TB Diagnostic Updates:

Implementation of Interferon Gamma-Release Assays

Interferon gamma-release assays (IGRA) are a method to detect the immune response to infection with *Mycobacterium tuberculosis*. IGRAs are based on the ability of specific *Mycobacterium tuberculosis* antigens (early secretory antigen target 6, ESAT-6 and culture filtrate protein 10, CFP-10) to stimulate host production of interferon-gamma. As these antigens are not present in nontuberculous mycobacteria (NTM) nor any BCG vaccine variant, IGRA provides greater specificity for the identification of TB infection. 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 an enzyme-linked immunosorbent assay, ELISA (e.g., QuantiFERON assays) to quantify total IFN-γ or enzyme-linked immunospot assay, ELISPOT (e.g., T-SPOT.*TB*) which counts the number of activated T-cells that secrete IFN-γ.

Commercially available, US Food and Drug Administration (FDA)-approved IGRAs in the US include:

- QuantiFERON-TB Gold Plus, QIAGEN (QFT-Plus) [Manual][1]
- LIAISON ® QuantiFERON-TB Gold Plus Solution (LIAISON QFT-Plus) [Automated][2]
- T-SPOT.TB, Oxford Immunotec (T-SPOT) [Manual][3]

Recommendations for IGRA Usage

- *Updated Guidelines for Using Inteferon Gamma Release Assays to Detect* Mycobacterium tuberculosis *Infection-US 2010* (US Centers for Disease Control and Prevention (CDC))
- *Tuberculosis Screening, Testing, and Treatment of U.S. Health Care Personnel: Recommendations from the National Tuberculosis Controllers Association and CDC, 2019* (CDC, National Tuberculosis Controllers Association (NTCA))
- *Joint Task Force on Implementation of the 2019 MMWR Recommendations* (NTCA, American College of Occupational and Environmental Medicine)
- *Testing and Treatment of Latent Tuberculosis Infection in the United States: Clinical Recommendations, February 2021* (NTCA)

Differences Between QFT-Plus and T-SPOT

QFT-Plus was FDA-approved in June 2017[1] and was developed to increase the sensitivity (as compared to their previous product QFT-Gold) of detecting latent TB infection and active TB disease. The LIAISON® QuantiFERON® TB Gold Plus, an automated version, was FDA-approved in November 2019.[2] The T-SPOT.TB test is an in vitro diagnostic test based on an enzyme-linked immunospot (ELISPOT) method.[3] While both tests, T-SPOT and QFT-Plus, detect CD4+ and CD8+ positive T cells, the detection of CD8+ T cell responses requires an additional tube for QFT-Plus. QFT-Plus offers two different specimen collection options (one tube vs. four tube)[4] compared to a single collection option (one tube) for the T.SPOT method (**Table 1**).

Table 1. Summary of Differences between the QFT-Plus and T-SPOT.TB [a,b]

	QFT-Plus (One Tube)	QFT-Plus (Four Tube)	T-SPOT.TB
Initial Process	Whole blood is collected into a single tube which must be transferred into the four QFT-Plus tubes for incubation within 16 hours of blood collection.	Process whole blood within 16 hours.	Process peripheral blood mononuclear cells (PBMCs) within eight hours If T-Cell Xtend® is used: process within 0-32 hours
Number of Tubes	One (lithium heparin), then transferred to four (see Four Tube)	Four (Mitogen, Nil, TB 1 Antigen, TB 2 Antigen)	One (lithium or sodium heparin tube)
Measurement	IFN-ɣ concentration	IFN-ɣ concentration	Number of IFN- γ producing cells (spots)
TB Antigens	Single mixture of synthetic peptides representing ESAT-6 and CFP-10	Single mixture of synthetic peptides representing ESAT-6 and CFP-10	Separate mixtures of synthetic peptides representing ESAT-6 and CFP-10
Specimen Collection	Blood draw: At least 5 mL into one lithium-heparin tube, then aliquot into four QFT-Plus tubes	Blood draw: 1 mL directly into four QFT-Plus tubes	Blood draw: Individuals ≥ 10 years old: 6 mL Children ≥ 2 and <10 years old: 4 mL Children < 2 years old: 2 mL

a. Table was modified from CDC Factsheet.[5]

b. Complete details on process, antigens and specimen collection are available from the manufacturer package inserts[1-3] and QFT-Plus Blood Collection Document.[4]

Considerations for Implementation

Laboratories will have to consider many factors when determining which FDA-approved assay is appropriate for use. The QFT-Plus and T-SPOT.TB are both manual assays; however, Qiagen and DiaSorin have partnered to develop an automated version of the QFT-Plus assay for the LIAISON® XL Analyzer.[6] The LIAISON QFT-Plus uses the QFT- Plus blood collection tubes which can be loaded directly into the LIAISON® XL Analyzer. This automated system allows the laboratory to connect up to nine LIAISON ® XL instruments and two LIS (Laboratory Information System) channels and can combine individual sample results (Nil, TB1, TB2, Mitogen) in one single patient report sent directly to the LIS or LIAISON® QuantiFERON® software (LQS).[6]

Note: If performing a single tube collection, the sample must be aliquoted into the four tubes prior to use on the LIAISON XL. If laboratories wish to use an alternative automated platform (e.g., Bio-Rad EVOLIS, Dynex DS2, Dynex DSX, Dynex Agility), consideration must be given on how to validate the automated method.

Reporting

Each laboratory will need to decide how to report results for the QFT-Plus and T-SPOT.TB. To help inform these decisions, find summarized results and interpretations in the tables below for the QFT-Plus (**Table 2**) and T-SPOT.TB (**Table 3**). Additionally, **Appendix A** includes information from manufacturers and example reports for 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 Codes currently used are: QFT-Plus test (71775-1-*Mycobacterium tuberculosis* stimulated gamma interferon panel—Blood) and T-SPOT.TB test (74281-7-*Mycobacterium tuberculosis* stimulated gamma interferon and spot count panel—Blood). See **Appendix B** for more information. California TB Control has also developed guidance for reporting IGRA results electronically.

Table 2: Interpretation of QFT-Plus Results[1]

Nil (IU/mL)	TB1 minus Nil (IU/mL)	TB2 minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)[a]	QFT-PLUS Result	Report/Interpretation
≤8.0	≥0.35 and ≥25% of Nil value	Any	Any	Positive[b]	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥25% of Nil value			
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	≥0.5	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	<0.5	Indeterminate[c]	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0[d]	Any				

a. 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.

b. 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.

c. Refer to the "Troubleshooting" section for possible causes.

d. In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

Table 3: Interpretation of T-SPOT.TB Results[3,7]

Spot count in Panel A minus Nil and/or Panel B minus Nil (IU/mL)[a]	T.SPOT Result	Report/Interpretation[d]
≥ 8 spots	Positive	*M. tuberculosis* infection likely
5, 6 or 7 spots	Borderline[b] (equivocal)	Test should be repeated; Likelihood of *M. tuberculosis* infection cannot be determined
< 4 spots	Negative	*M. tuberculosis* infection NOT likely
Nil well: >10 spots **Mitogen well: <20 spots** (unless Panel A-Nil and/or Panel B-Nil are Positive or Borderline)	Invalid[c]	Test should be repeated; Likelihood of *M. tuberculosis* infection cannot be determined

a. Results for the T-SPOT.TB test are interpreted by subtracting the spot count in the "Nil control" well from the spot count in each of the Panels, according to the package insert.

b. The inclusion of a borderline category is intended to reduce the likelihood of false-positive and false-negative results around the test cut-off. Note: It is recommended that borderline and invalid results be retested with a new specimen.

c. Quest Diagnostics[8]. Note: Repeat testing by collecting another specimen is recommended for invalid results.

d. The T-SPOT.TB assay does not include an interpretation in the package insert. Interpretations were developed by APHL to align with those used by the QFT-Plus Assay.

In addition to the reporting and interpretation information provided by the manufacturer, other resources exist such as the Online TST/IGRA Interpreter, which can be helpful for clinical providers to gain a better understanding of the result in the context of patient specific information and potential risk factors.[8]

References and Resources

1. QIAGEN GmbH. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert (1083163 Rev. 04) [Internet]. 2017 [cited 2021 Apr 21]. Available from: http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf

2. DiaSorin, Inc. LIAISON® QuantiFERON®-TB Gold Plus ([REF] 311020) [Internet]. 2019 [cited 2021 Apr 21]. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf18/P180047D.pdf

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5. US Centers for Disease Control and Prevention. Interferon-Gamma Release Assays (IGRAs) – Blood Tests for TB Infection [Internet]. 2011. Available from: https://www.cdc.gov/tb/publications/factsheets/testing/IGRA.pdf

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8. Quest Diagnostics. Test FAQs-T-SPOT®.TB [Internet]. Quest Diagnostics Education Center. [cited 2021 Apr 21]. Available from: https://education.questdiagnostics.com/faq/FAQ215

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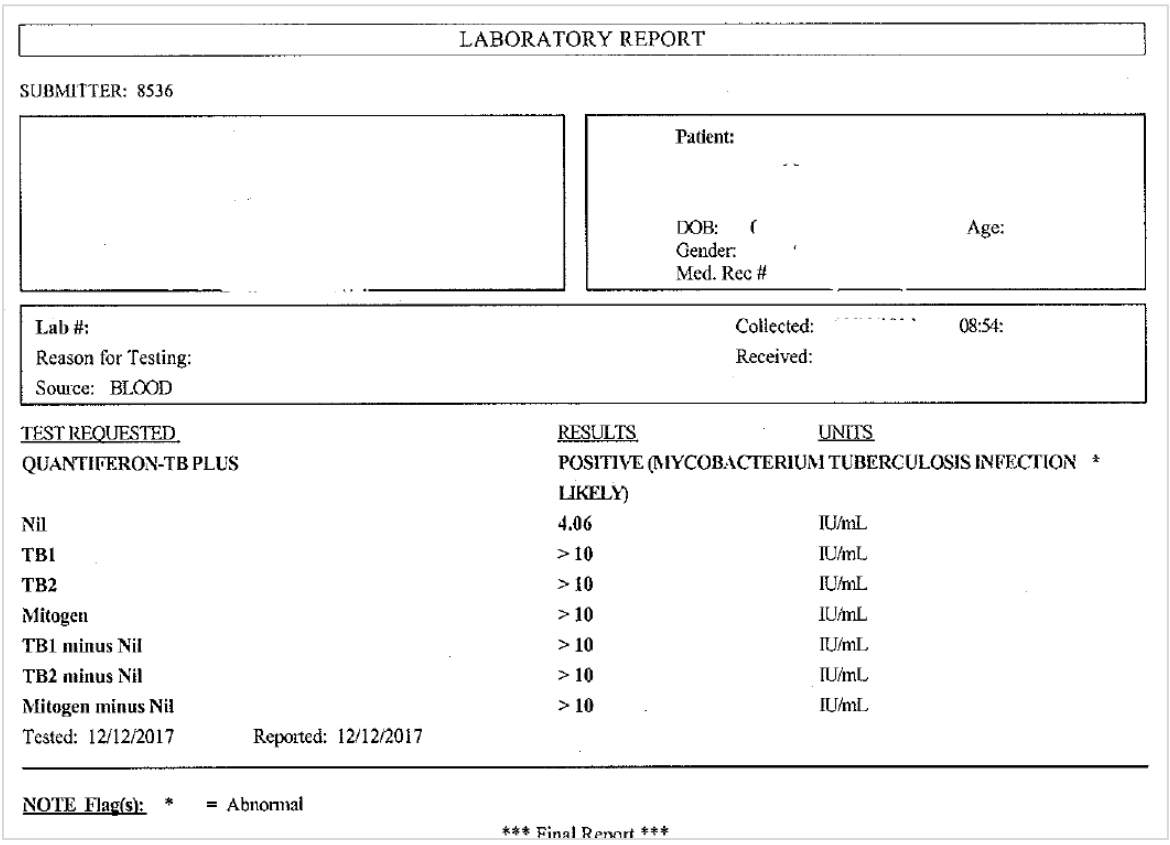
Appendix A: Example Reports (QFT-Plus)

Positive Result 1



LABORATORY REPORT

SUBMITTER: 8536

Patient:

DOB: Age:
Gender:
Med. Rec #

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

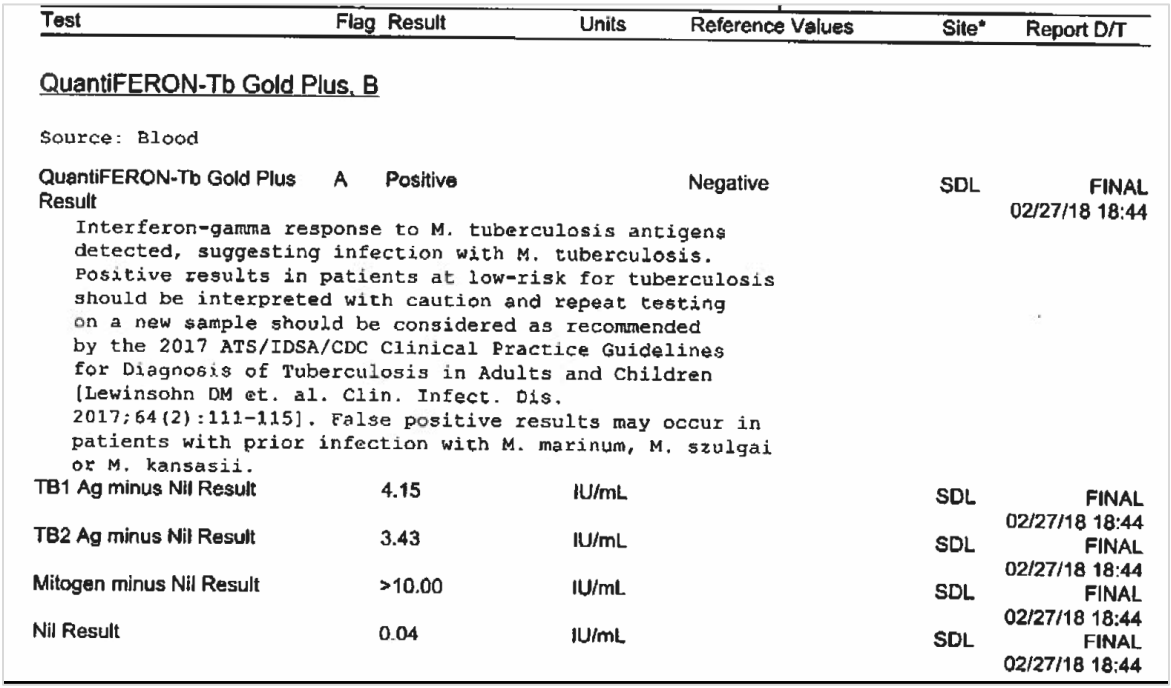
TEST REQUESTED	RESULTS	UNITS
QUANTIFERON-TB PLUS	POSITIVE (MYCOBACTERIUM TUBERCULOSIS INFECTION LIKELY) *	
Nil	4.06	IU/mL
TB1	> 10	IU/mL
TB2	> 10	IU/mL
Mitogen	> 10	IU/mL
TB1 minus Nil	> 10	IU/mL
TB2 minus Nil	> 10	IU/mL
Mitogen minus Nil	> 10	IU/mL

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 ***

Example Report (T-SPOT.TB)

oxford DIAGNOSTIC LABORATORIES®
A TRADING DIVISION OF OXFORD IMMUNOTEC LTD

Oxford Diagnostic Laboratories
115B Innovation Drive
Milton Park
Abingdon
Oxfordshire
OX14 4RZ

T-SPOT®.*TB* Test Results Report

ODL® ID : 991500001
Sample ID : *Example*

00/00/00

Collection Date : 19/02/2015
Receiving Date : 20/02/2015

Requesting Laboratory : ZZZZ01 Validation Location 1
Clinician : ZZV901 Stan ZZSmith

T-SPOT.*TB* Test Results

Approved By : KLO Date: 24/02/2015

Assay Result Negative

A test is Negative if both antigen panels minus the nil control is less than or equal to 5 spots. A Negative test indicates that Tuberculosis infection is unlikely.

Nil (Neg) Control Spot Count	0
Panel A Spot Count	0
Panel B Spot Count	0
Positive Control Spot Count	>20

Interpretation of Results
- The results should be used and interpreted in the context of the overall clinical picture
- A full breakdown of interpretation and Quality Control of the test is given at http://www.oxfordimmunotec.com
- A test is positive if either antigen panel minus the nil control is greater than or equal to 6 spots. A positive test indicates that Tuberculosis infection is likely
- A test is negative if both antigen panels minus the nil control are less than or equal to 5 spots. A negative test indicates that Tuberculosis infection is unlikely
- A test is borderline for spot counts around the cutoff (5, 6 or 7 spots) and a retest is recommended. This zone was created as a quality assurance to allow for the variability that can occur with every laboratory test and may be due to potential biological variation
- A test is indeterminate if the Nil Control spot count is in excess of 10 spots. Another sample should be collected from the individual and tested
- A test is also considered indeterminate where the Positive Control spot count is <20 spots, unless either Panel A or Panel B is 'Positive' as described in the Results Interpretation and Assay Criteria, in which case the result is valid

UKAS 4066

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MASTER DOCUMENT

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Appendix B: LOINC Codes for QuantiFERON-TB Gold Plus and T-SPOT.TB

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 and T-SPOT.TB assay.

LOINC Codes for 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 release by CD4+ T-cells [Units/volume] in Blood
64084-7	Mycobacterium tuberculosis stimulated gamma interferon release by CD4+ T-cells [Units/volume] corrected for background in Blood
88518-6	Mycobacterium tuberculosis stimulated gamma interferon release by CD4+ and CD8+ T-cells [Units/volume] in Blood
88517-8	Mycobacterium tuberculosis stimulated gamma interferon release by CD4+ and CD8+ T-cells [Units/volume] corrected for background in Blood
71773-6	Mycobacterium tuberculosis stimulated gamma interferon [Interpretation] in Blood Qualitative

LOINC Codes for T-SPOT.TB

LOINC #	LOINC Name
74281-7	*Mycobacterium tuberculosis* stimulated gamma interferon and spot count panel - Blood
74280-9	Mitogen stimulated gamma interferon positive control spot count [#] in Blood
74279-1	Gamma interferon negative control spot count [#] in Blood
74278-3	Mycobacterium tuberculosis stimulated gamma interferon ESAT-6 Ag spot count [#] in Blood
74277-5	Mycobacterium tuberculosis stimulated gamma interferon CFP10 Ag spot count [#] in Blood
71773-6	Mycobacterium tuberculosis stimulated gamma interferon [Interpretation] in Blood Qualitative