Abrogation of red blood cell G6PD enzyme activity through Heat treatment: development of survey material for the UK NEQAS G6PD scheme

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SUMMARY

Introduction: Participation in external quality assessment (EQA) is central to the maintenance of high-quality laboratory results in patient diagnosis and clinical trials. Laboratories in the TAF112582 DETECTIVE study (ClinicalTrials.gov identifier: NCT01376167) are enrolled in the United Kingdom National Quality Assessment Scheme (UK NEQAS) for glucose-6-phosphate dehydrogenase (G6PD) quantitative assay, which utilizes ovine (sheep) blood as a readily available source of apparently G6PD-deficient survey material. A substitute for sheep blood was sought because some non-UK sites in the study encountered participation difficulties due to the strict regulations on the import of sheep blood into their countries.

Methods: G6PD activity in normal human donor blood was abrogated by the action of heat under controlled conditions. Residual G6PD activity in the heated samples was measured by UK NEQAS using the Trinity Biotech 345 kit (Trinity Biotech) and a Jenway 6715 UV/Vis spectrophotometer with external temperature control to monitor enzyme kinetics and linearity over a set time. Heat-treated material was also assayed for G6PD activity and assessed for its acceptability as EQA survey material by selected UK laboratories.

Results: Blood heated at 45 °C for 15 h showed a reduction in G6PD activity of 76.3 ± 4.6% (n = 6) and was considered acceptable as EQA material in terms of appearance and behaviour by the majority of UK sites in the trial.

Conclusions: We have developed a simple heat-treatment procedure to produce EQA survey material with low/intermediate G6PD activity, similar to that found in females heterozygous for G6PD deficiency.
INTRODUCTION

The UK’s National External Quality Assessment Scheme (UK NEQAS) for Haematology sends a combination of human and ovine (sheep) whole blood samples for assessment of red cell glucose-6-phosphate dehydrogenase (G6PD) status to participating laboratories throughout the year to ensure continued high-quality results from this assay. Compared to humans, sheep appear naturally G6PD-deficient [1–3] making ovine samples a reliable source of deficient samples. However, during the set-up of tafenoquine study TAF112582 DETECTIVE (ClinicalTrials.gov identifier: NCT01376167), an international multicentred vivax malaria Phase III study with a G6PD entry criterion dependent upon the results of a quantitative G6PD assay, some non-UK laboratories encountered difficulties importing the animal (ovine) specimens due to their strict country regulations, often requiring additional steps to obtain clearance from the relevant authorities. For example, Brazilian law, through its National Agency of Sanitary Surveillance (ANVISA), requires permission from the Ministry of Agriculture, Livestock and Food Supply (MAPA) and the Ministry of Health (MS) to import animal and human specimens.

Subjects enrolled into the tafenoquine study were screened for G6PD deficiency by a quantitative spectrophotometric assay, and this analysis was used to determine subject eligibility [4]. This was necessary as 8-aminoquinoline antimalarial drugs (such as primaquine and tafenoquine) can induce serious haemolytic events [5]. Consequently there was a need to include an EQA service to ensure intra- and interlaboratory G6PD test reproducibility across and within the Phase III G6PD testing laboratories. As deficient specimens are critical to the EQA process, a practical approach using heated human (donor) blood samples was investigated as an alternative mechanism to facilitate importing via separate regulatory schemes, which would ensure a more timely analysis of the specimens.

Previously, chemicals such as urea [6] and guanidinium hydrochloride [7, 8] have been used experimentally to denature bacterial G6PD and other enzymes, often with total abrogation of enzyme activity. Apart from the health and safety aspect of handling such chemicals, it was felt a more straightforward approach was needed, without the use of exogenous agents, to produce intact whole blood samples for utilization in a variety of assay systems by different participants.

Therefore, we set out to produce a standardized system to heat denature blood as thermal stability studies of other enzymes (e.g. pyruvate kinase) at 56 °C [9] have been proven and heat-damaged but intact erythrocytes for splenic imaging using a 49.5 °C incubation [10] can be produced. A report by Choi et al. [11] found no significant morphological changes in erythrocytes after incubation of whole blood at 48 °C for 5 min, while exposure to a temperature ≥46 °C resulted in loss of anticoagulating property and induction of platelet clumping. Luzzatto & Afolayan [12] examined enzymatic properties including rates of heat inactivation of two rare G6PD variants. McCann et al. [13] found a temperature of 44 °C enabled them to study the rates of G6PD inactivation in partially purified mutant and control enzymes, and thus an incubation temperature of 45 °C was selected for preliminary experimentation.

MATERIALS AND METHOD

Initial experiment to define final incubation time for lowering of G6PD activity

A fresh donor unit of packed cells, blood group AB RhD+, in CPD anticoagulant (containing anhydrous glucose 129.0 mm/L; sodium citrate 89.4 mmol/L; citric acid monohydrate 15.6 mmol/L; sodium dihydrogen phosphate dihydrate 16.1 mmol/L) was obtained from National Health Service Blood and Transplant (NHSBT, Colindale, UK), and after thorough resuspension, the contents were transferred into a sterile glass 1 L Duran® bottle (VWR International Ltd., Lutterworth, Leicestershire, UK).

The initial haemoglobin concentration (Hb) of 189 g/L was abnormally high, and outside the levels typically provided in G6PD survey material, so group AB fresh frozen plasma (NHSBT) was used to dilute the blood 2 : 1 to achieve a final Hb concentration of 126 g/L.

Multiple 5 mL aliquots of blood were incubated in 5-mL capped polypropylene tubes (Becton Dickenson Falcon, Bedford, MA, USA) at 45 °C for 2, 4, 6, 8, 15 and 20 h, respectively, and then assayed for G6PD activity alongside the unheated blood using the Trinity Biotech 345 kit reagents (Trinity Biotech, Bray, Co
Wicklow, Ireland) according to the manufacturer’s instructions [14]. Briefly, after thorough resuspension, 10 μL blood volumes was pipetted from each sample tube in duplicate into clean 5-mL polypropylene tubes, each containing 1 mL lysing reagent (G-6-PDH assay solution) and vortex mixed without frothing. After a 5 min incubation at room temperature, 2 mL of substrate reagent was added and the contents mixed by gentle inversion. After transferring the reaction mixtures into disposable spectrophotometer cuvettes (VWR International Ltd), kinetic assays were performed at 30 °C on a Jenway 6715 UV/Vis spectrophotometer (Camlab Ltd., Over, Cambridge, UK) with temperature control via an external water bath and linear reaction rates over a 5-min period were obtained for each sample in duplicate.

Addition of antibiotics

To maintain the integrity of the blood, and prevent bacterial contamination, standard doses of penicillin (crystapen benzyl penicillin sodium, 600-mg vials; 5 μg/mL; Genus Pharmaceuticals, Linthwaite, Huddersfield, UK) and Cidomycin® (gentamicin; 50 μg/mL; Sanofi, Guildford, Surrey, UK) were added to all batches of survey material followed by thorough mixing on a roller mixer for 30 min.

Check of enzyme stability during posting

To evaluate stability of the product and mimic a typical survey, aliquots of the unheated, 2, 4, 6, 8 and 15-h incubated samples were posted, as per UK NEQAS Haematology standard practice, for return to the department for repeat testing and comparison with matched refrigerated (fridge) samples. Additionally, enzyme activities of the 15 hr incubated and unheated samples (both posted and on-site refrigerated) were rechecked 2 weeks after preparation and also after washing the cells three times in phosphate-buffered saline (PBS) solution to remove haemolysis.

Scale up for operational use

To produce enough material for a full UK NEQAS G6PD survey, approximately 400 mL of fresh donor whole blood was heated in a 1 L glass Duran bottle in a water bath for 15 h at 45 °C. Timing started after a 20-min delay to allow for effective warm-up of the larger blood volume (data not shown).

Reproducibility

We then checked reproducibility of our chosen method with additional normal blood donors. Aliquots of 20 mL were removed from six fresh donor ‘nonleucodepleted’ (NLD) whole blood units obtained for the preparation of UK NEQAS Full Blood Count survey material. However, aliquot NLD 4 formed clots and was discarded.

A 10 mL volume of blood from each aliquot was put into 30-mL plastic Sterilin ‘universal’ containers (R & L Slaughter Ltd., Upminster, UK) with temperature control via an external water bath and overnight for 15 h at 45 °C, as before. Samples were then mixed by gentle swirling and placed on a roller mixer for 10 min and assayed for G6PD activity.

Aliquots were removed from each of the heated and unheated samples and posted for return to the department for repeat assay. G6PD activity was measured in each of the returned (Day 6) postal aliquots, with additional checks being made on days 8, 12 and 15.

In view of the heat-induced haemolysis, the posted heated and unheated aliquots were subsampled and washed three times in PBS (3rd wash clear) and cells resuspended in 0.9% normal saline. G6PD assays on the washed cells were compared to the unwashed counterparts and showed little change after correction for differences in haematocrit (i.e. Hb) after resuspension.

To confirm the accuracy of the very low reaction rates, and overall assay linearity, assays were carried out on a range of sample volumes from the heated NLD 1 donor sample. In addition to the standard assay sample volume (10 μL), separate assays were performed with 5, 15 and 20 μL of blood.

In keeping with standard UK NEQAS policy, the ‘fluorescent spot test’ and ‘dye decolourisation time test’ (G-6-PDH Deficiency Screen Kits, Trinity Biotech) were also performed on the unheated and heated posted samples.

Long-term stability check of posted samples

Further G6PD assays were performed on the posted (unheated and heated) donor samples on days 8, 12 and 15 following preparation to ensure suitability as material in subsequent UK NEQAS surveys.
Pilot testing by additional UK laboratories

After heating (15 hrs at 45 °C), the red cells were washed ×3 in PBS solution, packed and resuspended in a volume of group-compatible fresh frozen plasma to achieve a normal haemoglobin level (124 g/L). Aliquots were prepared and refrigerated at 2–8 °C until dispatch.

Aliquots from this pool were anonymized, and a pair of specimens (1406G6EX1 and 1406G6EX2) was sent to each of six selected laboratories that assay with the same Trinity G6PD method used to develop the procedure. In addition, a short questionnaire was included to assess the acceptability of the samples as EQA material.

In a subsequent UK NEQAS survey, another fresh whole blood donor unit was obtained and prepared as before. Samples were labelled as 1501G6EX1 and 1501G6EX2 and distributed to another 24 UK participating laboratories to assay and comment upon.

STATISTICAL METHODOLOGY

Mean and standard deviation values were calculated using Excel software statistics package (Microsoft Office Excel® 2013), and an online statistics program was used to perform Student’s paired t-tests: http://www.socscistatistics.com/tests/ttestdependent/Default2.aspx. P-values <0.05 were considered significant.

RESULTS

Initial experiment to define final incubation temperature for lowering G6PD activity

G6PD activity declined exponentially with time when heated at 45 °C, up to an incubation time of 15 h (Figure 1). No data were generated beyond 15 h due to significant red cell lysis. Results of this initial experiment suggested that a 10-h incubation time would be sufficient to reduce G6PD activity to approximately 3.0 U/gHb; however, a 15-h incubation time was preferred as this allowed an overnight incubation, aligning the abrogation procedure with the survey material preparation workflow, without additional adverse effects on the material quality. An alternative 15-h incubation at 40 °C was also tried, but this did not reduce the G6PD activity sufficiently (data not shown).

Check of enzyme stability after posting

The unheated, and 8 and 15-h heated aliquots demonstrated the same G6PD activities after postage as the on-site refrigerated control samples when assayed in parallel (mean of duplicate assayed samples = 9.03, 3.46 and 1.54 U/gHb, respectively), indicating excellent stability throughout the postal journey (samples were out of the laboratory for about 24 h). The 2, 4 and 6-h samples were not retested.

The initial reduction in G6PD activity of the 15-h incubated sample was 83% (Table 1). There was an initial further fall in enzyme activity immediately after heating compared to the unheated sample, but this stabilized and the activity at Day 14 was 1.15 U/gHb for both posted and control incubated samples (a reduction of 87% compared to the unheated samples).

General sample quality

The 15-h incubated aliquots showed moderate haemolysis after centrifugation. A slight degree of haemolysis was also present in the 6 and 8-h
incubations. The unheated aliquots remained clear (Figure 2).

In view of the degree of haemolysis and the possibility that participants who perform G6PD assays on washed red blood cells might reject samples as unsuitable for analysis, the samples were washed ×3 in PBS solution (100 μL blood and 1.0 mL saline, with centrifugation in a microfuge for 30 s). Visual inspection after the third wash showed no detectable haemolysis in the supernatants with G6PD activity being maintained as in the ‘unwashed’ samples (data not shown).

**Scale up for operational use**

The larger volume (400 mL) of donor blood darkened in colour after incubation; however, acceptable redness returned after reoxygenation by thorough, gentle mixing. The G6PD activity in the scaled-up batch of survey material was 2.69 U/gHb.

Reproducibility

A mean reduction in G6PD activity of 75% (range 69–78%) was seen in the aliquots of five additional, fresh, whole blood units treated by this method, as shown in Table 2. When these heated samples were assayed again after posting, on days 6, 8, 12 and 15 after preparation, they showed a further significant drop in G6PD activity of 33–50% (Table 3). No significant difference (*t* = 0.04, *P* = 0.97) in activity was demonstrated between the posted, unheated aliquots on Day 6 and the refrigerated, unheated, control samples. Washing the red blood cells did not have a statistically significant effect on the G6PD activity, allowing for the difference in haematocrits after resuspension, with the unheated/unwashed mean = 9.95 U/gHb, the unheated/washed mean = 9.63 U/gHb (unheated: *t* = −1.32, *P* = 0.26), the heated/unwashed mean = 1.44 U/gHb and the heated/washed mean = 1.57 U/gHb (heated: *t* = 1.08, *P* = 0.34).

**Confirmation of the accuracy of low spectrophotometric reaction rates**

Individual enzyme assays performed with 5, 15 and 20 μL of blood yielded reaction rates (∆Abs/min) of 0.001, 0.005 and 0.007, respectively, and 0.003 for 10 μL. The reaction rates gave a straight line (R² = 1) when plotted against blood volume used (Figure 3), which indicated that the assay system was accurate at low reaction rates.

**G6PD screening tests demonstrated typical qualitative results**

Both the fluorescent spot test and dye decolourization time test gave the expected results: unheated posted

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**Table 1.** Mean enzyme activities of unheated and after 8 and 15-h heating of sample aliquots (both postal and on-site refrigerated) and 2 weeks after preparation.

| Sample                        | Day 0 G6PD (U/gHb) | Day 14 G6PD (U/gHb) |
|-------------------------------|--------------------|---------------------|
| Unheated (fridge)             | 9.03               | 8.87                |
| Unheated (postal)             | 9.03               | 8.83                |
| 8 h at 45 °C (fridge)         | 3.46               | nt                  |
| 8 h at 45 °C (fridge)         | 3.46               | nt                  |
| 15 h at 45 °C (fridge)        | 1.54               | 1.15                |
| 15 h at 45 °C (postal)        | 1.54               | 1.15                |

nt, not tested.

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**Figure 2.** Microfuge tubes displaying varying degrees of lysis in the refrigerated and posted sample aliquots after 2, 4, 6, 8 and 15-h incubations at 45 °C. Little difference can be seen between the on-site refrigerated and postal samples. U = unheated sample. (50 μL sample diluted with 750 μL saline, mixed and centrifuged).
samples demonstrated normal bright fluorescence while the heated posted samples showed no fluorescence. Similarly, the unheated samples had decolourized the dye within 25 min of incubation and the heated samples had failed to show any decolourization after 1 h, indicating normal and deficient G6PD activities, respectively.

### Long-term Stability Check of posted samples

The enzyme activities of four of the five heated samples remained stable between Day 6 and Day 15, despite the observed drop in activities between preparation day and Day 6. The fifth sample (NLD6) showed a 50% reduction in activity between days 6 and 15, although most of this occurred by Day 8 (Table 3). The unheated (refrigerated) samples remained stable throughout the survey period, with a reduction in activity seen in two of the donor samples by Day 15 (NLD5 3.0% drop; NLD6 3.4% drop).

### Pilot testing by additional volunteer UK Laboratories

Five of the initial six UK laboratories reported their samples as G6PD-deficient, and a median enzyme level of 2.6 U/gHb was in good agreement with the UK NEQAS results for 1406G6EX1 and 1406G6EX2 of 2.66 and 2.61 U/gHb, respectively.

Of the questionnaire comments received back from the second, larger group of trial participants, two laboratories commented that the samples showed (‘slight’ and ‘a degree of’) haemolysis: another laboratory reported that the samples were noticeably darker in appearance compared to the normal UK NEQAS G6PD samples, while a further laboratory commented that they appeared oxidized (this laboratory received the samples 4 days after posting). The mean score for ‘acceptability for EQA purposes’ was 4.5 (where 1 is unacceptable and 5 is very acceptable for EQA purposes). The median enzyme level of 3.60 U/gHb was in good agreement with the UK NEQAS result of 3.53 U/gHb for both aliquots (measured on the day of dispatch). None of the laboratories reported their samples as clotted (Table 4).

### Table 2. Abrogation (% drop) of G6PD activity in five additional donor samples, immediately after heating for 15 h at 45 °C

| Sample | Unheated G6PD (U/gHb) | Heated G6PD (U/gHb) | % drop |
|--------|------------------------|---------------------|--------|
| NLD 1  | 8.59                   | 2.66                | 69.0   |
| NLD 2  | 11.20                  | 2.85                | 74.6   |
| NLD 3  | 10.48                  | 2.38                | 77.3   |
| NLD 5  | 10.14                  | 2.22                | 78.1   |
| NLD 6  | 9.31                   | 2.22                | 76.1   |
| Mean   | 9.94                   | 2.47                | 75.0   |
| SD     | 1.02                   | 0.28                | 3.62   |

### Table 3. Summary of G6PD activities (U/gHb) of heated donor samples between preparation (Day 1) and Day 15

| Sample | Day 1  | Day 6 | Day 8 | Day 12 | Day 15 |
|--------|--------|-------|-------|--------|--------|
| NLD 1  | 2.66   | 1.33  | 0.89  | 0.79   | 0.82   |
| NLD 2  | 2.85   | 1.63  | 1.63  | 1.59   | 1.54   |
| NLD 3  | 2.38   | 1.59  | 1.59  | 1.59   | 1.64   |
| NLD 5  | 2.22   | 1.33  | 1.33  | 1.09   | 1.07   |
| NLD 6  | 2.22   | 1.33  | 0.89  | 0.64   | 0.69   |
| Mean   | 2.47   | 1.44  | 1.27  | 1.14   | 1.15   |
| SD     | 0.28   | 0.15  | 0.36  | 0.44   | 0.42   |

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Figure 3. Correlation of sample volume used in the Trinity assay against G6PD enzyme reaction rate using nonleucodepleted donor blood (NLD 1). Regression line and equation are shown, indicating good linearity of the assay system.
| UK Trial Labs | Specimen | Assay Temp (°C) | Correction factor | Results IU/gHb (corrected to 30C) | Interpretation | Overall acceptability for EQA purposes | Comments |
|---------------|----------|-----------------|------------------|----------------------------------|----------------|----------------------------------------|----------|
| 1             | 1501G6EX1 | 30 N/A          | 2.8              | Deficient                         | 5              |                                        |          |
| 2             | 1501G6EX2 | 23 1.55         | 4.3              | Intermediate                      | 4              |                                        |          |
| 3             | 1501G6EX1 | 30 N/A          | 2.8              | Deficient                         | ~              |                                        |          |
| 4             | 1501G6EX1 | N/A N/A         | 3.0              | Deficient                         | §              |                                        |          |
| 5             | 1501G6EX1 | 25 1.37         | 3.0              | Deficient                         | §              |                                        |          |
| 6             | 1501G6EX1 | 30 N/A          | 4.1              | Intermediate                      | 5              |                                        |          |
| 7             | 1501G6EX1 | 37 0.66         | 3.2              | Deficient                         | 5              |                                        |          |
| 8             | 1501G6EX1 | 26 1.28         | 2.8              | Deficient                         | 5              |                                        |          |
| 9             | 1501G6EX1 | N/A N/A         | 3.8              | Intermediate                      | §              |                                        |          |
| 10            | 1501G6EX1 | 30 N/A          | 4.2              | Intermediate                      | §              |                                        |          |
| 11            | 1501G6EX1 | 30 1            | 3.8              | Deficient                         | §              |                                        |          |
| 12            | 1501G6EX1 | 30 N/A          | 3.3              | Deficient                         | §              |                                        |          |
| 13            | 1501G6EX1 | 37 0.66         | 3.8              | Intermediate                      | §              |                                        |          |
| 14            | 1501G6EX1 | 37 0.66         | 3.5              | Deficient                         | §              |                                        |          |
| 15            | 1501G6EX1 | 30 N/A          | 3.5              | Deficient                         | §              |                                        |          |
| 16            | 1501G6EX1 | 30 N/A          | 4.1              | Deficient                         | §              |                                        |          |
| 17            | 1501G6EX1 | 30 N/A          | 3.8              | Deficient                         | §              |                                        |          |
| 18            | 1501G6EX1 | 37 0.66         | 3.5              | Intermediate                      | §              |                                        |          |
| 19            | 1501G6EX1 | N/A N/A         | 3.2              | Deficient                         | §              |                                        |          |
| 20            | 1501G6EX1 | 30 N/A          | 3.3              | Deficient                         | §              |                                        |          |
| 21            | 1501G6EX1 | 1.37            | 2.8              | Deficient                         | 4              |                                        |          |
| 22            | 1501G6EX1 | 37 0.66         | 3.2              | NotDeficient                      | 5              |                                        |          |
| 23            | 1501G6EX1 | 1.23            | 2.9              | Deficient                         | 3              |                                        |          |
| 24            | 1501G6EX1 | 1.76            | 3.4              | Deficient                         | 4              |                                        |          |
DISCUSSION

We undertook to develop a laboratory usable methodology to replace ovine blood samples with heat-treated human samples to allow easier international shipping of deficient samples for use in the UK NEQAS Haematology interlaboratory trials of G6PD quantitative assay.

The initial data suggested a 10-h incubation at 45 °C would achieve a final G6PD level of around 3.0 U/gHb, and we expected to observe a tail-off in activity over the course of a typical UK NEQAS survey (2 weeks to complete), which would better compare with the very low levels observed with ovine blood samples.

As it was difficult to achieve a 10-h incubation in routine practice, a 15-h incubation at a temperature of 40 °C was tried as an alternative. However, this only reduced the G6PD activity by 20% and we ideally wanted residual activities that approached those of ovine blood samples (0.65–1.54 U/gHb) [15] so we opted for 15-h incubations at 45 °C.

Loss in enzyme activity had previously been found to be quite significant in blood samples (albeit anticoagulated with EDTA) stored at room temperature, when after 15-day storage, G6PD levels fell by approximately 60% [16]. While storage-dependent changes in G6PD function have been described in leucoreduced donor blood units [17] additives such as that described by Kahn et al., [18] appeared to extend the functionality of intact red cells when analysed by flow cytometry, even though the overall G6PD level remained stable over a 21-day period.

Samples of the heated donor blood that had been posted back to ourselves were found to have kept their activity and appearance well. Following the initial heating of the samples, there was a further, slight fall in measured G6PD activity that occurred between the fourth and sixth day of storage. However, if this approach is adopted by UK NEQAS for future surveys, this would normally be before dispatch of the samples to participants and should not be an issue, as activity remains stable thereafter until the survey closing date 10 days later.

Scale up to the larger blood volume of 400 mL (which would be required for a survey for all UK NEQAS registered participants) yielded a G6PD level of 2.69 U/gHb which was not as diminished as seen previously with the smaller aliquot (70% vs. 83%). However, this still represented a satisfactory reduction in activity for the EQA process.

We sent samples in a pilot study to a selected group of UK participants for user acceptability, and they returned an average score of 4.5 of 5 for ‘acceptability for EQA purposes’, which largely indicated their approval of such samples.

Heat treatment of human blood specimens to produce G6PD deficient cells for EQA specimens seems to work well and had high levels of acceptability with participants. The resultant enzyme activities (UK laboratories’ mean values of 2.6 and 3.6 U/gHb) by this procedure were higher than those observed with sheep blood in previous NEQAS surveys, returning a level of approximately 1.0 U/gHb at 30 °C; similar to a hemizygous deficient individual. However, we believe that such an ‘intermediate’ G6PD level will

| UK Trial Labs | Specimen  | Assay Temp (°C) | Correction factor | Results IU/gHB (corrected to 30°C) | Interpretation | Overall acceptability for EQA purposes (Comments) |
|---------------|-----------|----------------|------------------|-----------------------------------|---------------|-----------------------------------------------|
| a             | 1501G6EX1 | 30             | N/A              | 3.56                              | Deficient     |                                               |
|               | 1501G6EX2 |                |                  | 3.56                              | Deficient     |                                               |
| b             | 1501G6EX1 | 30             | N/A              | 3.53                              | Deficient     |                                               |
|               | 1501G6EX2 |                |                  | 3.53                              | Deficient     |                                               |
| c             | 1501G6EX1 | 30             | N/A              | 3.29                              | Deficient     |                                               |
|               | 1501G6EX2 |                |                  | 3.21                              | Deficient     |                                               |

a, preparation day; b, dispatch day; c, survey closing day; §, only screening tests performed. Median 3.60; EstSD 0.63; N 40; CV 17.5%.
prove useful in subsequent UK NEQAS surveys as intermediate values are seen in females, due to heterozygosity for deficiency [19, 20].

One limitation of our work is that the majority of UK registered participants in the red cell enzyme G6PD scheme utilize commercial kits such as those sold by Trinity Biotech, where the use of whole blood is required. It would be interesting to obtain feedback from the few laboratories performing ‘in-house’ assays based on the ICSH/WHO procedures, where prewashing and lysing of the red blood cells is required.

In summary we have produced a laboratory methodology for heat treatment of human red blood cells to give low G6PD assay results whilst retaining red cell membrane integrity for use in an EQA program that has enabled us to send samples internationally, thus supporting a key safety entry criterion in a clinical trial of a new antimalarial tafenoquine.

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CONFLICT OF INTEREST

Laboratory work was sponsored by GSK and writing performed by David Roper. JG is employed by and holds stock in GlaxoSmithKline. All other authors state that they have no interests that might be perceived as posing a conflict or bias.