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TB Diagnostic Updates:

Discontinuation of QuantiFERON-TB Gold In-Tube & Implementation of QuantiFERON-TB Gold Plus

*** Modified May 17, 2018 (changes highlighted in yellow)**

**** Modified July 5, 2018 (changes highlighted in blue)**

Interferon gamma-release assays (IGRA) are a method to detect the immune response to infection with *Mycobacterium tuberculosis*. Most people who become infected with *M. tuberculosis* generate specific immune cells in response to that infection, "primed memory T-cells", which upon exposure to certain TB antigens will be activated and stimulated to release an immunological marker, interferon gamma (IFN-γ). IGRAs measure this immunological marker by either a traditional enzyme-linked immunosorbent assay, ELISA (e.g. QuantiFERON assays) or enzyme-linked immunospot assay, ELISPOT (e.g. T-SPOT.*TB*).

Commercially available, FDA-approved IGRAs as of May 2018 include:

- QuantiFERON-TB Gold In-Tube, QIAGEN (QFT-Gold)
- QuantiFERON-TB Gold Plus, QIAGEN (QFT-Plus)
- T-SPOT.*TB*, Oxford Immunotec

The last order date for the QuantiFERON-TB Gold In-Tube assay will be June 29, 2018 and will be discontinued and no longer be available for purchase as of June 30, 2018. If your laboratory is currently utilizing this QFT-Gold product, a decision will need to be made whether to switch to the QFT-Plus, to discontinue testing, or to consider an alternative test. In any case, laboratories should communicate these changes with the respective TB Control Programs and submitting healthcare providers who are utilizing this test.

Differences Between the QFT-Gold and the QFT-Plus

QFT-Plus was FDA-approved in June 2017[1] and was developed to increase the sensitivity of detecting latent TB infection and active TB disease. The assay includes TB antigens to stimulate both CD4+ and CD8+ T Cells (QFT-Gold is optimized to stimulate CD4+ T Cells only). The detection of CD8+ T cell responses requires an additional tube and the QFT-Plus uses 4 tubes rather than 3 (Table 1). Another significant change with the QFT-Plus is two different specimen collection options.[1,2]

QFT-Plus comes with the new option for single tube sample collection (Please refer to package insert[1] and workflow documents[2] for additional references).

1. Direct draw (same process as QFT-Gold): Draw 1ml of blood directly into each of the 4 QFT-Plus tubes. The sample tubes can be held at room temperature for up to 16 hours (with a minimum of 15 minutes) before a 16-24 hour incubation at 37°C. After incubation, ELISA can be performed, or tubes can be held at between 4°C and 27°C for up to 3 days prior to testing.
2. Draw into lithium-heparin single tube: Draw a minimum of 5ml of blood into a lithium-heparin tube. After collection there are two options:

a. Tube can be held at room temperature for up to 12 hours (with a minimum of 15 minutes) prior to transfer to the 4 QFT-Plus blood collection tubes, followed by a 16-24 hour incubation at 37°C before performing the ELISA.

b. Tube can be held at room temperature for a minimum of 15 minutes and up to 3 hours after collection, then must be stored/shipped at 2-8°C for 16-48 hours. The tube must then be brought back up to room temperature for no more than 2 hours before transferring 1mL of blood to each of the 4 QFT-Plus tubes for the 16-24 hour 37°C incubation and then the ELISA.

While all of the new options offer additional flexibility to the program and laboratory workflow, the laboratory must work closely with submitting providers to ensure the pre-analytical considerations regarding test collection, handling and shipping are clearly outlined. QIAGEN is actively working to simplify the collection protocols and changes are likely forthcoming. If changes are made, we will provide an update to members.

Table 1: Summary of Differences between the QFT-Gold and QFT-Plus

	QFT-Gold [a]	QFT-Plus
Stimulates	Primarily CD4+ T Cells	Both CD4+ and CD8+ T Cells
Number of Tubes	3 (Mitogen, Nil, TB Antigens)	4 (Mitogen, Nil, TB 1 Antigen, TB 2 Antigen)
TB Antigens	Long Peptides (MHC Class II) ESAT-6, CFP-10, TB7.7	TB1: Long Peptides (MHC Class II) ESAT 6, CFP10; TB2: Long Peptides (MHC Class II) ESAT 6, CFP10, Short Proprietary Peptides (MHC Class I)
Specimen Collection [b]	Draw 1 mL of blood directly into the 3 QFT-Gold tubes	Draw 1 mL of blood directly into the 4 QFT-Plus tubes OR Draw at least 5 mL of blood into 1 lithium-heparin tube and then aliquot into the 4 QFT-Plus tubes
a. QFT-Gold will be discontinued June 30, 2018. Last order date is June 29, 2018 b. Complete details on specimen collection are available from the manufacturer (package insert[1], fact sheet[2])		

Considerations for Implementation

As with any other new FDA-approved assay, a verification study will need to be performed to meet regulatory requirements. QIAGEN can provide a training panel upon request through a sales representative. The training panel consists of known positive and negative plasma samples. Laboratories may choose to utilize the QIAGEN training panel or use their own known positive and negative TB samples for their studies. A direct comparison of the QFT and QFT-Plus using the same sample is challenging given the number of blood tubes that would need to be drawn on the patient (3-1 mL tubes for the QFT, and 4-1mL tubes for the QFT-Plus).

Options for Validation/Verification Studies:

- Accuracy Studies: A number of known positives and negatives tested over a number of days/runs and analyzed for agreement.
- Precision Studies: A smaller number of known positives and negatives tested each day for several days to ensure reproducibility.
- Reportable Range: Dilution series tested in duplicate or triplicate spanning the reportable range.

Additional Considerations:
Laboratories should consider how they plan to validate/address the pre-analytical aspects of the test prior to implementation – especially if planning to implement single tube draw followed by aliquoting into the 4 collection tube.

FDA approval for the QFT-Plus was based on performance data from a manual test method. Laboratories that want to perform the QFT-Plus on an automated platform (e.g. Bio-Rad EVOLIS, Dynex DS2, Dynex DSX, Dynex Agility) should consider how they plan to validate the automated method (Of note, the QFT-Gold assay was also FDA-approved based on manual test method data).

Qiagen manufactures external controls for the QFT-Plus to control performance between runs and operators, but they are not available for purchase in the US at this time.

Reporting

Each laboratory will need to decide how to report results for the QFT-Plus. APHL has gathered information from the manufacturer as well as a few example reports (Appendix A) with an overall result that includes both the result and interpretation from the package insert followed by the result from each individual tube and the corrected results. For laboratories that utilize standardized coding, the LOINC (Logical Observation Identifiers Names and Codes) Panel Code currently used for QFT (71775-1-*Mycobacterium tuberculosis* stimulated gamma interferon panel – Blood) can still be used for the new QFT-Plus test. Regenstrief, which manages LOINC codes, has added 2 more elements to this panel (and adjusted others) to account for the updates between QFT-Gold and QFT-Plus (Appendix B). California TB Control has also developed guidance for reporting IGRA results electronically.

Table 2: Interpretation of QFT-Plus Results (From Package Insert)

<table>
<tr><th>Nil (IU/mL)</th><th>TB1 minus Nil (IU/mL)</th><th>TB2 minus Nil (IU/mL)</th><th>Mitogen minus Nil (IU/mL)*</th><th>QFT-PLUS Result</th><th>Report/Interpretation</th></tr>
<tr><td rowspan="4">≤8.0</td><td>≥0.35 and
≥ 25%
of Nil value</td><td>Any</td><td rowspan="2">Any</td><td rowspan="2">Positive†</td><td rowspan="2">M. tuberculosis infection likely</td></tr>
<tr><td>Any</td><td>≥0.35 and
≥ 25%
of Nil value</td></tr>
<tr><td><0.35 or
≥0.35 and
<25% of Nil value</td><td><0.35 or
≥0.35 and
<25% of Nil value</td><td>≥0.5</td><td>Negative</td><td>M. tuberculosis infection NOT likely</td></tr>
<tr><td><0.35 or
≥0.35 and
<25% of Nil value</td><td><0.35 or
≥0.35 and
<25% of Nil value</td><td><0.5</td><td rowspan="2">Indeterminate‡</td><td rowspan="2">Likelihood of M. tuberculosis infection cannot be determined</td></tr>
<tr><td>>8.0§</td><td colspan="3">Any</td></tr>
<tr><td colspan="6">* Responses to the Mitogen positive control (and occasionally TB Antigens) can be outside the range of the microplate reader. This has no impact on test results. Values >10 ml are reported by the QFT-Plus software as >10 IU/ml.
† Where M. tuberculosis infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QFT-Plus ELISA. If repeat testing of one or both replicates is positive, the individual should be considered test positive.</td></tr>
</table>

‡ Refer to the "Troubleshooting" section for possible causes.
§ In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

References and Resources

1. QuantiFERON-TB Gold Plus Package Insert: http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf
2. QIAGEN Guide to QFT-Plus Specimen Collection; http://www.quantiferon.com/wp-content/uploads/2017/10/PROM-11180-001_1107787_FLY_Workflows_0717_ONLINE.pdf
3. Mayo Medical Laboratories Hot Topic Webinar; https://news.mayomedicallaboratories.com/2018/01/22/quantiferon-tb-gold-plus-hot-topic/

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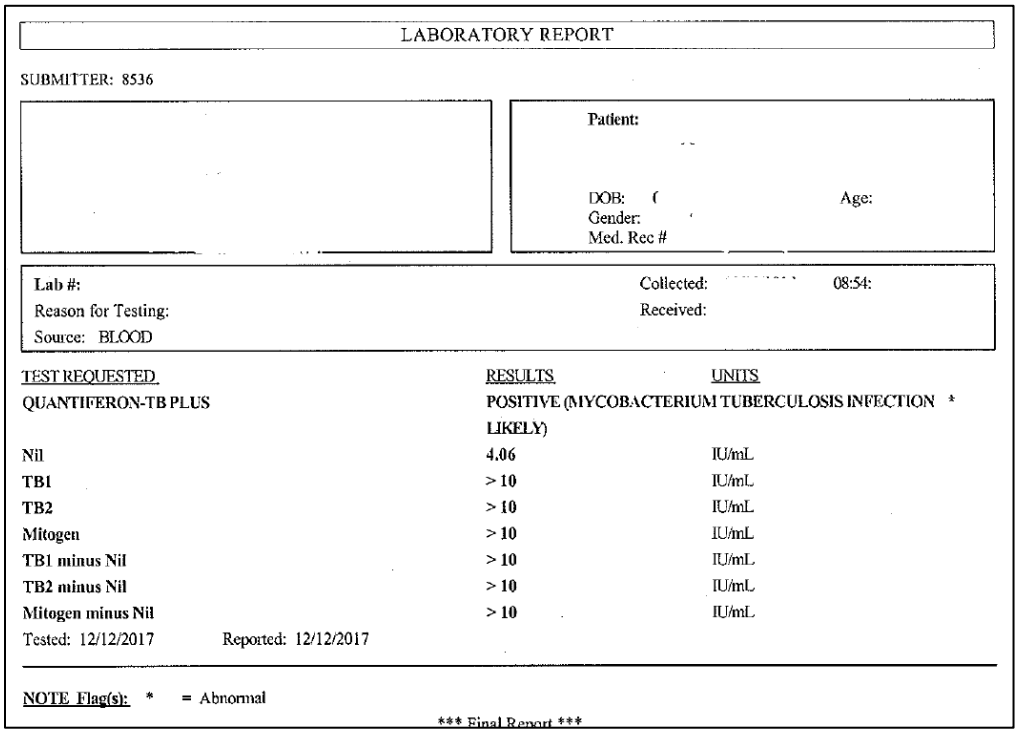
Appendix A: Example Reports

Positive Result 1



LABORATORY REPORT

SUBMITTER: 8536

Patient:

DOB: (Age:
Gender:
Med. Rec #

Lab #: Collected: 08:54:
Reason for Testing: Received:
Source: BLOOD

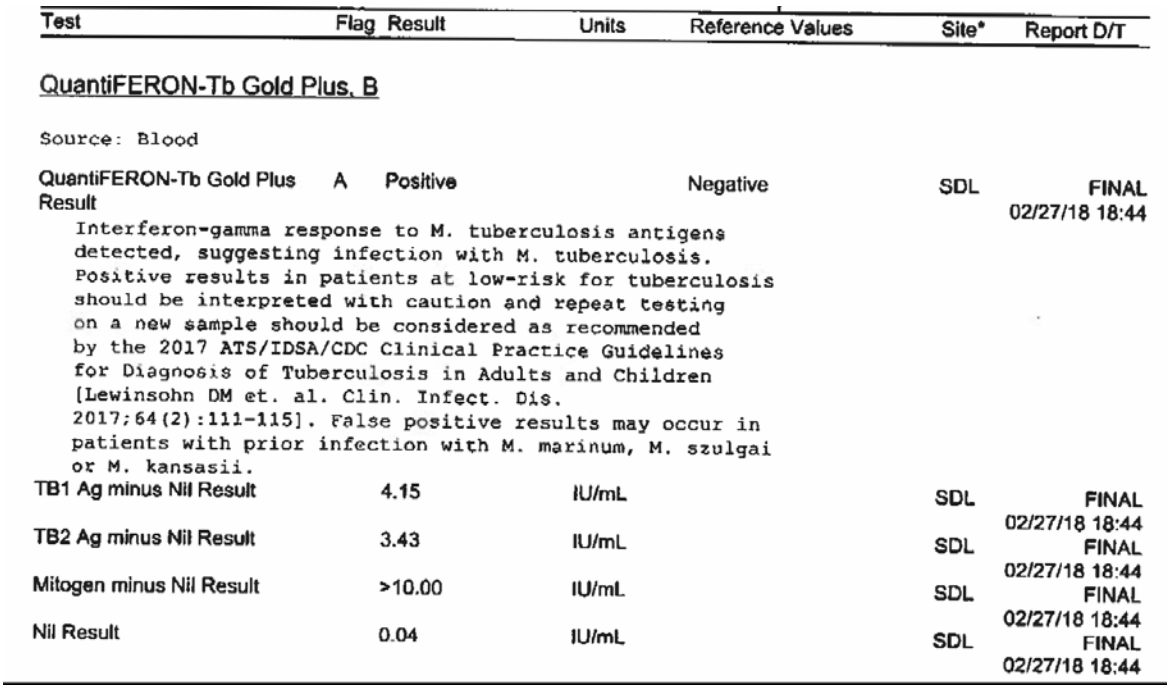
TEST REQUESTED	RESULTS	UNITS
QUANTIFERON-TB PLUS	POSITIVE (MYCOBACTERIUM TUBERCULOSIS INFECTION LIKELY) *	
Nil	4.06	IU/mL
TB1	> 10	IU/mL
TB2	> 10	IU/mL
Mitogen	> 10	IU/mL
TB1 minus Nil	> 10	IU/mL
TB2 minus Nil	> 10	IU/mL
Mitogen minus Nil	> 10	IU/mL

Tested: 12/12/2017 Reported: 12/12/2017

NOTE Flag(s): * = Abnormal

*** Final Report ***

Positive Result 2



Test	Flag	Result	Units	Reference Values	Site*	Report D/T
QuantiFERON-Tb Gold Plus, B						
Source: Blood						
QuantiFERON-Tb Gold Plus Result	A	Positive		Negative	SDL	FINAL 02/27/18 18:44
Interferon-gamma response to M. tuberculosis antigens detected, suggesting infection with M. tuberculosis. Positive results in patients at low-risk for tuberculosis should be interpreted with caution and repeat testing on a new sample should be considered as recommended by the 2017 ATS/IDSA/CDC Clinical Practice Guidelines for Diagnosis of Tuberculosis in Adults and Children [Lewinsohn DM et. al. Clin. Infect. Dis. 2017;64(2):111-115]. False positive results may occur in patients with prior infection with M. marinum, M. szulgai or M. kansasii.						
TB1 Ag minus Nil Result		4.15	IU/mL		SDL	FINAL 02/27/18 18:44
TB2 Ag minus Nil Result		3.43	IU/mL		SDL	FINAL 02/27/18 18:44
Mitogen minus Nil Result		>10.00	IU/mL		SDL	FINAL 02/27/18 18:44
Nil Result		0.04	IU/mL		SDL	FINAL 02/27/18 18:44

Negative Result

LABORATORY REPORT

SUBMITTER: 8536

Patient:

DOB: Age:
Gender:
Med. Rec #

Lab #:
Reason for Testing:
Source: BLOOD

Collected: 08:53:
Received: 12/08/2017

TEST REQUESTED	RESULTS	UNITS
QUANTIFERON-TB PLUS	**NEGATIVE (MYCOBACTERIUM TUBERCULOSIS INFECTION NOT LIKELY)**	
Nil	**0.02**	IU/mL
TB1	**0.01**	IU/mL
TB2	**0.01**	IU/mL
Mitogen	**> 10**	IU/mL
TB1 minus Nil	**-0.01**	IU/mL
TB2 minus Nil	**-0.01**	IU/mL
Mitogen minus Nil	**> 10**	IU/mL

Tested: 12/12/2017 Reported: 12/12/2017

*** Final Report ***

Appendix B: LOINC Codes for QuantiFERON-TB Gold Plus, QIAGEN (QFT-Plus)

LOINC (Logical Observation Identifiers Names and Codes) are commonly used in standardized electronic reporting. The table below includes the LOINC and the LOINC name for the items associated with the QFT-Plus assay. All but the last two were used for the QFT-Gold assay, with the last two, newly developed codes created to accommodate the new results for the QFT-Plus.

LOINC #	LOINC Name
71775-1	Mycobacterium tuberculosis stimulated gamma interferon panel – Blood
71776-9	Gamma interferon background [Units/volume] in Blood by Immunoassay
71772-8	Mitogen stimulated gamma interferon [Units/volume] in Blood
71774-4	Mitogen stimulated gamma interferon [Units/volume] corrected for background in Blood
46217-6	Mycobacterium tuberculosis stimulated gamma interferon [Units/volume] in Blood
64084-7	Mycobacterium tuberculosis stimulated gamma interferon [Units/ volume] corrected for background in Blood
71773-6	Mycobacterium tuberculosis stimulated gamma interferon [Presence] in Blood
88518-6*	Mycobacterium tuberculosis stimulated gamma interferon release by helper CD4 and cytotoxic CD8 cells [Units/volume] in Blood
88517-8*	Mycobacterium tuberculosis stimulated gamma interferon release by helper CD4 and cytotoxic CD8 cells [Units/volume] corrected for background in Blood

*Created on February 2, 15, 2018, In Development for Next Release, https://loinc.org/prerelease/