QFT™
QuantiFERON-TB Gold

QuantiFERON®-TB Gold Analysis Software (v2.62*) Instructional Guide

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

The software may be downloaded from the Cellestis website. Alternatively, contact your authorised QuantiFERON distributor to obtain a copy via email or CD-ROM.

Customers will be advised by Cellestis 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 Analysis Software. It is recommended that you read these instructions before referring to the Software Quick Guide, available at www.cellestis.com

CONTENTS

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.
- Quality Control analysis of Standard Curve.
- Export data and results to other applications.
- Selection of reporting options.

Features addressed in this version—

- Correction of file loading error.

*For non-USA use only

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Installation

FROM WEBSITE/EMAIL

- **Save** the **QFT_v2.62_setup.zip** file to an appropriate location on the computer's hard drive.

Alternatively, from the **Start Menu** select **Run** and then **Browse** to locate the Analysis Software .zip file. Ensure that **All Files** is chosen in the **Files of Type** field, then select **OK**.

If not currently installed, the program Winzip can be obtained from the website: **www.winzip.com**

- Using **My Computer**, locate the QFT_v2.62_setup.zip file and double-click on it. This will open the program Winzip®, which can be used to unzip the QFT Analysis Software **installation files**.

- Unzip the installation files to an appropriate location on the computer's hard drive—for example, the **temp** folder.

- Using My Computer, locate and run the file **QuantiFERON_Startup.exe**. An installation screen will appear, as described in the following section.

FROM SELF-INSTALLING CD-ROM

- Insert the QuantiFERON-TB Gold Analysis Software CD-ROM into the CD-ROM drive. An installation screen will automatically be displayed.

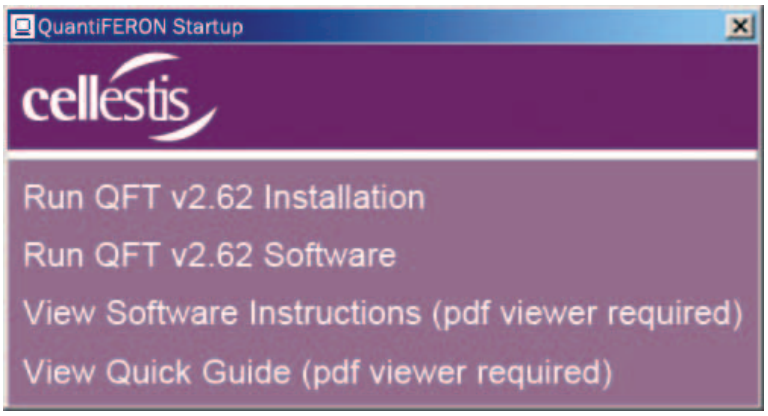


Figure 1 Installation screen.

The software and support files can also be accessed directly from the **CD-ROM** by selecting the other options on the installation screen.

- Select the option **Run QFT v2.62 Installation**. Follow the prompts to install the software and support files. A folder **Program Files\ QuantiFERON** is created for this purpose.

  Shortcuts to the Analysis Software are created on the desktop and in the **Start Menu.**

Getting Started

- Click on the **QFT v2.62 Software** shortcut to open the QuantiFERON-TB Gold Analysis Software.

  The program will open to the first of 4 screens that sequentially progress through the calculations. These 4 screens are

1. **Run Details**
   Enter general test details such as the **Run date**, **Run number**, **Kit batch number** and **Operator**.
2. **Raw Data**
   Enter Optical Density (OD) values and apply a format that defines the standards and samples.
3. **Standards Results**
   View Standard Curve results, which indicate the validity of the ELISA.
4. **Subject Results**
   View test results for each sample. Save, print and export data and results.

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

RUN DETAILS screen

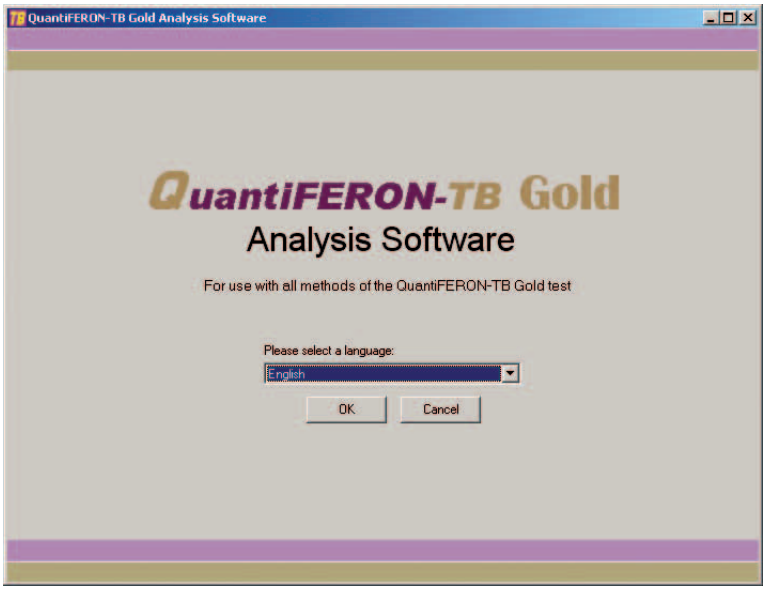


Figure 2 Language Selection screen.

- Select appropriate language.

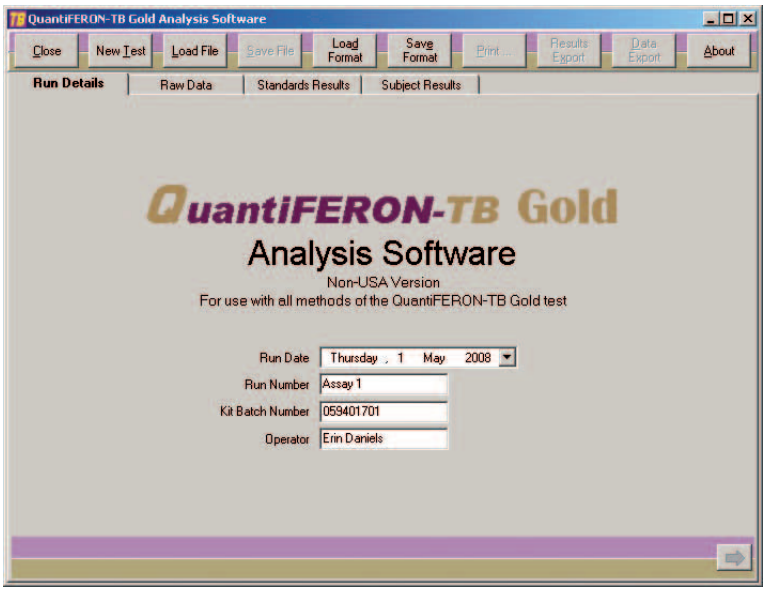


Figure 3 Run Details screen.

- Enter the following information in the fields provided:
  - Run Date (drop-down calendar)
  - Run Number
  - Kit Batch Number (shown on QuantiFERON ELISA box)
  - Operator

- Select the **Raw Data** tab to advance to the next screen.

RAW DATA screen

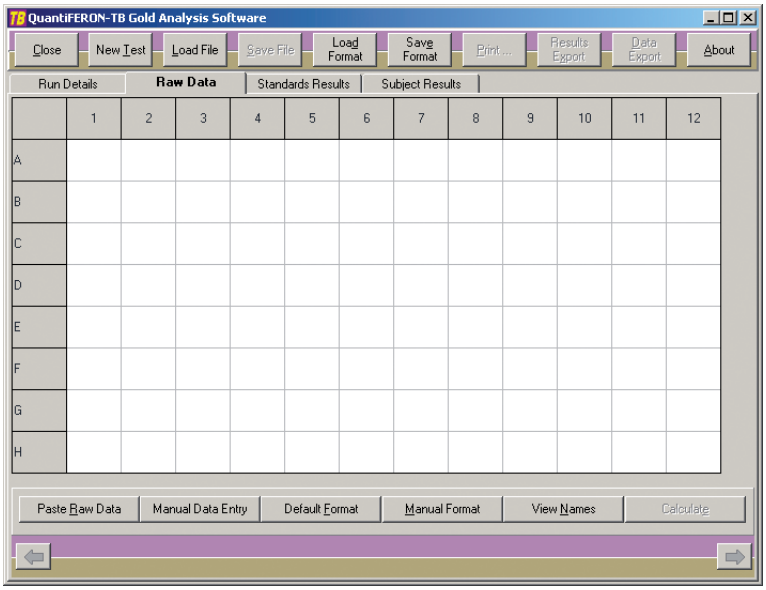


Figure 4 Raw Data screen.

DATA ENTRY

The QuantiFERON-TB Gold 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

1. Automatic Data Entry

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

- Select the **Paste Raw Data** button—the data will be entered into the program's data cells.

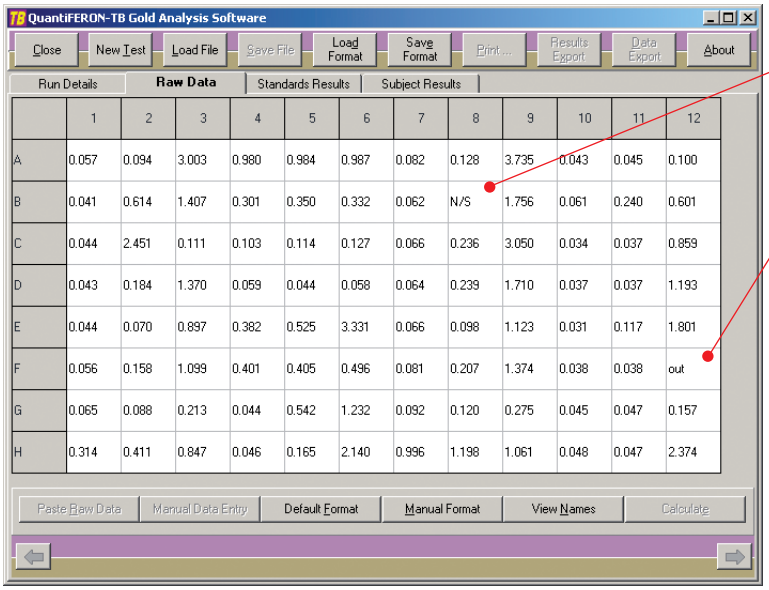


Figure 5 Raw Data screen after pasting raw data.

Data from plates with less than 12 strips can be analysed, however each strip of data pasted must contain 8 values (including empty cells, if necessary).

If for any reason data is **absent** from a cell, the cell is denoted by **N/S** (No Sample) and takes no further part in the analysis.

If a cell contains **text** (*****, out,** etc), the software interprets the OD value as being off-scale and the sample is given a final calculated value of **>10 IU/mL**.

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 analysed. Negative OD values are not normally obtained for the QuantiFERON ELISA, and may indicate the need to service the plate reader.

Use ↑/↓ arrows—or the mouse—to navigate between cells.

RAW DATA screen

2. Manual Data Entry

- Select the **Manual Data Entry** button. Click on a cell to enter data manually, to three decimal places. Press **Enter**—or click on another cell—to store the value.

- When all data has been entered, select the **Complete** button on the **Manual Data Entry toolbar** to proceed.

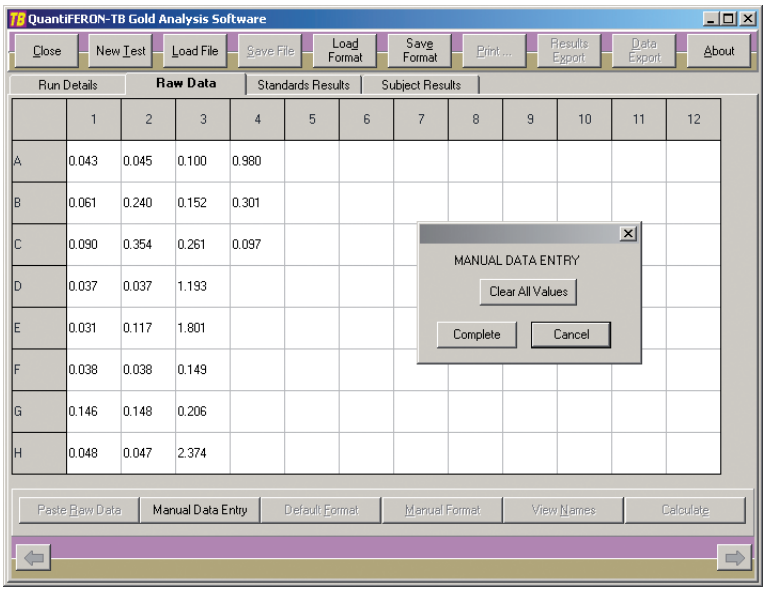


Figure 6 Raw Data screen while manually entering raw data.

RAW DATA screen

ANALYSIS FORMAT

Before data can be analysed, a **format** must be applied to nominate which cells are **samples** and which are **standards**.

- There are **two** methods for assigning a format

1. Default Format

- Select the **Default Format** button to automatically assign the relevant *Cellestis-recommended* testing layout to the data. The standards and samples will be set out in the same configuration as outlined in the relevant QFT Package Insert.

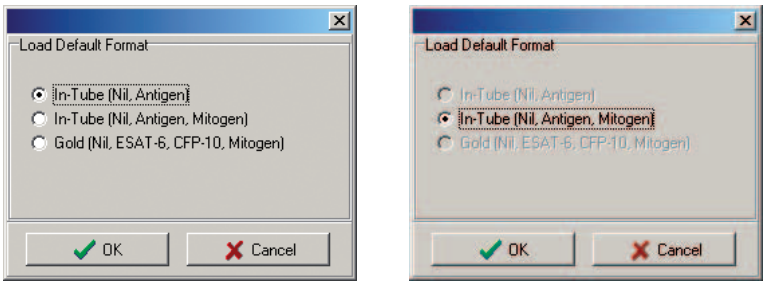


Figure 7 Load Default Format selection menu.

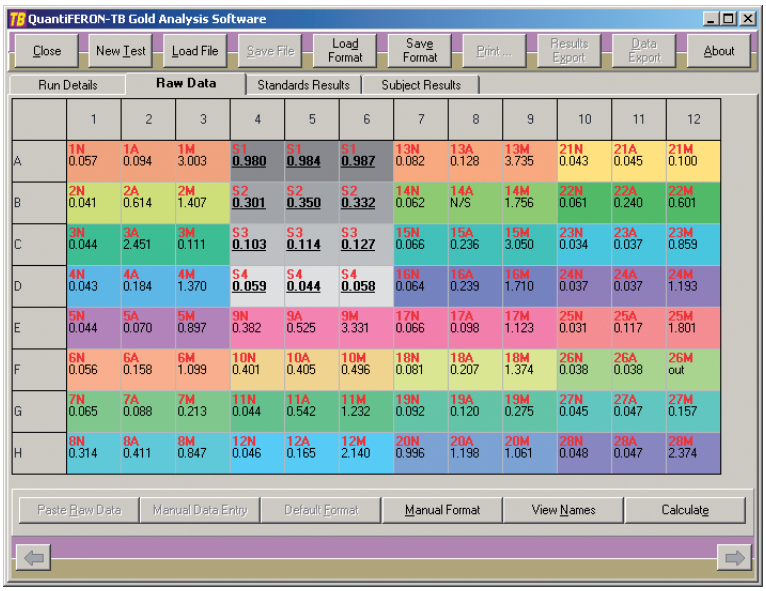


Figure 8 Raw Data screen after Default Format has been applied.

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 QFT test method (Gold, In-Tube).

The following table indicates the number of strips of data required for the Default Format option to be available.

QuantiFERON-TB Gold test method	Number of strips required
In-Tube (Nil, Antigen)	7+
In-Tube (Nil, Antigen, Mitogen)	6, 9, 12
Gold (Nil, ESAT-6, CFP-10, Mitogen)	7+

If the Default Format option is not available, the format can be applied manually.

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

By **default**, the toolbar opens in **Standards** mode with standards ready to be assigned in a **vertical** orientation. These settings can be changed by selecting the appropriate radio buttons.

RAW DATA screen

2. Manual Format

- Select the **Manual Format** button to open the Manual Formatting Toolbar. This toolbar is used to manually assign both standards and subject samples to the data's format.

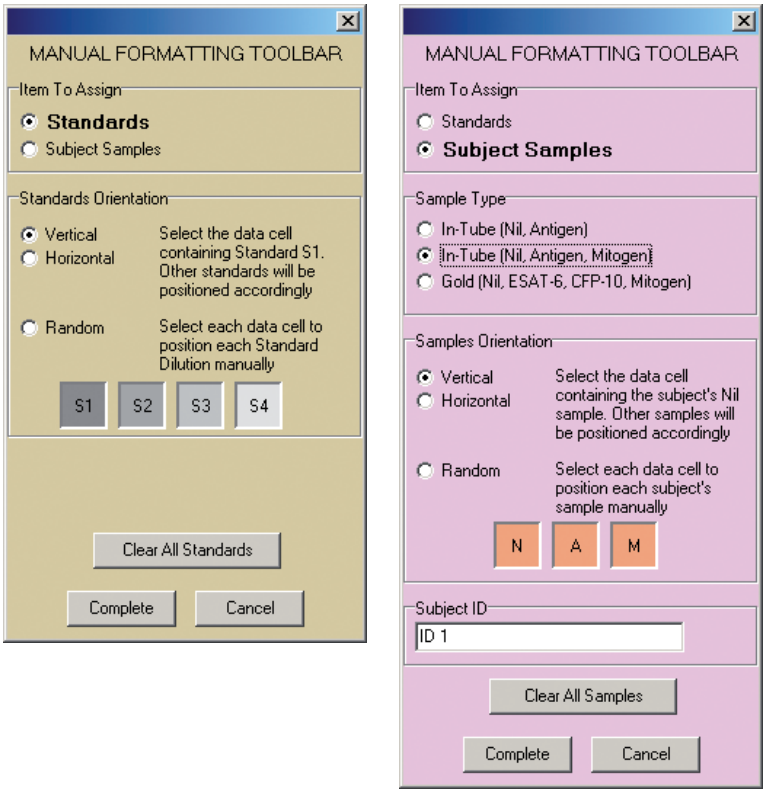


Figure 9 Manual Formatting Toolbar, in 'Standards mode' and 'Subject Samples mode'.

RAW DATA screen

Standards

- To assign a set of **standards** (S1, S2, S3, S4)—either **vertically** or **horizontally**—click on the cell 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, each of the standards S1 to S4 must be positioned manually by clicking on the appropriate cells, in order.

Standard S1 is the highest standard, containing 4 IU/mL of IFN-γ. Standard S4 is the lowest standard, containing 0 IU/mL of IFN-γ.

Once the entire 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.

- To **delete** a single set of standards, right-click on the coloured block and select **Delete Block** from the menu.

  Alternatively, to delete **all** standards, select the **Clear All Standards** button on the Manual Formatting Toolbar.

Subject Samples

- In order to assign **subject samples** to the data, select the **Subject Samples radio button** on the Manual Formatting Toolbar.

By default, **Subject Sample** mode opens with **Nil, Antigen and Mitogen** samples ready to be assigned in a **horizontal** orientation. Settings can be changed by selecting the appropriate radio buttons.

- To assign subject samples—either **vertically** or **horizontally**—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.

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 coloured differently in order 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 sample types.

- 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 the instructions in the next section.

- To **delete** a single subject sample, right-click on the coloured block and select **Delete Block** from the menu.

  Alternatively, to delete **all** subject samples, select the **Clear All Samples** button on the Manual Formatting Toolbar.

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

- Once the standards and subject samples have been applied, finish by selecting the **Complete** button.

  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.

  Refer to the **Saving/Loading Files** section for further details.

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

RAW DATA screen

SUBJECT NAMES

As subject names can be up to 15 characters in length, they are not displayed on the Raw Data screen. Instead the stored subject names can be viewed via the **View Names** button.

- Subject names can be changed at any stage by left-clicking on the coloured block for each subject and typing the new name in the pop-up box.

- Alternatively, **multiple** Subject Names (IDs) can be changed more easily by selecting the **View Names** button. If all subject names are to begin with an identical prefix (eg. A009) these characters can be entered into the **ID Prefix** field. Afterwards, click on each subject's name in the list to add the remainder of the name manually.

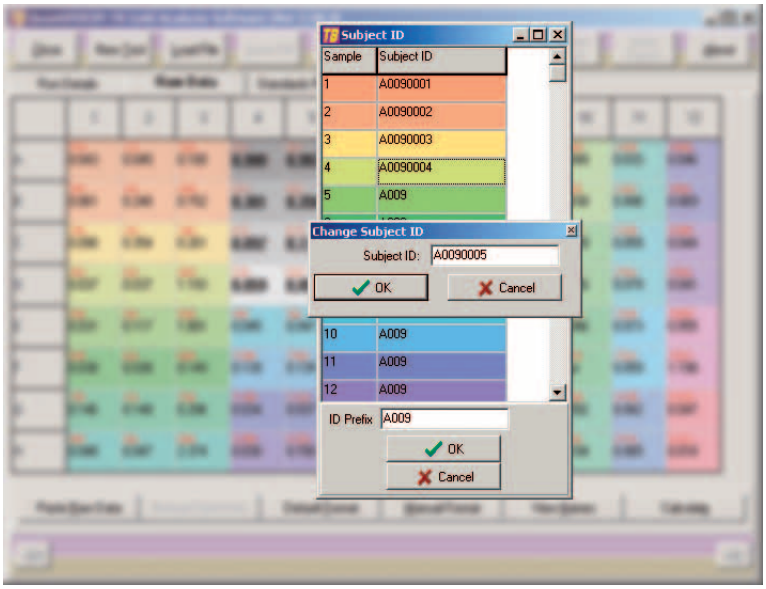


Figure 10 Renaming Subject Samples using the View Names button.

In order for the **Calculate** button to be enabled, *at least* **2 blocks of Standards** and **one Subject Sample** block must be assigned.

- Once the format has been generated, select the **Calculate** button. The standard curve for the assay will be automatically analysed and the **Standards Results** screen displayed.

STANDARDS RESULTS screen

The accuracy of test results is dependent on the accuracy of the standard curve. The software automatically performs Quality Control analysis of the standard curve prior to interpreting test sample results.

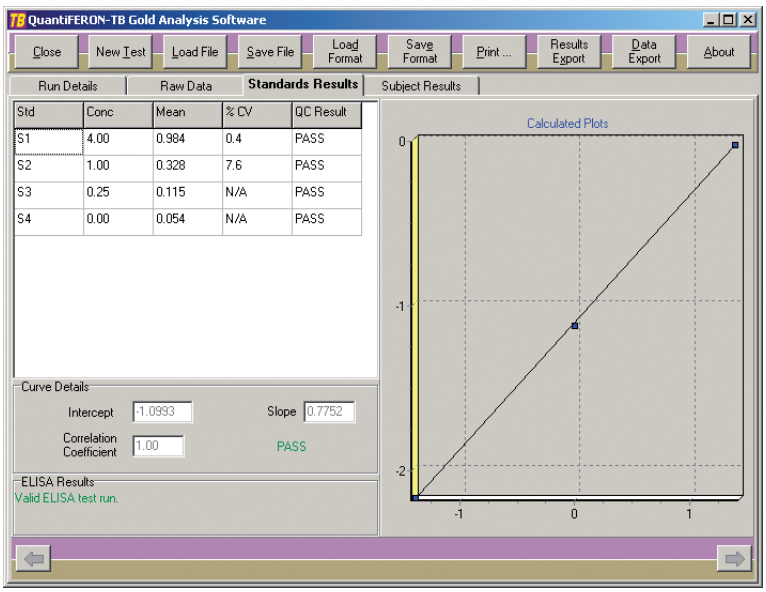


Figure 11 Standards Results screen.

- 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 of the replicate standards
  - Correlation Coefficient of OD values and known IFN-γ concentrations.

  The results of the Quality Control acceptance criteria for the Standard Curve are shown as **PASS** or **FAIL**.

For further details of the **acceptance criteria**, refer to the 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.

- A statement indicating whether the ELISA test is **Valid** or **Invalid**—based on the QC criteria—is shown at the bottom 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 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.

The Package Insert contains additional information regarding **high background** results.

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

SUBJECT RESULTS screen

Refer to the Package Insert for more information on the calculation of QuantiFERON-TB Gold Results.

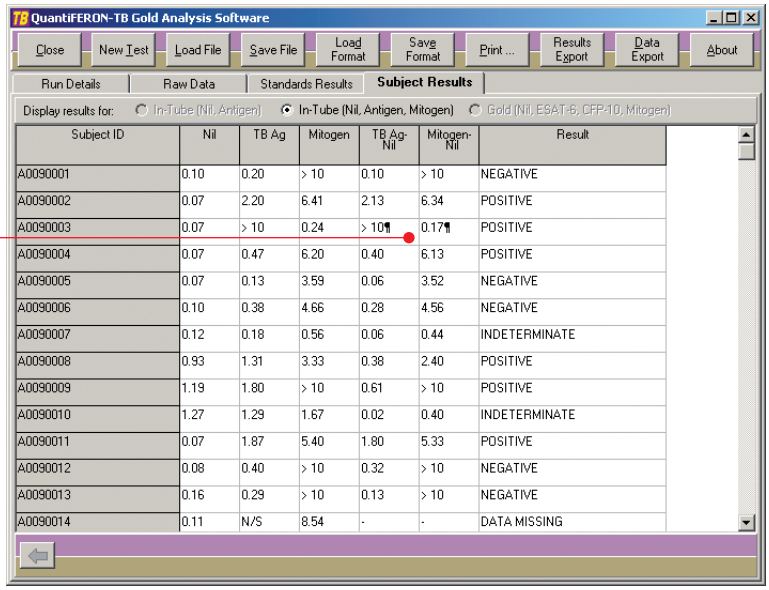
The Standard Curve is used to calculate a value (IU/mL of IFN-γ) for each subject's samples. The software **subtracts** the value of the **Nil** plasma sample from each of the other samples—based on these values, the **Result** for each Subject is reported.

- Subjects are grouped according to the QuantiFERON-TB Gold test method used for the samples.

  To view results for each QuantiFERON-TB Gold test method, select the relevant radio button. For example **In-Tube (Nil, Antigen)** in the example below..

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

In the unlikely event that a subject's result is reported as **positive** and their **Mitogen minus Nil result is less than 0.35 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.



Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
A0090001	0.10	0.20	> 10	0.10	> 10	NEGATIVE
A0090002	0.07	2.20	6.41	2.13	6.34	POSITIVE
A0090003	0.07	> 10	0.24	> 10¶	0.17¶	POSITIVE
A0090004	0.07	0.47	6.20	0.40	6.13	POSITIVE
A0090005	0.07	0.13	3.59	0.06	3.52	NEGATIVE
A0090006	0.10	0.38	4.66	0.28	4.56	NEGATIVE
A0090007	0.12	0.18	0.56	0.06	0.44	INDETERMINATE
A0090008	0.93	1.31	3.33	0.38	2.40	POSITIVE
A0090009	1.19	1.80	> 10	0.61	> 10	POSITIVE
A0090010	1.27	1.29	1.67	0.02	0.40	INDETERMINATE
A0090011	0.07	1.87	5.40	1.80	5.33	POSITIVE
A0090012	0.08	0.40	> 10	0.32	> 10	NEGATIVE
A0090013	0.16	0.29	> 10	0.13	> 10	NEGATIVE
A0090014	0.11	N/S	8.54	-	-	DATA MISSING

Figure 12 Subject Results screen.

DATA EXPORT

This optional step is not required to obtain QuantiFERON-TB Gold results. It may be employed by the user for the purpose of pooling and trending data.

Care should be taken when pasting data into spreadsheet programs, due to the possibility of the spreadsheet's default formatting affecting the presentation of the data.

- If desired by the user, the **results** and/or **data** can be exported to external applications, such as Microsoft Excel.

  To export the results, select the **Results Export** button. The user can then choose to export the **assay details and results** to either the Windows Clipboard or a text file.

  Similarly, selecting the **Data Export** button offers the user the choice of exporting the **assay details, raw data and QC results** to either the Windows Clipboard or a text file.

Reports

Selecting the Print button will display a printing 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.

- The **Sample Type** options allow the user to print separate reports for all, or some, of the QuantiFERON-TB Gold test methods used.

  To print results for a specific test method, select the appropriate check box. Alternatively, select **Print All Reports** to print a separate report for all of the QuantiFERON-TB Gold test methods used.

If multiple test methods are selected for printing, the lower screen will display **only one** of the reports.

- The **Report Type** options allow the user to print various reports as follows:

  1. **All Subjects (Group Report)** prints the results for **all** subjects on **one** page.
  2. **All Subjects (Individual Report)** prints the results for **each** subject on a **separate** page.
  3. **Single Subject Report** prints the results for **one subject**, as selected from the drop-down box.

On the **All Subjects (Grouped)** report, the Raw OD values used to generate the Standard Curve are **highlighted** (bold and underlined).

- The **Print Standard Curve and Plate Formatting** option generates an additional report page containing the original raw data, plate layout and standard curve.

- Alternatively, reports can be saved as **PDF files**, as described in the **Saving/Loading Files** section.

  Once the desired type of summary report is selected, clicking on the **Print** button will print the report to the computer's **default** printer.

  Selecting the **Close Print Window** button will close the printing screen and return to the main software.

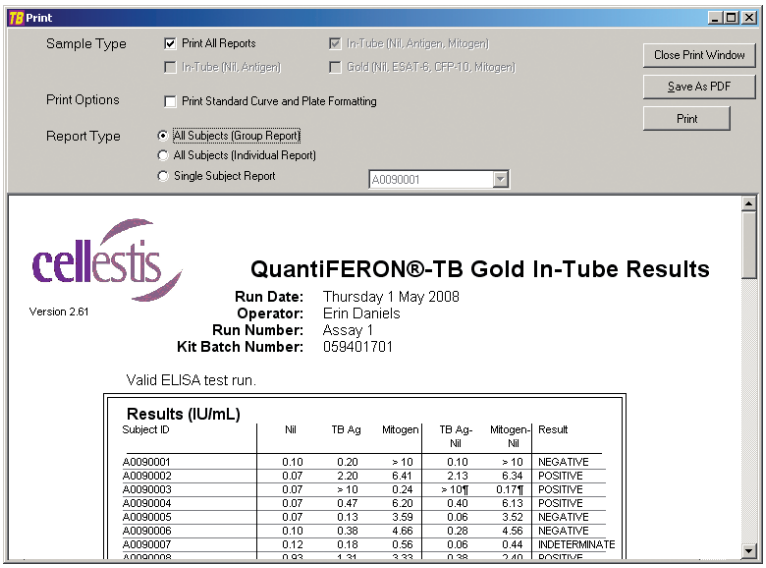


Figure 13 Summary report.

- The analytical range of the QuantiFERON-TB Gold ELISA is between zero and 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 subject's TB antigen or Mitogen value to be reported as ">10 IU/mL", yet their "minus Nil" value be **less than** 10 IU/mL.

Saving / Loading Files

SAVING FILES

- Upon opening the QFT Analysis Software for the first time, a folder called **My Documents\QuantiFERON** is created. By default, all files are saved to sub-folders within this folder, and are given default file names as per the following table:

File Type	File Extension	Sub-Folder Name	Default File Name
Format	.qff	Format	OperatorDate
Results	.qdf	Save	Date_RunNumber
PDF Results	.pdf	PDF	Date_RunNumber

Run Details information *is not* retained within a saved **format** file.

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

Run Details information *is* retained within a saved **result** file.

- **Results files.** Select the **Save File** button to save a copy of the Results to file, which can be reloaded for further analysis.

- **PDF files.** Select the **Save As PDF** button 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.

LOADING FILES

- **Format files** can be reloaded within the QFT Analysis Software by selecting the **Load Format** button.

After reloading a results file, the **Calculate** button must be selected in order to re-generate results.

- **Results files** can be reloaded by selecting the **Load File** button at any time.

END OF ANALYSIS

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.

- Selecting the **New Test** button clears all entered information, enabling new assay data to be analysed.

- Selecting the Close button will close the program.

Frequently Asked Questions

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

A. You can use your own spreadsheet to calculate QuantiFERON-TB Gold test results. However, the calculations required to obtain the correct IFN-γ values are logarithm based. Therefore, it is essential that your calculations, including Quality Control checks, are **validated** against the Analysis Software to ensure that the QuantiFERON-TB Gold test result obtained for each subject is correct.

The QFT software has already been rigorously validated to ensure that the Quality Control checks—and the results obtained—are accurate and reproducible.

The QFT 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 QFT 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 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 Cellestis 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 QFT software **results file** (.qdf) which by default is located in the folder My Documents\QuantiFERON\Save.

The easiest way to provide this information is via email, with 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 In-Tube results, blank values or text may reduce the quality of the standard curve.

Q. *When I open the 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 set up for the software. Under Start>Control Panel>Display>Settings>Advanced, make sure that the **Display DPI setting** is set to **Normal size (96 DPI)**.

Specifications

SOFTWARE SPECIFICATIONS

QuantiFERON-TB Gold Analysis Software

Version 2.62

Catalogue Number 05990026.

SYSTEM REQUIREMENTS

Intel® Pentium® processor, or equivalent

Microsoft® Windows® 98 or higher

16 MB RAM

5 MB available hard-disk space

Screen resolution set to 800 x 600 pixels, or higher.

Contact Information

For further information on QuantiFERON-TB Gold please contact

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Alternatively, contact your authorised QuantiFERON distributor.

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