M. tuberculosis* negatives (stocks V005 and V007) were reported. These findings were most likely due to variable amounts of bacteria being spotted onto each DCS due to problems with retaining the cells in a homogenous suspension during the postmanufacture spotting process. Earlier bulk batches showed increased clumping during the inactivation process, which was subsequently corrected for all new batches.

The use of DCSs has also proven to be accurate (with accept-

able variability) and feasible for the verification of GeneXpert instruments in clinics, at the point of care (POC), by non-laboratory-trained personnel, although it highlights the need for the verification program to supply extra consumables (50-ml Nunc tubes and a pipette to push out the perforated DCSs) at an additional cost. Furthermore, a comparison between operators or settings with the newer G4 assay version cartridge would be beneficial.

The robustness of the DCS program was further demonstrated by its use at an outdoor NDoH campaign on World TB Day 2012, held at a mining community in Carltonville, South Africa. DCSs were used to verify 10 GeneXpert instruments situated in 5 mobile vehicles, but due to a power failure, the DCSs already prepared for use could not be tested following the 15-min incubation time. The lower mean $C_T$ value observed was due to the increased number of bacteria resuspended off the filter paper after the longer incubation time. This effect was similarly shown in the lower $C_T$ value that was observed for the laboratories where a vortex was used to resuspend the bacteria off the filter paper compared to the clinic, where hand shaking was used ($C_T$ values of 24.1 versus 21.6, respectively).

The data presented in this study show the suitability of DCSs for GeneXpert instrument verification in all settings across a national program of broad geographic coverage and by all cadres of testing staff.

## ACKNOWLEDGMENTS

This work was supported by the U.S. Agency for International Development (USAID) and Grand Challenges Canada (grant no. 0007-02-01-01). B.K. was supported through grants from the National Research Foundation, the South African Medical Research Council, and the Howard Hughes Medical Institute.

The contents of this article are the responsibility of the authors and do not necessarily reflect the views of the USAID or the U.S. Government.

We thank Sebaka Molapo and Elizabeth Prentice (National Priority Programme of the NHLS) for help with implementing DCSs into the National GeneXpert program, Saloshini Ramsamy for assistance with preparation of the DCSs, and Braimoh Bello (Centre for Statistical Analysis and Research, Johannesburg, South Africa) for assistance with statistical analysis.

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