

# BD ProbeTec™ CT/GC Q<sup>x</sup>

## Technical Bulletin



## Understanding the BD ProbeTec™ CT/GC Q<sup>x</sup> Amplified DNA Assays MaxRFU Score

### *What is MaxRFU?*

The MaxRFU (Maximum Relative Fluorescent Units) score is a metric used to assess the magnitude of signal generated as a result of the reaction. The presence or absence of the analyte is determined by calculating the peak fluorescence (MaxRFU) over the course of the amplification process and comparing this measurement to a predetermined threshold value.

### *How is MaxRFU calculated?*

The BD Viper™ System with XTR™ technology reads fluorescent signals (raw data) from the microwells once per minute over the course of a 60-minute assay.

The signal is related to the amount of amplification products in the microwell.

- An increase in signal equal to or above the threshold value indicates an increase in the amount of amplification products. As a result the reaction will be considered positive.
- Any increases in signal that remain below the threshold value results in a negative reaction.

***There is no equivocal zone for the BD ProbeTec™ CT/GC Q<sup>x</sup> Amplified DNA Assays.***

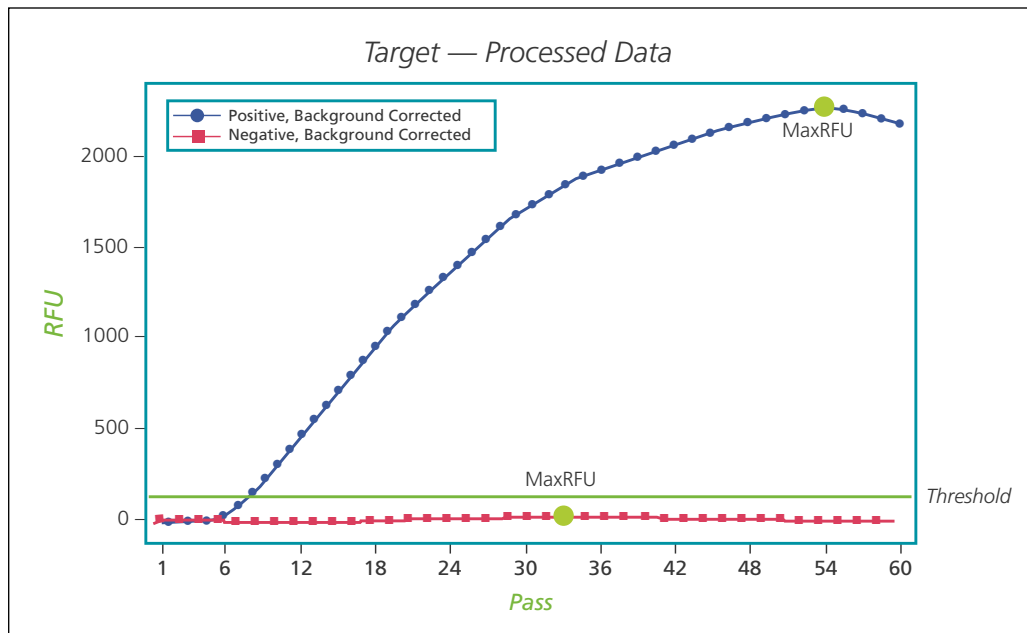
MaxRFU represents the maximum value from the background corrected array of relative fluorescence values.

- Raw data is filtered and normalized to remove optical artifacts and then background corrected.
- Background correction removes the baseline fluorescence caused by the optical system, the assay components, and the sample matrix.
- The MaxRFU value is determined after smoothing the data from the Fluorescein amidite (FAM) optical channel using a three-point running median filter to eliminate outliers.



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An example of processed data from a positive (blue line) and negative (red line) result is shown in **Figure 1**. In this example, the MaxRFU score for the positive result is 2348 and the negative is 76.



**Figure 1:**  
BD Viper™ MaxRFU  
Signal Algorithm  
Sample

### Why isn't MaxRFU Quantitative?

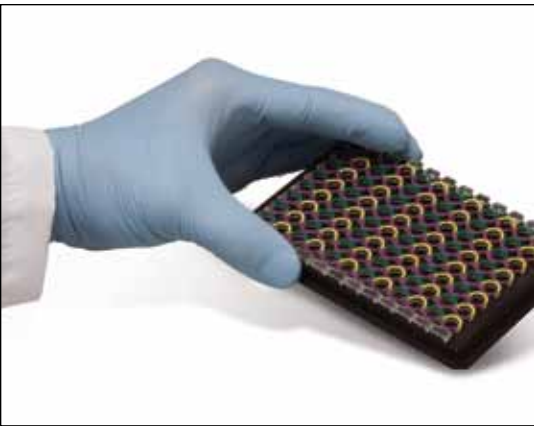
MaxRFU is not a quantitative measurement that can be used to infer the level of target nucleic acid. In reporting of diagnostic results, MaxRFU scores are regarded as purely qualitative.

Interpretation of MaxRFU — MaxRFU is influenced by:

- the amount of target in the sample
- the sample matrix
- subtle reagent, hardware, environmental and workflow variations
- kinetics associated with primer/target/enzyme interactions

Since the amount of target in the sample is only one of many factors that influence MaxRFU, the magnitude of the MaxRFU score is not indicative of the level of organism in the specimen.

# Understanding the BD ProbeTec™ CT/GC Q<sup>x</sup> Amplified DNA Assays Extraction Control



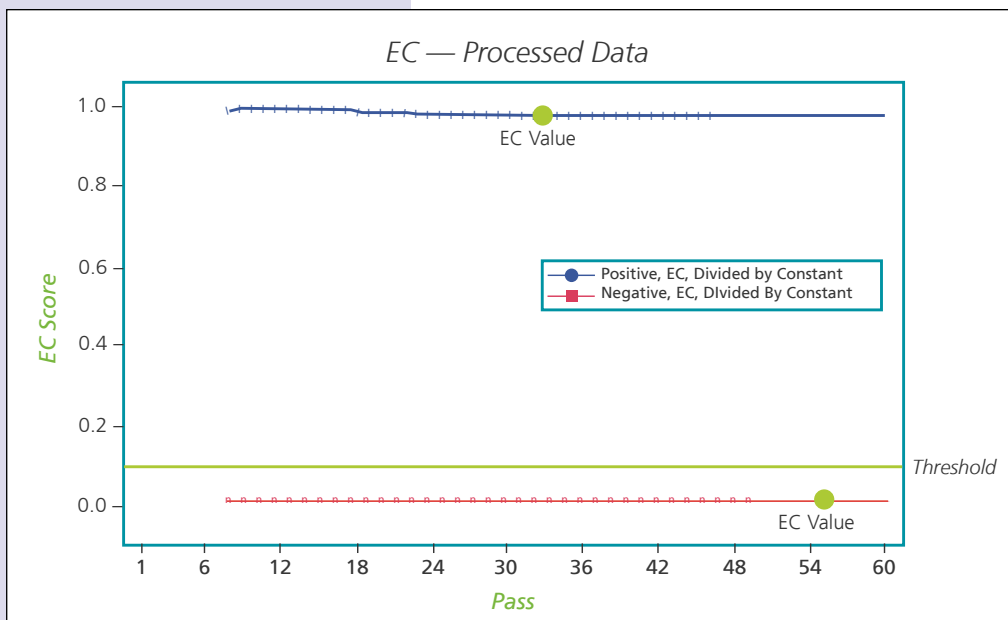
## What is the Extraction Control?

In addition to the fluorescent probe used to detect amplified *C. trachomatis* or *N. gonorrhoeae* target DNA, a second labeled oligonucleotide is incorporated in each reaction. The Extraction Control (EC) oligonucleotide is labeled with a different dye than that used for detection of the analyte specific target and is used to confirm the validity of the extraction process.

## How does it work?

The EC is dried in the Extraction Tubes and is rehydrated upon addition of the specimen and extraction reagents. The EC is a fluorescently-labeled DNA oligonucleotide that binds to and is released from the ferric oxide particles under the same pH conditions as target DNA from the lysed CT and GC organisms. Recovery of the EC is used to monitor the extraction process and the extraction reagents. The EC is not amplified during the course of the SDA reaction but instead is detected directly by virtue of its 5' fluorescent tag. At the end of the extraction process, the EC fluorescence is monitored by the BD Viper System and an automated algorithm is applied to both the EC and assay specific signals to report results as positive, negative, or EC failure.

An example of processed data from a valid (blue line) and invalid (red line) extraction control result is shown in **Figure 2**.



**Figure 2:**  
BD Viper™  
Extraction Control  
(EC) Algorithm  
Sample




## What should I do with an Extraction Control failure (⊗)?

- Inspect Extraction Tube for Iron Oxide (black precipitate) presence.
- For urines or swabs, repeat from the processed sample tube.
- If repeat result is either positive or negative, the sample will be reported as positive or negative, respectively.
- If results repeat as Extraction Control failure, a new specimen should be requested.

## What factors influence Extraction Control failure?

Extraction Control is influenced by:

- the sample matrix
- subtle reagent, hardware, environmental and workflow variations

Result	Q* Max RFU	Report	Interpretation	Result
	≥ 125	Organism plasmid DNA detected by SDA	Positive for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> .	Positive
	< 125	Organism plasmid DNA not detected by SDA	Presumed negative for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> .	Negative
	< 125	Extraction control failure. Repeat test from initial specimen tube or obtain another specimen for testing.	<i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> , if present, are not detectable.	Extraction Control failure

For any questions, please contact BD Technical Services at (800) 638-8663, selection 2 for assistance. Outside the United States and Canada, contact your local BD Representative.



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