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QuantiFERON®-TB Gold Plus Analysis Software (v2.71) Instructional Guide

For installation, setup and use of the QuantiFERON-TB Gold Plus Analysis Software

IVD For in vitro diagnostic use

CE

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Sample to Insight

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Contents

1 Introduction

This guide contains all the information required to download QuantiFERON-TB Gold Plus (QFT®-Plus) Analysis Software, Version 2.71. QuantiFERON-TB Gold Plus Analysis Software is a PC-based program for calculating QuantiFERON-TB Gold Plus (QFT-Plus) test results. The software may be downloaded from the www.QuantiFERON.com website. Alternatively, contact your authorized QuantiFERON distributor to obtain a copy via email.

Customers will be advised by QIAGEN or their QuantiFERON distributor as new editions of the software are made available.

This guide provides detailed step-by-step instructions on the use of QuantiFERON-TB Gold Plus Analysis Software. It is recommended that you read these instructions before referring to the Software Quick Guide, available at www.QuantiFERON.com.

Table 1. Release information

Parameter	Version
QuantiFERON-TB Gold Plus Analysis Software Version	2.71(including all sub-versions)

1.1 General information

1.1.1 Technical assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding the QuantiFERON-TB Gold Plus Analysis Software or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance, contact QIAGEN Technical Services (see back cover).

For up-to-date information about QuantiFERON-TB Gold Plus, visit www.QuantiFERON.com.

1.1.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

1.2 Intended use of QuantiFERON-TB Gold Plus Analysis Software

QuantiFERON-TB Gold Plus Analysis Software is for optional use with the QuantiFERON-TB Gold Plus ELISA.

1.3 Requirements for QuantiFERON-TB Gold Plus Analysis Software

System requirements are shown in Table 2.

Table 2. System requirements

Description	Minimum requirement
Operating system	Microsoft® Windows® 7, 8 or 10
Processor	Intel® Pentium® processor, or equivalent 1-GHz processor or higher, dependent on operating system
Main memory	1 GB RAM or higher
Hard disk space	5 MB available hard disk space
Monitor	Minimum screen resolution set to 800 x 600 pixels, but higher resolution is recommended

1.4 Software specifications

QuantiFERON-TB Gold Plus Analysis Software, Version 2.71 (including all sub-versions).

2 Installation

2.1 Software installation from web site

The most recent version of QuantiFERON-TB Gold Plus Analysis software is available for download at www.QuantiFERON.com under Laboratory Resources. In order to download the software you must enter your contact information, read and accept the terms of the End User License Agreement, and submit. The download screen will then appear and the software zip file can be saved to an appropriate location on the computer's hard drive. First download the zip file from the website and save (e.g., "QFT_TBGoldPlus_v.2.71.2.exe") to a location on your hard drive. In addition, you may create a shortcut on your desktop. Start the software by double clicking on the file (e.g., "QFT_TBGoldPlus_v.2.71.2.exe") or shortcut. During the very first startup, the software will create a folder "QuantiFERON" and subfolders on your personal directory (e.g., "My Documents\QuantiFERON", depending on your computer's operating system).

3 Software Features

QuantiFERON-TB Gold Plus Analysis Software is a PC-based program for calculating QuantiFERON-TB Gold Plus (QFT-Plus) ELISA results.

Software features:

- Record test-related information
- Automatically import or manually enter raw data
- Highlight standards and samples to create an Analysis Format
- Save Analysis Format for use with future tests
- Assign subject's identity to each sample
- Obtain quality control analysis of standard curve
- Export data and results to other applications
- Select from an array of reporting options

4 Getting Started

4.1 Starting the QFT-Plus v2.71 software

Double-click on the "QFT-Plus v2.71 Software" shortcut, or directly on the *.exe file, to open the QuantiFERON-TB Gold Plus Analysis Software.

The program will open to the first of four screens that sequentially progress through the calculations. These four screens are:

- Run Details: Enter general test details such as the Run Date, Run Number, Kit Batch Number, and Operator.
- Raw Data: Enter Optical Density (OD) values and apply a format that defines the standards and samples.
- Standards Results: View standard curve results, which indicate the validity of the ELISA.
- Subject Results: View test results for each sample. Save, print, and export data and results.

The four screens are described in more detail below and on the following pages.

4.2 Screens in the QFT-Plus v2.71 software

4.2.1 Run Details screen

First, enter the appropriate language using the drop-down menu. Click "OK" to advance to the next screen.

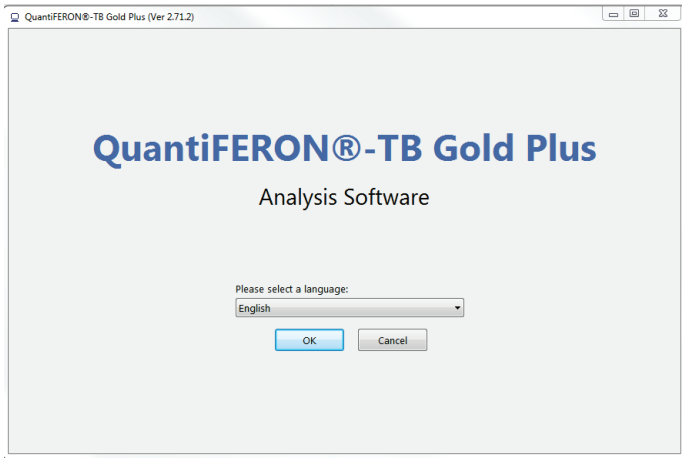

Figure 1. Language selection screen.

On the "Run Details" screen, enter the run date using the drop-down calendar, the kit batch number (shown on the QuantiFERON-TB Gold Plus ELISA outer box label), the Run Number and the Operator. Select the "Raw Data" tab or click the arrow in the lower right corner to advance to the next screen.

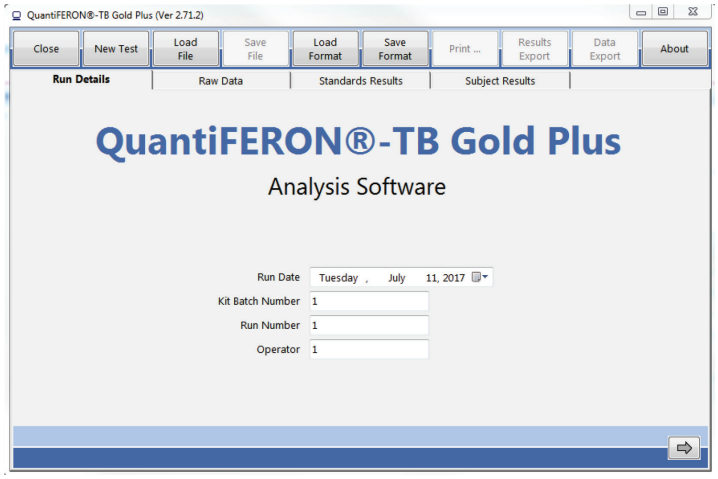

Figure 2. "Run details" screen.

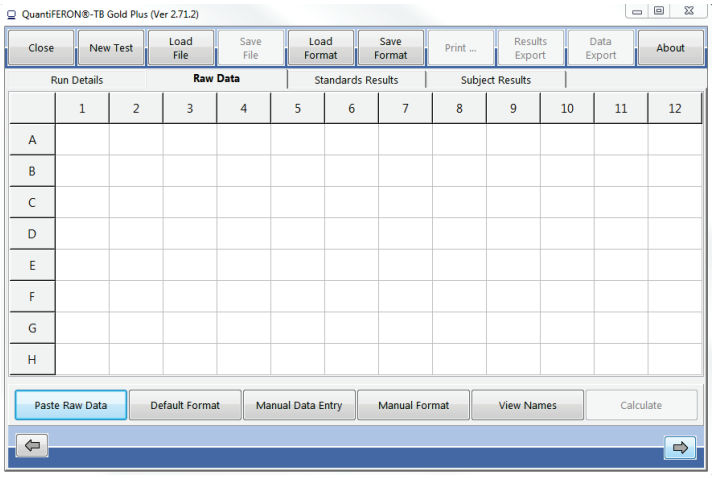

Figure 3. "Raw data" screen.

4.3 Data entry

QuantiFERON-TB Gold Plus Analysis Software uses optical density (OD) values as the basis for all calculations. The user does not need to perform any calculations prior to using the software, simply enter the raw data from the plate reader into the software.

There are two methods of data entry: automatic data entry and manual data entry.

4.3.1 Automatic data entry

Copy the raw data (OD values) to be analyzed from the ELISA plate reader program. Some plate reader programs require the data to first be exported into a spreadsheet.

Select "Paste Raw Data". The data will be entered into the program's data cells.

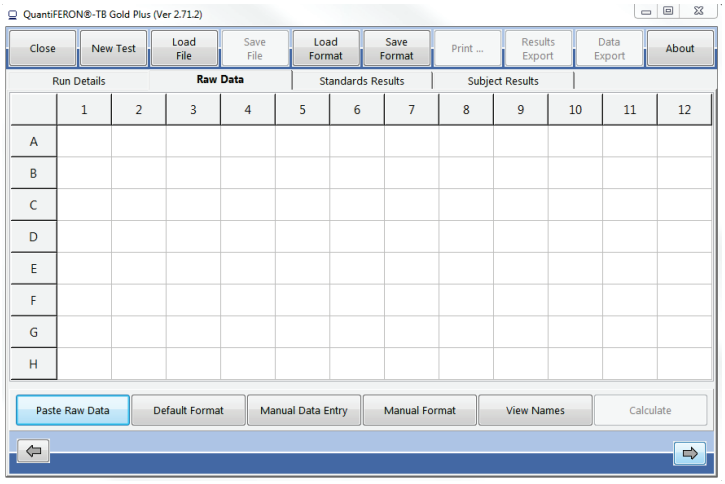

Figure 4. "Raw data" screen. 1 = "Paste Raw Data".

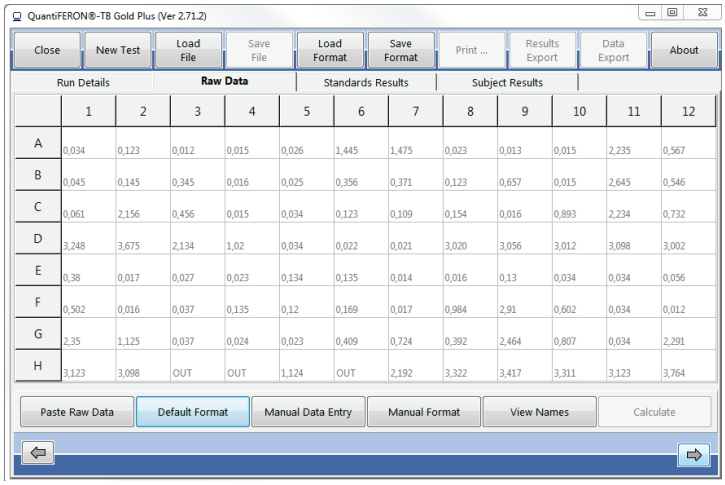

Figure 5. "Raw data" screen after pasting raw data. If a cell is missing data, the cell is denoted "N/S" (no sample) and takes no further part in the analysis. If a cell contains text, such as "***", "Out", "OVRFLW", etc.), the software interprets the OD value as being off-scale and the sample is given an OD value of 4.000 units.

Data from plates with less than 12 strips can be analyzed: however, each strip of data pasted must contain eight values (including empty cells, if necessary). Data cells for standards cannot be blank or contain text. If such a situation arises, the analysis software will report this as an Invalid ELISA.

Due to the logarithmic calculations performed by the software, negative OD values cannot be analyzed. Negative OD values are not normally obtained for the QuantiFERON-TB Gold Plus ELISA, and may indicate the need to service the plate reader.

4.3.2 Manual data entry

Select "Manual Data Entry". Click on a cell to enter data manually. To store the value, click "Enter". Alternatively, use the ↑ and ↓ arrows or the mouse to navigate to another cell or simply click on another cell.

When all data have been entered, click "Complete" on the "Manual Data Entry" toolbar to proceed.

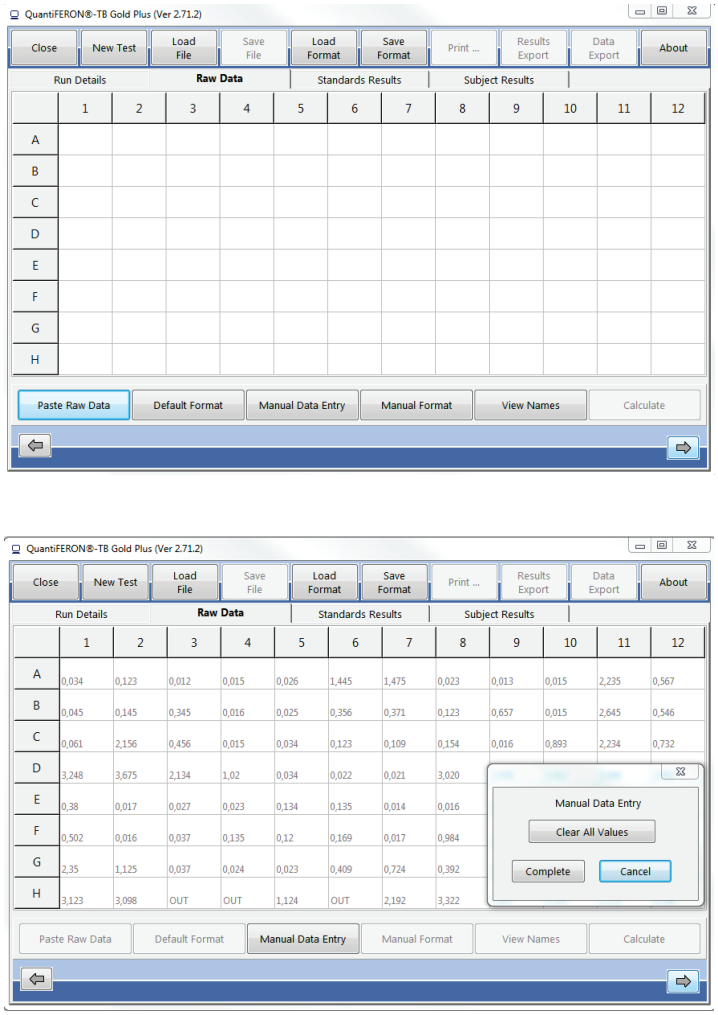

Figure 6. "Raw data" screen during manual data entry. 1 = Click "Complete" to end manual data entry. Note: Manual entry is more prone to errors, and the user should take additional care to ensure data entry accuracy.

Important: It is critical to compare the original raw OD data/format with data on the report, as errors in manual data entry (or copy/paste errors) can cause incorrect report results.

4.4 Analysis format

Before data can be analyzed, users must apply a format to nominate the cells that contain samples and those that are standards. There are two methods for assigning a format.

4.4.1 Default format

Select "Default Format" to automatically assign the relevant QuantiFERON-recommended testing layout to the data. The standards and samples will be set out in the same configuration as outlined in the *QuantiFERON-TB Gold Plus ELISA Package Insert*.

The format can be applied either before or after data entry. This allows formats to be prepared prior to obtaining the ELISA results. Depending on the number of strips of data entered, the "Default Format" option may or may not be available, due to the location/orientation of samples and standards for each QuantiFERON-TB Gold Plus ELISA method.

Figure 7. "Default Format" selection menu.

Figure 8. "Raw Data" screen after "Default Format" is applied.

Once "Default Format" has been applied, it can be edited by selecting "Manual Format" and following the instructions outlined below.

4.4.2 Manual format

The "Manual Formatting Toolbar" is used to manually assign both standards and subject samples to the data's format. By default, the toolbar opens in "Standards" mode with standards ready to be assigned in a vertical orientation. The settings can be changed by selecting the appropriate radio buttons.

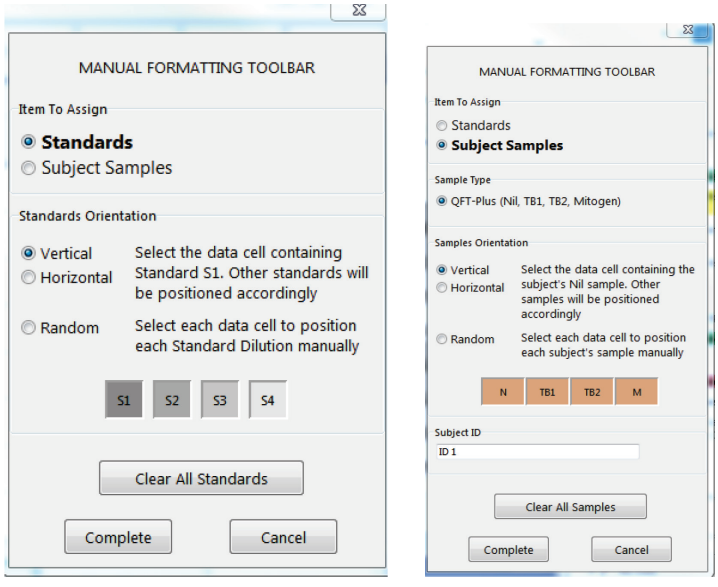

Figure 9. "Manual Formatting Toolbar" in "Standards" mode (left) and "Subject Samples" mode (right).

4.5 Standards

Standard S1 is the highest standard, containing 4.0 IU/ml of interferon-gamma (IFN-γ). Standard S4 is the lowest standard, containing 0 IU/ml of IFN-γ.

Once the set of standards, S1 to S4, has been assigned, the toolbar resets, ready to automatically assign another set of standards.

The standard orientation can be adjusted at any time, allowing replicates of standards to have different orientations in the one format.

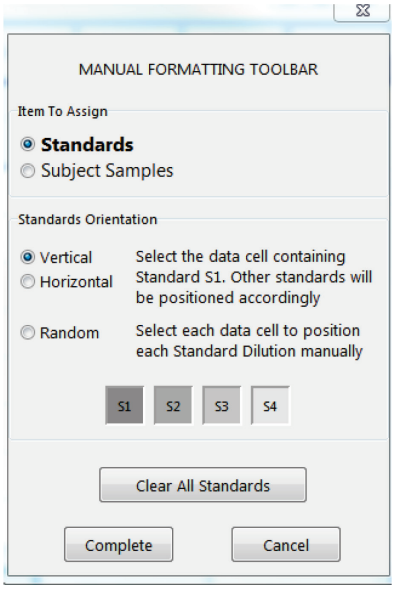

Figure 10. "Manual Formatting Toolbar" in "Standards" mode.

To assign a set of standards (S1, S2, S3, S4), within "Standards" mode, select the radio button that corresponds to your choice of either vertical or horizontal standards orientation, then click on the cell in the "Raw Data" screen that contains the data for standard S1. The chosen cell will be designated as S1, and the other standards will be appropriately positioned in adjacent cells in order.

To assign a set of standards in a random manner, select the "Random" radio button, and then manually position each of the standards S1 to S4, in order, by clicking on the appropriate cells within the "Raw Data" screen.

To delete a single set of standards, right-click on the colored block and select "Delete Block" from the pop-up menu. Alternatively, to delete all standards, select "Clear All Standards" on the "Manual Formatting Toolbar".

4.6 Subject (patient) samples

To assign subject samples to the data, select "Subject Samples" on the "Manual Formatting Toolbar".

To assign subject samples (either vertically or horizontally) select the corresponding radio button in the "Samples Orientation" section, then click click on the cell that contains the data for the subject's Nil sample. The chosen cell will be designated as "Nil", and the other samples will be appropriately positioned in adjacent cells, in order.

To assign subject samples in a random manner, each of the samples must be positioned manually by clicking on the appropriate cells.

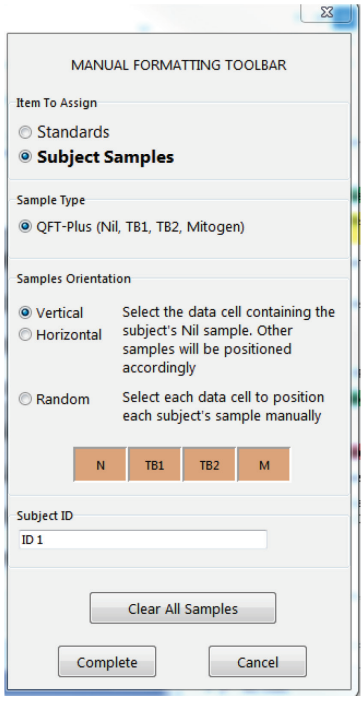

Figure 11. "Manual Formatting Toolbar" in "Subject samples" mode.

Prior to assigning a sample to the data, the subject's name/ID can be entered into the "Subject ID" field on the toolbar. Alternatively, subject naming can be performed according to "Raw Data" screen: Subject names" on page 16.

To delete a single subject sample, right-click on the colored block and select "Delete Block" from the pop-up menu. Alternatively, to delete all subject samples, select "Clear All Samples" on the "Manual Formatting Toolbar".

Once the standards and subject samples have been assigned, finish by selecting "Complete". Upon completing a format, it can be saved as a file and reloaded for analysis of future data, allowing the user to create just a few format files for all of their analysis needs.

See "Saving and Loading Files" on page 23 for more information.

By default, "Subject Sample" mode opens with Nil, TB1, TB2 and Mitogen samples ready to be assigned in a vertical orientation. Settings can be changed by selecting the appropriate radio (round) buttons.

Once the entire subject sample has been assigned, the toolbar is automatically ready to assign another sample of the same type. Subsequent subject samples are colored differently to assist recognition of individual subjects.

The "Sample Type" and "Sample Orientation" can be adjusted at any time in order to create a format containing a mixture of different QuantiFERON-TB Gold Plus sample layouts.

To delete all standards and subject samples, right-click on any colored block and select "Clear Format" from the menu.

Non-format information, such as run details and subject (patient) names, is not retained as part of the saved format file. These details are, however, retained as part of all saved result files.

4.7 "Raw Data" screen: Subject names

Subject names can be up to 15 characters in length. For this reason, they are not displayed on the "Raw Data" screen. Instead, the stored subject names can be viewed via "View Names".

Subject names can be changed at any stage by left-clicking on the colored block for each subject and typing the new name in the "Change Subject ID" dialog box that appears.

To change multiple subject names (IDs), select "View Names". If all subject names are to begin with an identical prefix (e.g., A009), then these characters can be entered into the "ID Prefix" field. Afterward, left-click on each subject's name in the list to add the remainder of the name manually.

To assign subject samples in a random manner, each of the samples must be positioned manually by clicking on the appropriate cells.

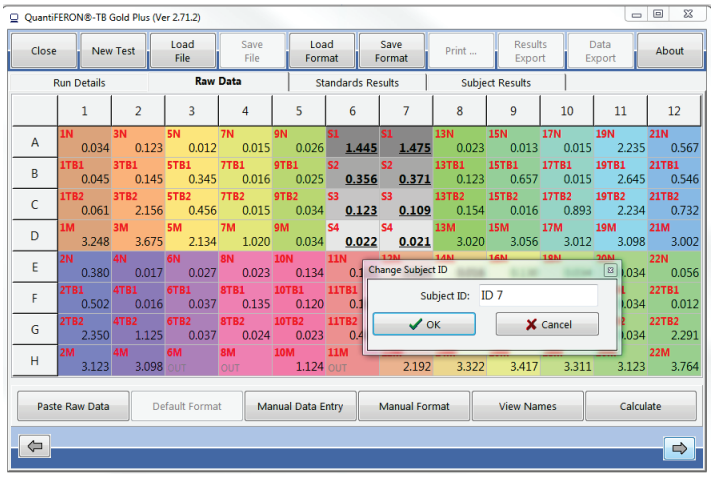

Figure 12. Renaming subject samples using "View Names".

Once the format has been generated, select "Calculate". The standard curve for the assay will be automatically analyzed, and the "Standards Results" screen will be displayed. For the "Calculate" function to be enabled, at least two blocks of Standards and one Subject Samples block must be assigned.

4.8 "Standards Results" screen: Quality control of standard curve

The accuracy of test results is dependent on the accuracy of the standard curve. The software automatically performs quality control (QC) analysis of the standard curve prior to interpreting test sample results.

The "Standards Results" screen provides information that is directly related to the acceptance criteria of the ELISA:

- Mean of the replicate standards
- Coefficient of variation (%CV) of the replicate standards
- Correlation coefficient of OD values and known IFN-γ concentrations (Conc)

The results of the QC acceptance criteria for the Standard Curve are shown as PASS or FAIL. For further details of the acceptance criteria, see the *QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert*.

The following information is also displayed:

- A graph of the Standard Curve, including linear regression line
- Intercept and slope of the linear regression

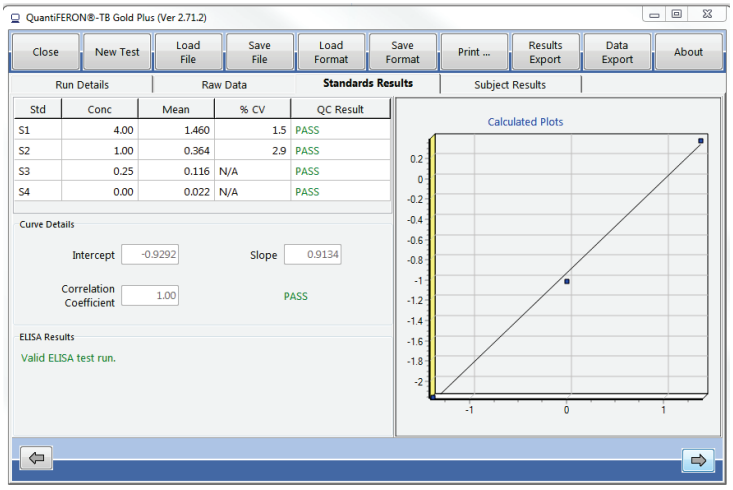

Figure 13. "Standards Results" screen.

A statement indicating whether the ELISA is "Valid" or "Invalid", based on the QC criteria, is provided in the "ELISA Results" section (bottom left corner of the screen). This statement is also displayed on all printed and PDF reports.

- If any of the QC criteria are not met, the ELISA test run is "Invalid" and MUST be repeated.
- In the event that the Mean value of the zero standard (zero IFN-γ) is greater than 0.150 OD units, a statement is displayed suggesting that ELISA plate washing procedures be investigated. This statement is also displayed on all printed and PDF reports.

Select the "Subject Results" tab to proceed to the next screen.

4.9 Standard curve

The standard curve is used to calculate a value (IU/ml of IFN-γ) for each patient's samples. The software multiplies the value of the plasma sample calculated from the standard curve by the dilution factor assigned at the sample formatting step; based on these values, the result (concentration of IFN-γ) for each patient is reported.

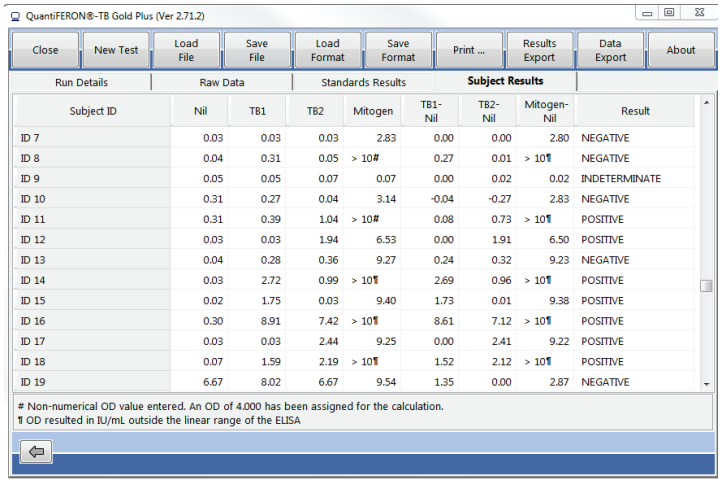

Figure 14. "Subject Results" screen. ¶ = Sample result is outside the linear range of the assay.

In the unlikely event that a patient's result is reported as positive and their Mitogen minus Nil result is less than 0.5 IU/ml, the software will flag the result as a possible sample mix-up using the "*" symbol. This warning helps to limit the possibility of a false-positive result due to a mix-up of the TB antigen and Mitogen samples.

The result "Data Missing" is reported if any of a patient's plasma samples display the value N/S (No Sample).

Samples that have results beyond the linear range of the assay are reported as ">10 IU/ml" and are flagged using the "¶" or "#" symbols. "¶" indicates that the result is outside the linear range of the assay. "#" indicates that a value outside the plate reader range was used to determine the result — non-numerical characters include "OUT" or "***". In the case of non-numerical entries, an OD of 4.000 is used to calculate the IU/ml result.

For further information regarding the calculation of QuantiFERON-TB Gold Plus ELISA results, see the *QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert.*

5 Data Export

If desired by the user, the user can export results and/or data via Windows Clipboard or structured text file to external spreadsheet applications, such as Microsoft Excel® software.

To export results, select "Results Export". An "Export Type" pop-up window appears with the two options for file export "Export to Clipboard" (default) and "Export to File". If "Export to Clipboard" is selected by clicking "OK", a "Results Copied to Windows Clipboard" pop-up window appears. After clicking "OK" on this pop-up window, data can be pasted into a spreadsheet.

Alternatively, if the "Export to File" option is manually selecting by clicking "OK" in the "Export Type" window, another pop-up window appears, allowing you to save the results as a file on your computer. After clicking "Save" on this pop-up window, data are saved as a text file.

Similarly, selecting "Data Export" offers you the choice of exporting the assay details, raw data and QC results to either the Windows Clipboard or a text file. The process for exporting data using "Data Export" is the same as that described above for "Results Export".

Note: The optional step of exporting data is not required to obtain QuantiFERON-TB Gold Plus results. It may be employed by the user for pooling and trending data. Take care when pasting data into spreadsheet programs, due to the possibility of the spreadsheet's default formatting affecting the presentation of the data.

6 Reports

Selecting "Print" will display a print screen that is divided into two sections. The upper section displays the various printing options available, while the lower section displays a summary report of the ELISA details and results.

IMPORTANT: It is critical to compare the original raw OD data/format with data on the report, as errors in manual data entry or copy/paste errors can cause incorrect report results.

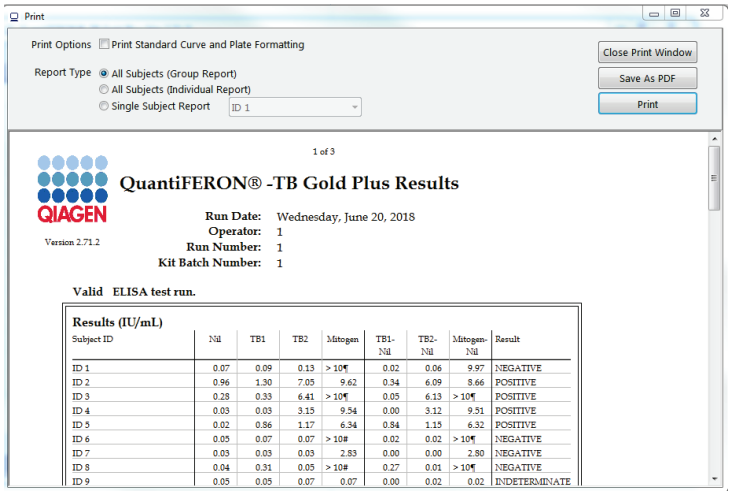

Figure 15. Summary report.

Select a "Report Type" radio button to print a particular report:

- "All Subjects (Group Report)" prints the results for all subjects on one page. The Raw OD values used to generate the Standard Curve are highlighted (bold and underlined) in this report.
- "All Subjects (Individual Report)" prints the results for each subject on a separate page.
- "Single Subject Report" prints the results for one subject, as selected from the drop-down box.

Select "Print Standard Curve and Plate Formatting" to generate an additional report page that includes the plate layout and standard curve.

Select the "Close Print Window" to close the printing screen and return to the main software.

Alternatively, reports can be saved as PDF files by selecting "Save As PDF", as described in "Saving and Loading Files", page 23.

Once the desired type of summary report is selected, click "Print" to print the report to the computer's default printer.

The upper range of the QuantiFERON-TB Gold Plus ELISA is 10 IU/ml. Therefore, samples determined to have an IFN-γ concentration greater than this range are reported as >10 IU/ml.

Although values above 10 IU/ml are reported as >10 IU/ml, the calculations for subtracting the Nil control value are based on the original value. Therefore it is possible for a patient's TB1, TB2 or Mitogen value to be reported as ">10 IU/ml", yet their "minus Nil" value be less than 10 IU/ml.

7 Saving and Loading Files

7.1 Saving files

Upon opening the QFT-Plus Analysis Software for the first time, the software creates a folder called "My Documents\QuantiFERON" or "Documents\QuantiFERON", depending on your Windows operating system. By default, all files are saved to subfolders within this folder, and are given default file names (Table 3).

Table 3. File names and extensions

File type	File extension	Sub-folder name	Default file name
Format	.qft	Format	OperatorDate
Results	.qdf	Save	Date_RunNumber
PDF results	.pdf	PDF	Date_RunNumber

File type and description:

- Format files: Select "Save Format" to save a completed format to file, which can be reloaded for use with future analysis.

  "Run Details" information is not retained within a saved format file.
- Results files: Select "Save File" to save a copy of the results to file, which can be reloaded for further analysis.

  Run Details information is retained within a saved result file.
- PDF files: Select "Save As PDF" to save the results report in PDF format, for electronic viewing by others. It is recommended that PDF files be used for record-keeping purposes.

  PDF files contain all of the information available in the printed report.

7.2 Loading files

- Format files can be reloaded within the QFT-Plus Analysis Software by selecting "Load Format".
- Results can be reloaded by selecting "Load File" at any time.
- After reloading a results file, "Calculate" must be selected in order to regenerate results.

8 End of Analysis

- The software allows the user to work on one run at a time (single session mode).
- Using the "New Test" function, the user can work on a second run without having to restart the software.
- Selecting "New Test" clears all entered information, enabling new assay data to be analyzed.
- Selecting "Close" will close the program.

For convenience, the information previously entered into the "Run Date", "Kit Batch Details" and "Operator" fields on the "Run Details" screen is retained as default until the software is closed. These details can be modified as required.

9 Frequently Asked Questions

Q. Why do I need to use the QuantiFERON-TB Gold Plus Analysis Software? Can I use my own spreadsheet to calculate results instead?

A. You can use your own spreadsheet to calculate QuantiFERON-TB Gold Plus test results. However, the calculations required to obtain the correct IFN-γ values are logarithm based. Therefore, it is essential that you follow the instructions in the "Calculations and Test Interpretation" section of the QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert.

The QuantiFERON-TB Gold Plus Analysis Software has already been validated to ensure that the quality control checks – and the results obtained – are accurate and reproducible. The QuantiFERON-TB Gold Plus Analysis Software also has the added flexibility of simple one-click formatting of standards and samples, allowing for the format to be easily updated as changes to your ELISA test layout arise.

Q. When a newer version of the software is available, should I uninstall the old version of the QuantiFERON-TB Gold Plus Analysis Software? How do I do this?

A. Yes, you should always uninstall obsolete versions of the software before installing the new software. The new version of the QFT-Plus software may contain changes to the test criteria; therefore, it is essential that only the current version of the software be available for use.

To uninstall the old software, simply locate the default QuantiFERON folder in the Start Menu (Start >QuantiFERON) and select "Uninstall".

Alternatively, locate and remove the software using Start > Control Panel > Add/Remove Programs.

Q. I would like to contact QIAGEN to discuss my data/results/technique. What information should I provide in order to obtain a prompt reply?

A. It is best to provide the QuantiFERON-TB Gold Plus Analysis Software results file (*.qdf) which by default is located in the folder "My Documents\QuantiFERON\Save". It is best to provide a detailed outline of your enquiry, kit lot number and any other information you feel is relevant.

Q. Why can't data cells for standards be blank or contain text?

A. Because the standard curve is used to derive QuantiFERON-TB Gold Plus ELISA results, blank values or text may reduce the quality of the standard curve.

Q. When I open the QuantiFERON-TB Gold Plus Analysis Software, some of the text appears to be missing, as though it is covered by other text. What is the problem?

A. The computer's Display Settings may be incorrectly setup for the software. Make sure that the Display settings are set to "Default".

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Notes

Notes

QuantiFERON-TB Gold Plus Analysis Software is for use with QuantiFERON-TB Gold Plus ELISA and Blood Tubes. For comprehensive instructions for use, please refer to the *QuantiFERON-TB Gold Plus ELISA Package Insert*, available in up to 27 different languages, at www.QuantiFERON.com.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.