

## Use of the Amplification Control

The Amplification Control (AC) is available as an option for use with the BDProbeTec™ ET CT/GC Assays. Each lab should assess the value of the AC in the population/specimens being tested.

### What is the Amplification Control?

The Amplification Control is a linearized plasmid containing a GC target sequence dried inside the wells of the Amplification Control priming plates. The purpose of this control is to verify that proper conditions exist for amplification and to indicate lack of inhibition so that amplification could occur if target is present. Use of the amplification control may help to verify negative results and to identify results which are falsely negative due to the presence of inhibitory substances in the sample matrix.

### How does it work?

Each sample and control are tested in up to three discrete microwells: one for CT, one for GC, and one for AC. When reaction conditions are normal, the AC should amplify; however, if inhibitors are present, the AC may not amplify. If this occurs when the CT or GC results are negative, the BDProbeTec ET system will report an indeterminate result (Table 1).

### What should I do with an indeterminate result?

- For urines, repeat from the original specimen. If original specimen is not available, repeat from the processed sample tube.
- For swabs, repeat from the processed sample tube.
- If repeat result is either positive or negative, the sample will be reported as positive or negative. If results repeat as indeterminate, a new specimen should be requested.

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## Important Phone Numbers and Contact Information

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**Instrument Repair**  
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**Certificate of Analysis (Bactofax)**  
US only: 800.343.2655  
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[www.bdreghdocs.com](http://www.bdreghdocs.com)

**Material Safety Data Sheet (MSDS)**  
Tel: 800.638.8663, selection 4  
e-catalog: [www.bd.com](http://www.bd.com) "products"

**Technology Training Center (TTC)**  
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## Technology Training Center

BDDS Service and Support Systems

- Use of Amplification Control
- BDProbeTec™ ET MOTA Score

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## Technical Understanding of the BDProbeTec™ ET MOTA Score

### What is MOTA?

The MOTA (Method Other Than Acceleration) 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 relating the MOTA score for the sample or control to pre-determined cutoff values.

### How is MOTA calculated?

The BDProbeTec ET instrument 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 indicates an increase in the amount of amplification products and thus a positive reaction.

A negative reaction does not produce amplification products, so no change in signal occurs.

An example of raw data from a positive microwell is shown in Figure 1.

MOTA represents the area under a background-corrected signal vs. time curve.

- 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.
- An example of processed data from the positive result previously described is shown in Figure 2.

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## Technical Understanding of the BDProbeTec™ ET MOTA Score (continued)

MOTA represents the area under this processed signal vs. time curve. In this example, the MOTA score is 30472 (gray shaded area in **Figure 3**).

### Why Isn't MOTA Quantitative?

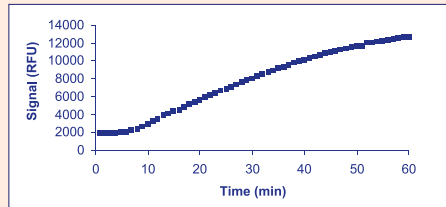
MOTA is an integrated metric that is related not only to the amount of amplification products but also the rate at which amplification products are produced.

MOTA is influenced by:

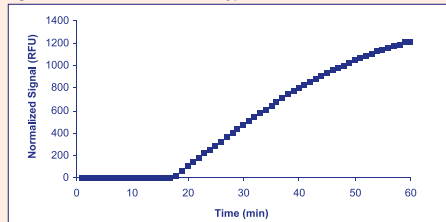
- the amount of target in the sample
- the sample matrix
- subtle reagent, hardware, and workflow variations
- random events associated with primer/target/enzyme interactions

Since the amount of target in the sample is only one of many factors that influence MOTA, the magnitude of the

**Figure 1:** Raw Data from a Typical Positive Result



**Figure 2:** Processed Data from a Typical Positive Result



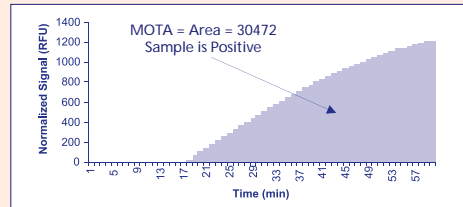
MOTA score is not indicative of the level of organism in the specimen.

An example of two positive samples with different MOTA scores is shown in **Figure 4**. In this case, samples A and B generate similar amounts of amplification products but have very different MOTA scores because the amplification reactions had very different kinetics. The reason that sample A produced signal earlier than sample B could be any of the factors discussed above.

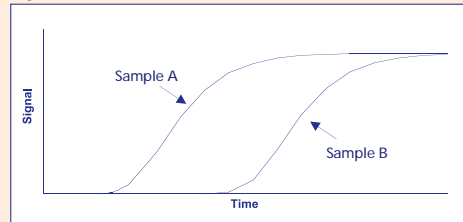
Most amplification metrics are not quantitative in that they do not accurately predict sample target load. Predicting sample target load generally requires a competitive amplification system with a series of run calibrators.

If you have any questions, please contact BD Technical Service at 800/638-8663, selection 2 for assistance. Outside the United States and Canada, contact your local BD Representative.

**Figure 3:** MOTA from a Typical Positive Result



**Figure 4:** Two Positives with Different MOTA Scores



## Use of Amplification Control (continued)

### What is the “expected” or “normal” rate of indeterminate results when using the AC?

Indeterminate rates can vary from lab to lab, but the overall initial indeterminate rate for BDProbeTec ET CT and GC DNA Amplification Assays is about 5%. Expected indeterminate rates are higher in urine specimens than in swab specimens. The frequency of initial and repeat indeterminate results as demonstrated during clinical trials for all samples tested is shown in **Table 2**.

### What factors influence the indeterminate rate?

Different sample types may exhibit different rates of inhibition. Female specimens usually demonstrate higher inhibition rates than male specimens. Swabs (endocervical swabs or male urethral swabs) demonstrate an overall lower inhibition rate than urines. In addition to sample type differences, other known factors which can increase inhibition frequency, include:

- Poor cleaning of the cervix prior to sample collection
- Incomplete decanting of urine after centrifugation
- Pipetting sediment or debris into the microwells
- Presence of inhibitors such as blood (>5% v/v), leukocytes, bilirubin, phenazopyridine, or other unidentified substances

### How do I decide whether or not to use the AC?

Each lab should determine the use of the AC based on their needs and unique population.

For example, using the AC may identify inhibitory specimens and prevent reporting some false negative results. During the clinical study which included 2109 patients and 4131 specimens, there were four (4) patients infected with CT that would have been reported as false negative without the AC. Additionally, there was one (1) patient infected with GC that would have been reported as false negative without the AC. The AC may identify specimen collection and processing anomalies as discussed above.

However, the AC wells can be a potential source of contamination (please see BD Technical Bulletin #49: “Minimizing the Risk of Contamination with Amplified Technologies” for more information). Use of the AC well with each sample also decreases throughput.

In making a decision to use the AC, laboratories should consider these factors as well as:

- laboratory experience with amplified technologies
- type of sample to be tested
- historical indeterminate rate
- regulatory/accrediting requirements

The AC is optional to give you flexibility in your testing routine. Some labs choose to run the AC all of the time, some labs use the AC with selected sample types (i.e. urine specimens) and some labs don't use the AC at all.

Should you have additional questions, please do not hesitate to contact Technical Service at 800/638-8663, selection 2. Outside the United States and Canada, contact your local BD Representative.

**Table 1:** The role of the AC in result reporting

CT or GC Result	AC Amplified	BDProbeTec ET Report	Comment
Positive	Yes	Positive	Both the target and the AC amplified. The sample will be reported as positive.
Positive	No	Positive	The target amplified but the AC did not. It is possible for target amplification to occur in the presence of inhibitors. The sample will be reported as positive.
Negative	Yes	Negative	The analyte did not amplify but the AC did. No inhibitors were detected. The sample will be reported as negative.
Negative	No	Indeterminate	Both the analyte and AC did not amplify. Inhibitors may be present. The sample will be reported as indeterminate.

**Table 2:** Frequency of initial and repeat indeterminate results

Specimen Type	n	CT Indeterminate Rate		GC Indeterminate Rate	
		Initial	After Repeat Testing	Initial	After Repeat Testing
Female Swab	1419	0.6% (8/1419)	0.1% (1/1419)	0.6% (8/1411)	0.1% (1/1411)
Female Urine	1338	12.1% (161/1338)	6.1% (81/1338)	11.6% (154/1331)	6.5% (86/1331)
Male Swab	678	0.3% (2/678)	0% (0/678)	0.3% (2/680)	0% (0/680)
Male Urine	675	5.3% (36/675)	1.9% (13/675)	6.2% (42/680)	2.6% (18/680)
Total	4108	5.1% (208/4108)	2.1% (85/4108)	5.0% (206/4105)	2.6% (105/4105)