Investigation of False-Positive Results Given by the QuantiFERON-TB Gold In-Tube Assay

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We investigated a sudden increase in the rate of positive QuantiFERON-TB Gold In-Tube results from 10% to 31% at a U.S. academic institution. Direct comparison of the TB antigen tubes with tubes from a different lot number identified that a potential problem with the TB antigen vials in a certain tube lot was the likely cause of the elevated positive rate. The underlying defect remains unknown. This finding warrants refinement of quality control programs by the manufacturer and users.

Diagnosing latent tuberculosis infection (LTBI) relies on the host T cell response to *Mycobacterium tuberculosis* antigens. Historically, the tuberculin skin test (TST) has been used, but more recently, *in vitro* gamma interferon (IFN- γ) release assays (IGRAs) have become more widespread. IGRAs are based on measuring IFN- γ released from predominantly CD4 T cells upon stimulation with *M. tuberculosis*-specific antigens (2). The QuantiFERON-TB Gold In-Tube test (QFT-GIT) (Cellestis Limited, Carnegie, Victoria, Australia) is an IGRA that is increasingly used for LTBI surveillance of health care employees in the United States. Blood is collected in 3 tubes specially designed for QFT-GIT that contain *M. tuberculosis* antigens (TB antigen [TB Ag] tube), mitogen, or neither TB antigen nor mitogen (nil tube). After incubation, the IFN- γ concentration in plasma from the nil tube, the TB Ag tube, and the mitogen tube are determined by enzyme-linked immunosorbent assay (ELISA). The TB response is calculated by subtracting the nil tube value from the TB Ag value (TB Ag–nil). The threshold for a positive result is a TB Ag–nil response of 0.35 IU/ml.

The Stanford Hospital and Clinics clinical microbiology laboratory implemented the QFT-GIT test in 2008. This assay is used to annually screen new employees and employees with prior negative tests for LTBI (>10,000 tests per year). Due to transient increases in the daily positivity rate in the past, the laboratory implemented a surveillance program in 2010 for tracking daily positive rates. The baseline positive rate has consistently ranged from 5% to 15% (mean, 9%). However, the proportion of positives significantly increased, to an average of 31% ($P < 0.001$, chi-squared test), during the period of 10 to 20 November 2011 (Fig. 1). Means of the nil, TB Ag, mitogen, and TB Ag–nil values for the 10-day suspect period ($n = 370$) were compared by using the Student t test to means for the 10-day period preceding 10 November 2011 (presuspect period) ($n = 435$) (Table 1). Age distribution was similar across the two periods ($P = 0.31$, chi-square test). The mean TB Ag–nil values were 0.77 IU/ml and 0.23 IU/ml for the suspect and presuspect periods, respectively, and the mean TB Ag values were 0.87 IU/ml and 0.33 IU/ml, respectively. Both the TB Ag–nil and TB Ag values during the suspect period were significantly elevated compared to the respective presuspect period values ($P < 0.001$). The mean nil tube values were not significantly different between the suspect and presuspect periods at 0.12 IU/ml and 0.1 IU/ml, respectively ($P = 0.99$) (Table 1). The mean mitogen value was significantly higher

in the presuspect period than in the suspect period, with values of 9.2 IU/ml and 8.69 IU/ml, respectively ($P < 0.001$).

Given the negative impact of false-positive results for occupational health and infection control departments, an investigation to identify the cause of the significant rise in the positive rate was initiated. The fact that 92% (107/116) of the health care workers with positive results during the suspect period had no history of positive test results or TB exposure between their last QFT-GIT result and the current test suggested that their current result was a false positive. Therefore, all aspects of the assay, including preanalytic, analytic, and manufacturer-related defects, were examined to determine the potential cause of false positives. There was no change in the patient populations tested. Preanalytic evaluation of the phlebotomy procedure, incubation of blood, and harvesting of plasma was conducted during a visit by the manufacturer's technical manager. There was a slight deviation from the updated manufacturer's recommended blood collection protocol (1) of a flick-of-the-wrist motion rather than inversion of the sample during mixing. However, this collection method had been in place for months prior to the detection of the sudden increase in the positive rate. The IFN- γ ELISA procedure was also examined by the manufacturer. Since only the TB Ag value and not the nil or mitogen value was positively affected, the ELISA seemed an unlikely source of error. However, 26 positive patient plasma samples were retested by the manufacturer's technical staff using the existing software/hardware setup and reagents in use in the laboratory. All positive results were reproduced when stored plasma was retested, with the exception of two samples: one was borderline negative, with a TB Ag–nil value of 0.33 IU/ml, and the other had a pipetting error due to instrument failure, which occurred during substrate addition. The fact that essentially all of the results were reproducible confirmed that the laboratory processes or the instrumentation was not the source of the problem.

We then investigated manufacturer-related defects. We dis-

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FIG. 1. The QFT-GIT surveillance graph showing daily positive rates. The histogram displays the proportion of total QFT-GIT tests that were positive on each day during November 2011. The arrows indicate the date that the increase in positive test rates was noted and the date that TB antigen tube lot A110401T was switched to a new lot (A11030U2).

covered that all of the TB Ag tubes giving false results were from a single lot number (A110401T) that had been introduced around the time of the sharp increase in positive test results. We therefore considered the possibility that a manufacturing-related defect could have caused the sudden elevation in the positive test rate and switched to a new TB Ag tube lot number (A11030U2) on 21 November 2011. In the 10-day period following the TB Ag tube replacement, the proportion of positives dropped from 31% to 7% ($n = 705$) (Fig. 1). The mean TB Ag values declined from 0.87 IU/ml to 0.26 IU/ml ($P < 0.001$), while the nil tube value changed from 0.12 IU/ml to 0.16 IU/ml ($P = 0.23$). The mean TB Ag–nil values were significantly different at 0.77 IU/ml and 0.1 IU/ml for the suspect and postsuspect periods, respectively ($P < 0.001$). To provide direct evidence of the TB Ag tubes being the source of the problem, 463 employees presenting to Occupational Health for annual LTBI screening with QFT-GIT underwent paired testing with the suspect TB Ag tubes (lot A110401T) and a new lot of TB Ag tubes (lot A11030U2). Results clearly demonstrated a significant difference in the positive rate between the suspect lot (31%) and the new lot (5%) ($P < 0.001$, McNemar's test) (Fig. 2). The mean TB Ag–nil values were significantly higher in the suspect lot (0.58 IU/ml) than in the new lot (0.06 IU/ml; $P < 0.001$). While 32% of subjects ($n = 46$) had a borderline-positive result (TB Ag–nil value ≥ 0.35 but < 0.7 IU/ml) with the suspect TB antigen tube, 53% ($n = 77$) had a robust response (TB Ag–nil value > 1 IU/ml) (Fig. 2). Additionally, in a subset of the employees ($n = 81$) who underwent retesting after the initial false-positive results during the suspect period, 91% tested negative with the new lot of TB Ag tubes.

FIG. 2. Within-subject comparison of QFT-GIT results with the suspect lot and a new lot of TB Ag tubes. The dot plot displays the TB Ag–nil values, in IU/ml, for the QFT-GIT performed with paired blood samples from 463 subjects using the suspect lot (A110401T) and a new lot (A11030U2) of TB Ag tubes. The cutoff for positive results is marked with a dashed line. Each symbol represents one subject. The mean was significantly higher for the suspect lot (0.58 IU/ml) than for the new lot (0.06 IU/ml) by paired t test.

Extensive efforts were undertaken by the laboratory in collaboration with the manufacturer to discover and remove the underlying cause. The manufacturer conducted an investigation of the tubes from the suspect lot number. No deviations had occurred during manufacturing of tubes, and endotoxin contamination was ruled out. Their final conclusion was that the root cause of the discrepant results that we observed was not discovered. We also excluded contamination of TB Ag tubes with live culturable organisms. The FDA was contacted with our findings through our communication with the CDC. The underlying mechanism of increased false-positive results remains unclear at this point.

The elevated QFT-GIT percent positive rate described in this report caused a resource burden on the Occupational Health Clinic and had an emotional impact on the employees. The sequelae of the false-positive results highlight the need for more rigorous quality control measures by the manufacturer to guarantee batch-to-batch consistency. This could be accomplished by parallel testing of new lots with old lots with adequate numbers of subjects with and without LTBI to ensure proper results. In addition to the manufacturer's quality control program, we recommend that each clinical laboratory institute a surveillance system

TABLE 1 QFT-GIT results for the periods preceding, during, and following the increase in the daily positive rate

Parameter	Presuspect period ^a	Suspect period (lot A110401T)	Postsuspect period (lot A11030U2)	<i>P</i> values (Pre, Post) ^b
No. of tests	435	370	705	
Mean nil value (IU/ml)	0.1	0.12	0.16	0.99, 0.23
Mean TB Ag value (IU/ml)	0.33	0.87	0.26	<0.001 , <0.001
Mean mitogen value (IU/ml)	9.2	8.69	7.9	<0.001 , <0.001
Mean TB Ag–Nil value (IU/ml)	0.23	0.77	0.1	<0.001 , <0.001
% positive results	11	31	6.8	<0.001 , <0.001

^a The lot number for the presuspect period was not recorded.

^b *P* values are for comparison of results from suspect lot to pre- and postsuspect lots using the Student t test.

to monitor and detect sudden changes in daily or weekly positive and indeterminate rates. The surveillance system at our institution was implemented in 2010 in response to a 3-day interval of elevated daily QFT-GIT positivity rate (from 10% to 42%) that resolved spontaneously without any intervention. Similar spikes with spontaneous resolutions have since been observed. Although the causes of these spikes were not investigated, we now question whether these events and the current episode are related. The positivity rates over the 2 months prior to the described episode were analyzed, and we recommend a daily positive rate greater than 2 standard deviations above the mean lasting longer than 3 days as the threshold prompting investigation. We validated this recommendation with 2 years of QFT-GIT surveillance data. Another benefit of a surveillance system is to monitor the indeterminate rate, although it was not affected in the current episode. The Veterans Affairs Palo Alto Health Care System recently reported a sharp increase in the indeterminate rate of their QFT-GIT results, caused by a faulty lot of mitogen tubes (3). An ensuing investigation traced the transportation route of the tubes through the Panama Canal, possibly resulting in heat damage of the mitogen peptides and altering results. This investigation highlighted the need for temperature-controlled transport of QFT-GIT tubes, which

led to implementation of refrigerated transportation of tubes by the manufacturer.

In summary, we identified that a problem with the TB antigen vials in a certain lot was the likely cause of the elevated false-positive rate. This finding underscores the need for more rigorous quality control measures by the manufacturer and implementation of surveillance programs by clinical laboratories.

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