Short Communication

Seasonal variation in the performance of QuantiFERON-TB Gold In-Tube assays used for the diagnosis of tuberculosis infection

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This study aimed to determine whether there are seasonal changes in the performance of QuantiFERON-TB Gold In-Tube (QFT-GIT) assays, an interferon-gamma release assay widely used for the diagnosis of tuberculosis infection. Results of 31,932 QFT-GIT assays performed at a large independent, accredited diagnostic service provider in London, UK over a 4.5-year-period were analysed. The proportion of positive results was significantly lower in autumn (14.8%) than in spring (16.0%; p=0.0366) and summer (17.5%; p<0.0001), but similar to winter (15.2%; p=0.4711). The proportion of indeterminate results was significantly higher in autumn (8.2%) than in spring (6.2%; p<0.0001), summer (4.8%; p<0.0001), and winter (6.2%; p<0.0001). The highest proportions of indeterminate results were observed in October (8.4%) and November (8.8%), the lowest in June (4.5%). Our data show that significant seasonal variation occurs in the performance of QFT-GIT assays in a temperate climate setting. Potential underlying mechanisms, including host and environmental factors, are discussed.

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INTRODUCTION
Interferon-gamma release assays (IGRAs) detect interferon-gamma responses following in vitro stimulation of blood with mycobacterial antigens [1], and are used widely throughout the U.S. and Europe for the detection of tuberculosis (TB) infection [2]. Of the two commercially available IGRAs, QuantiFERON-TB Gold assays are more commonly used in routine clinical practice than T-SPOT.TB assays [2].

IGRAs have several limitations including significant proportions of indeterminate assay results in certain patient populations and suboptimal sensitivity in patients with active TB [3-5]. Additionally, numerous studies indicate that IGRAs are not particularly robust, with minor variations in sampled blood volumes, intensity of assay tube shaking and delays in sample incubation having considerable impact on interferon-gamma responses and consequently assay results [3].

Data from a recent ex vivo study suggest that the performance of IGRAs is also influenced by environmental temperatures to which assay tubes are exposed prior to incubation [6]. As ambient temperature is not usually controlled during sample transport in routine clinical settings, we hypothesized that IGRA performance may vary between seasons in temperate climates. This study aimed to determine whether there is seasonal variation in the performance of QuantiFERON-TB Gold In-Tube (QFT-GIT) assays.

METHODS
Materials
We retrospectively studied data from QFT-GIT assays (Cellestis/Qiagen, Carnegie, Australia) processed routinely at a large independent, accredited diagnostic service provider in London between December 2009 and May 2014. The QFT-GIT samples originated from external healthcare providers or from the in-house phlebotomy services. Samples from the former are transported to the laboratory by car or motorcycle without temperature monitoring. All samples were incubated within 16 hours, as per manufacturer’s instructions. Assays were processed and interpreted according to the most current version of the manufacturer’s instructions [7]. All data analyses were performed by independent researchers with no access to personal or identifiable data.
Statistical analysis
Categorical variables were compared using two-tailed chi square tests and quantitative variables with Kruskal Wallis tests (comparisons of multiple groups) or two-tailed Mann Whitney U tests (two-group comparisons). 95% confidence intervals around proportions were calculated with the Wald method. To determine potential associations between temperature and assay results, linear regression analyses were performed and Spearman’s rank correlation coefficients with two-tailed significance values were calculated. All statistical analyses were done with Prism (V7.0; GraphPad, La Jolla, U.S.).

RESULTS
A total of 31932 QFT-GIT assays were included in the analyses. Of these, 8044 were performed in spring (March - May), 7376 in summer (June - August), 8996 in autumn (September - November), and 7516 in winter (December - February).

Analysis of positive QFT-GIT results
The result was positive in 5051 (15.8%) QFT-GIT assays. The proportion of positive versus other assay results (i.e. negative or indeterminate) was significantly lower in autumn (n=1332; 14.8%) than in spring (n=1285; 16.0%; p=0.0366) and summer (n=1290; 17.5%; p<0.0001), but similar to the proportion in winter (n=1144; 15.2%; p=0.4711). The lowest proportions of positive results were observed in October (12.8%), November (14.7%), and December (13.6%), while the highest proportion of positive results occurred in June (18.5%) (Figure 1A).

Analysis of indeterminate QFT-GIT results
The result was indeterminate in 2052 (6.4%) QFT-GIT assays. The majority of indeterminate results were due to inadequate positive control responses (n=1923; 93.8%) rather than high interferon-gamma concentrations in the negative control samples (n=129; 6.3%). The proportion of indeterminate compared with determinate (positive or negative) assay results was significantly higher in autumn (n=734; 8.2%) than in spring (n=497; 6.2%; p<0.0001), summer (n=354; 4.8%; p<0.0001), and winter (n=467; 6.2%; p<0.0001). The highest proportions of indeterminate results were observed in October (8.4%) and November (8.8%), while the lowest proportions were observed in June (4.5%), July (5.0%) and August (4.8%).
Indeterminate assay results due to inadequate positive control responses and high interferon-gamma concentrations in the negative control both contributed to the high proportion of indeterminate results observed in autumn (n=670 and n=64, respectively, corresponding to 7.4% and 0.71% of tests during that season) (Figure 1B&C). Compared with autumn, the proportion of indeterminate results due to inadequate positive control responses was significantly lower in spring (n=464; 5.8%; p<0.0001), summer (n=330; 4.5%; p<0.0001), and winter (n=459; 6.1%; p=0.0008). Furthermore, compared with autumn, the proportion of indeterminate results due to high interferon-gamma concentrations in the negative control was significantly lower in spring (n=33; 0.41%; p=0.0122), summer (n=24; 0.33%; p=0.0011) and winter (n=8; 0.11%; p<0.0001).

Analysis of background-corrected positive control responses (i.e. interferon-gamma concentration in the positive control sample minus concentration in the negative control sample) showed that median responses were significantly lower in assays performed in autumn (11.94 IU/mL; IQR: 6.32 - 15.00 IU/mL) than in those performed in spring (13.60 IU/mL; IQR: 8.19 - 17.54; p<0.0001), summer (12.62 IU/mL; IQR: 8.17 - 15.19 IU/mL; p<0.0001) and winter (13.07 IU/mL; IQR: 8.28 - 15.88 IU/mL; p<0.0001).

Relationship between temperature and categorical QFT-GIT results

To determine whether there was a potential association between temperature and categorical assay results, regression analyses using average seasonal temperatures recorded in central London (St. James’s Park) were performed. There was a non-significant trend for a positive correlation between average temperature and the proportion of positive assay results (Figure 2A). In addition, there was a statistically significant, weak inverse correlation between average temperature and the proportion of indeterminate assay results due to failed positive controls (r = -0.4742, p = 0.0468; Figure 2B).

DISCUSSION

To our knowledge, this is the first study to show significant seasonal variation in the performance of QFT-GIT assays, and it is one of the largest studies investigating IGRAst to date.
We found that positive QFT-GIT results were significantly more common in spring and summer. Furthermore, the proportion of indeterminate results was significantly higher in autumn. This finding is consistent with a Swiss study that analysed 1429 T-SPOT.\textit{TB} assays (which being ELISPOT-based assays differ from QFT-GIT assays) performed over a single year [8]. Indeterminate T-SPOT.\textit{TB} results were significantly more common in autumn and winter combined, compared with spring and summer. In addition, indeterminate results were more common in young children and elderly patients, consistent with our previously reported observations in QFT-GIT assays [4,9].

Seasonal variation in the performance of QFT-GIT assays may result either from host factors or from environmental conditions directly impacting the assay. Significant seasonal variations in the human immune system have recently been described, including the cellular composition of peripheral blood and the expression of pro-inflammatory genes [10]. However, environmental factors might also play a substantial role, with indeterminate results increasing during colder months due to samples being exposed to cooler ambient temperatures during transport. We have previously shown, in a controlled laboratory setting, that QFT-GIT positive control responses are significantly reduced when samples are maintained below 22°C prior to incubation [6]. This hypothesis is supported by the fact that in the present study the majority of indeterminate assay results were due to inadequate positive control responses and were more common in autumn, when mean monthly temperatures in central England ranged from 5.2°C to 15.1°C during the study period [11]. Furthermore, our analyses revealed that there was indeed a weak correlation between average seasonal temperatures and the proportions of indeterminate test results. The positive finding is more striking as the diurnal and day-to-day temperature variations, which can be considerable in the U.K., were not accounted for in these analyses, which may have resulted in an underestimate of the impact of temperature.

Indeterminate responses due to high interferon-gamma concentrations in negative control samples were also significantly more common in autumn than in other seasons. Although the underlying mechanism remains uncertain, this may relate to viral respiratory infections being more common in autumn than in warmer seasons, as these can cause increased interferon-gamma concentrations in peripheral blood [12]. Alternatively this could reflect the immune system of Europeans showing a pro-inflammatory bias during autumn and winter [10].
The observation that the proportion of positive QFT-GIT results also varied between seasons, with the highest proportion occurring in spring and summer, is more difficult to explain. However, several studies have shown seasonal variation in the incidence of active TB as well as non-tuberculous mycobacterial disease in temperate climates with peaks occurring in spring and troughs in autumn [13,14]. Furthermore, in the U.K. population the levels of vitamin D, which plays a crucial role in anti-mycobacterial immune responses, are highest during summer months due to increased sun exposure [15]. This potentially results in higher interferon-gamma responses during that season, thereby leading to a greater number of positive QFT-GIT assay results. The alternative explanation is that, analogous to positive control responses, antigen-stimulated interferon-gamma responses are also impaired by colder temperatures, resulting in more false-negative results during cooler months.

The main limitation of this study is that no clinical data were available. Therefore, the number of individuals with risk factors for TB, confirmed active TB, or immunocompromise, all of which can impact on individual QFT-GIT results, is unknown. However, given the large size of the dataset capturing data from a 4.5-year period, it is unlikely that observed differences are attributable to coincidental changes in the patient population. Additionally, official population figures suggest that the population of Greater London showed little variation over the study period with regards to age and gender distribution [16]. A second limitation of the study is that we were not able to determine the precise number of samples that were transported from external healthcare providers to the diagnostic service provider (vs. samples obtained in-house). However, according to information provided by the diagnostic service provider, annually more than 80% of the QFT-GIT samples originate from external providers, confirming that the majority of samples have been exposed to ambient temperatures.

In conclusion, there is significant seasonal variation in the performance of QFT-GIT assays in a temperate climate setting. Both environmental and host factors are potentially causally implicated in the high proportion of indeterminate results during autumn and winter. Further research is required to determine whether temperature control during sample transport can improve assay performance during colder periods of the year, generating more reliable results for patient care.
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Conflict of interests: MT received QuantiFERON-TB Gold assays at reduced cost for another research project from the manufacturer (Cellestis/Qiagen). The manufacturer had no influence on the study design, data interpretation, writing of the manuscript or decision to submit the data for publication.

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Contributors: Study concept: MT and SM-J. Data analyses: MT, NC, VC, PE and SM-J. Data interpretation: MT, NC, VC, CF-T, NK, KF, SM, PE and SM-J. Drafting of the manuscript: MT, NC, PE and SM-J. All authors critically read, commented on, and approved the final version of the manuscript.
References


**Figure 1.** Proportions of (A) positive results, (B) indeterminate results due to insufficient interferon-gamma responses in the positive control sample, and (C) indeterminate results due to high interferon-gamma concentrations in the negative control sample in QuantiFERON-TB Gold In-Tube assays according to month and season. The whiskers represent the 95% confidence intervals. The p-values shown were calculated with Kruskal Wallis tests.
Figure 2. Relationship between average seasonal temperatures and categorical QuantiFERON-TB Gold In-Tube assay results. (A) Positive assay results; (B): indeterminate assay results due to failed positive controls. The graphs show the regression line (black) with 95% confidence intervals (grey), the Spearman’s rank correlation coefficient ($r$) and the corresponding p-value ($p$).