



C. difficile and Group B *Streptococcus* Results Interpretation

Process Improvement Project
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Learning Objectives

After completing this BD MAX training session, the user will be able to:

- 1- Identify the correct viewing mode to interpret test results.
- 2- Describe the use of internal control in BD MAX test.
- 3- Identify invalid test results.

Nature of the problem

Technologists' interpretation of the results from the system generated graph may be different than the system interpretation. The graph doesn't show scale on the Y axis which poses a problem. Positive results might be falsely interpreted as negative results.

The goal of this project is to illustrate to laboratory technologists the correct approach to read the graphs and give multiple scenarios to ensure accurate reading of results.

PCR technology is an accurate method of testing. This project was chosen to ensure the staff understanding of the test results.

Plan of Action

The project will address the following points:

- Group B *strep* and *Cdiff* assays methodologies
- Real time polymerase chain reaction curve analysis
- Results interpretation based on curve analysis



An MTS module will be created with review questions for the staff to complete as part of their mandatory assignments in the lab.

Test Methodologies

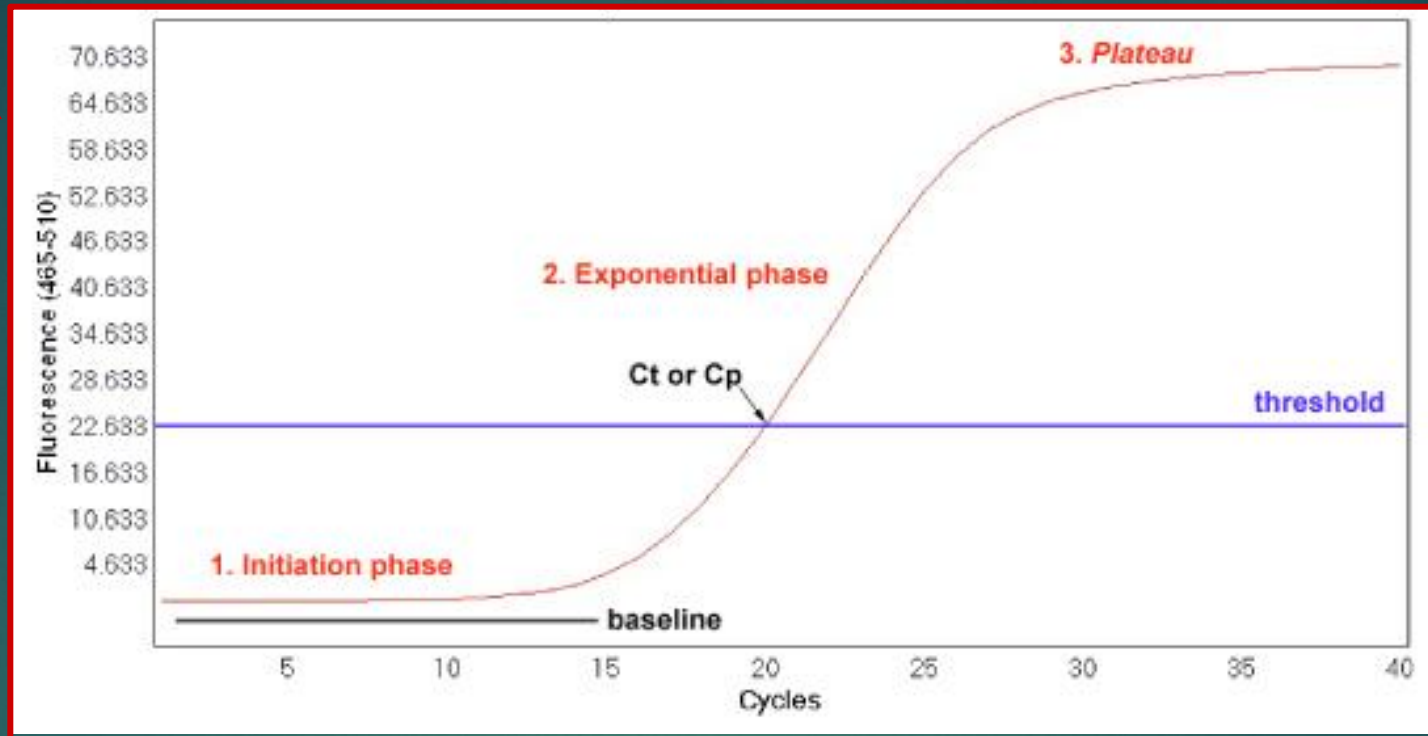
BD MAX™ Cdiff Assay

The test, performed directly on the specimen (human liquid or soft stool) utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA

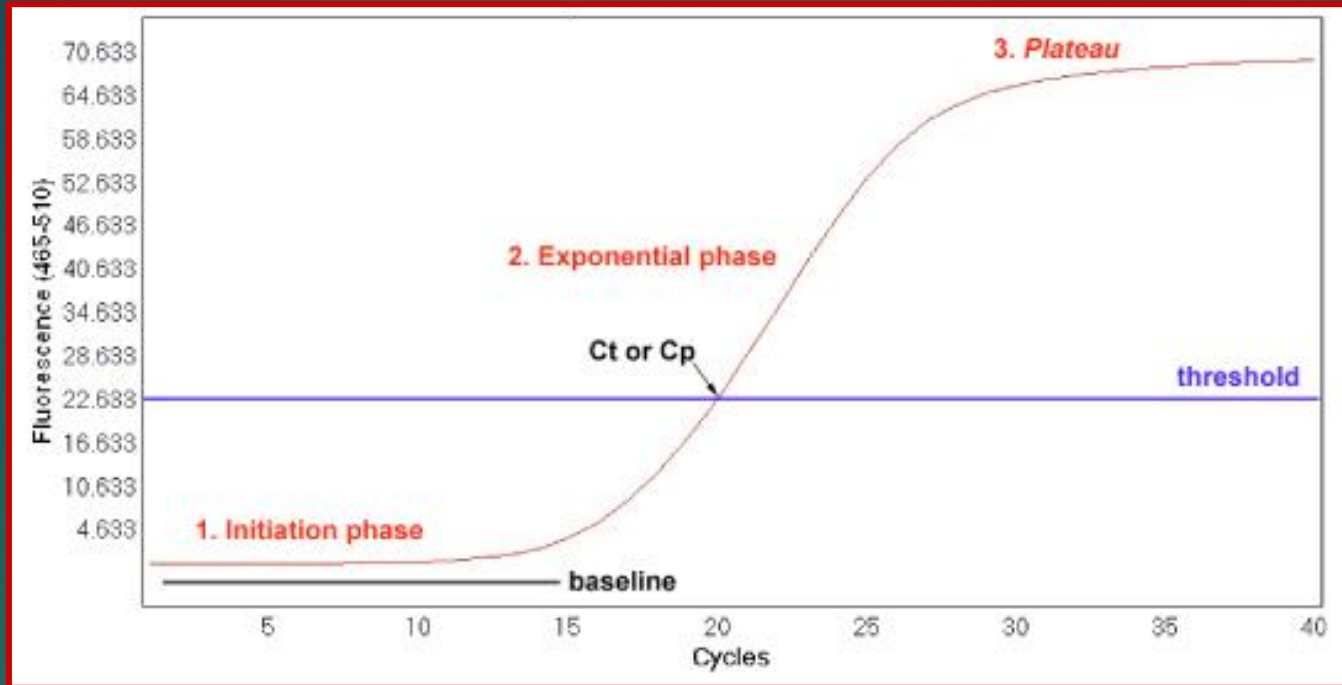
BD MAX™ GBS Assay

The test incorporates automated DNA extraction to isolate the target nucleic acid from the specimen (Lim Broth culture of vaginal-rectal swab specimens from antepartum pregnant women) and real-time polymerase chain reaction (PCR) to detect a 124 bp region of the *cfb* gene sequence of the *Streptococcus agalactiae* chromosome.


Real Time PCR Curve



- The fluorescence emission during qPCR is proportional to the synthesized DNA, and can be visualized as an amplification plot.
- **Threshold** is the level of signal that reflects a statistically significant increase over the calculated baseline signal
- **Crossing threshold (CT)** value is the cycle at which fluorescence achieves a defined threshold. CT value is inversely related to the starting amount of target.



- **Baseline** is the signal level during the initial cycles of PCR, usually cycles 3 to 15, in which there is little change in fluorescent signal.
- **Initiation phase**: it occurs during the first PCR cycles where the emitted fluorescence can not be distinguished from the baseline
- **Exponential phase**: at every cycle, the amount of product is doubled (assuming 100% reaction efficiency).
- **Plateau**: No more product accumulates due to exhaustion of reagents and enzyme.



An **Internal Control** is provided in each BD MAX Assay. This internal control monitors the efficacy of the DNA extraction and PCR amplification processes. A test result may be called as NEG (negative), POS (positive), or IND (Indeterminate) based on the amplification status of the target and Internal Control.



Each curve should be checked individually. Every specimen should have two curves, one for the Internal control and one for the patient. The graph labeled **475/520** is the patient graph and the one labeled **585/630** is the internal control.

Internal control curve can take various shapes (sometime flat line) when the patient is positive and becomes irrelevant to the interpretation of the result.

On the other hand, internal control curve should be valid (a smooth curve in an upwards "s-shaped) when the patient result is negative.

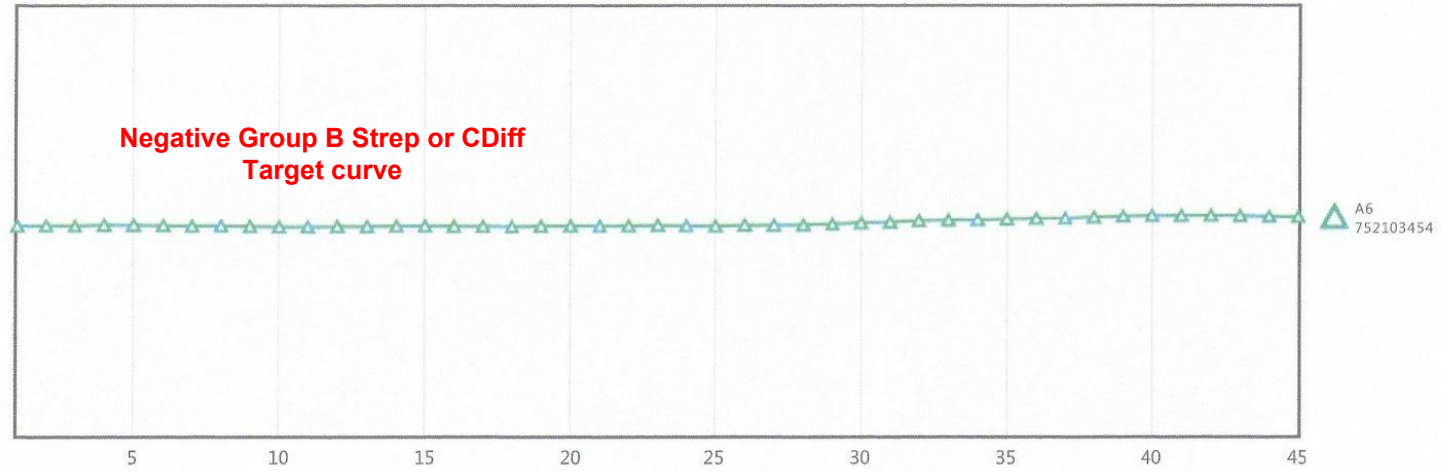
Negative Test Result

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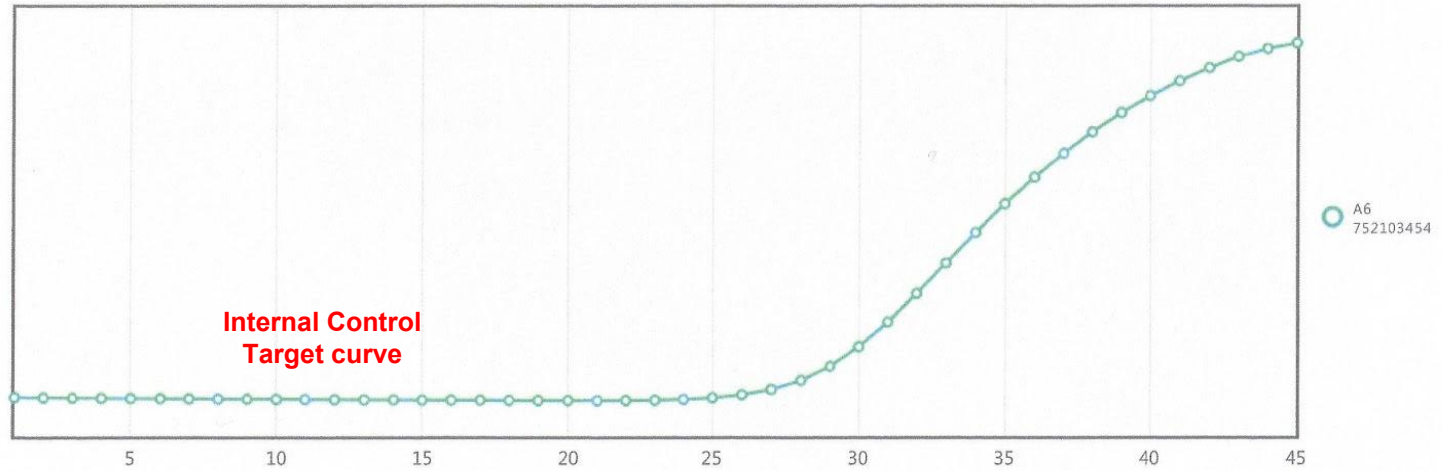
Run Report: 3975

Instrument #:
Version #:

BD MAX 475/520



BD MAX 585/630

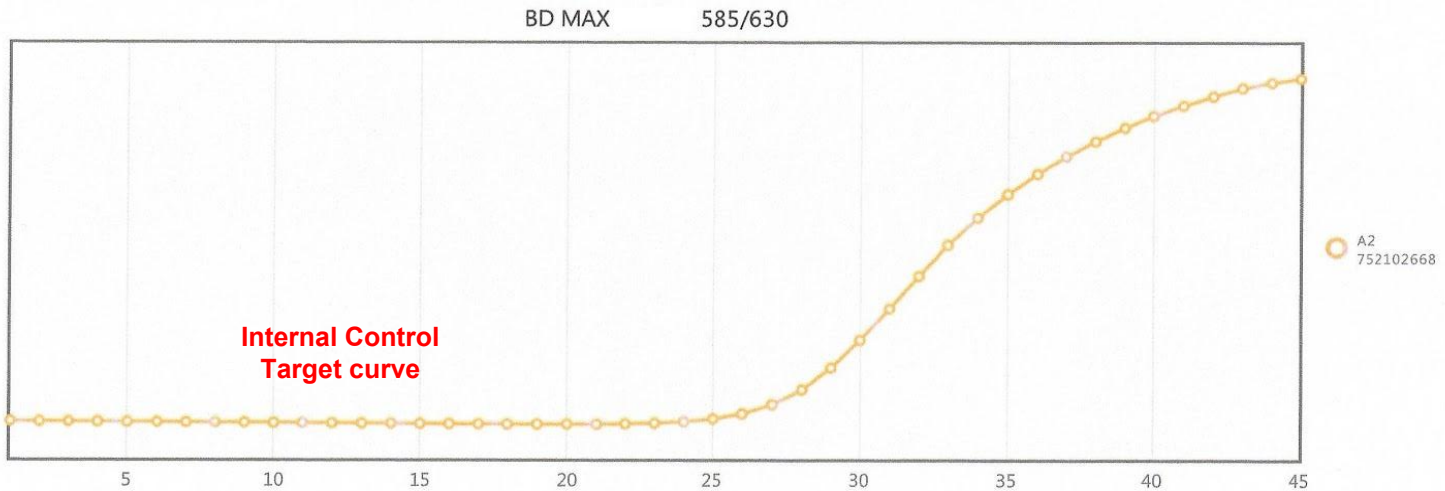
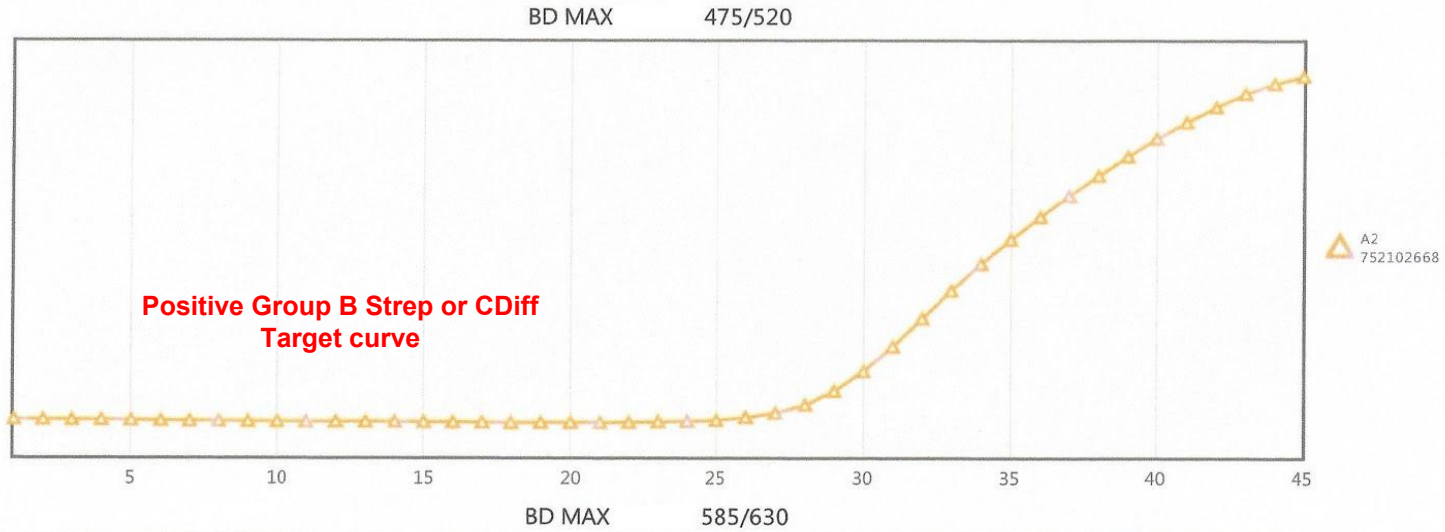


Positive Result. Example 1: Both Patient and Internal Control Have Positive Curves.

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Version #:



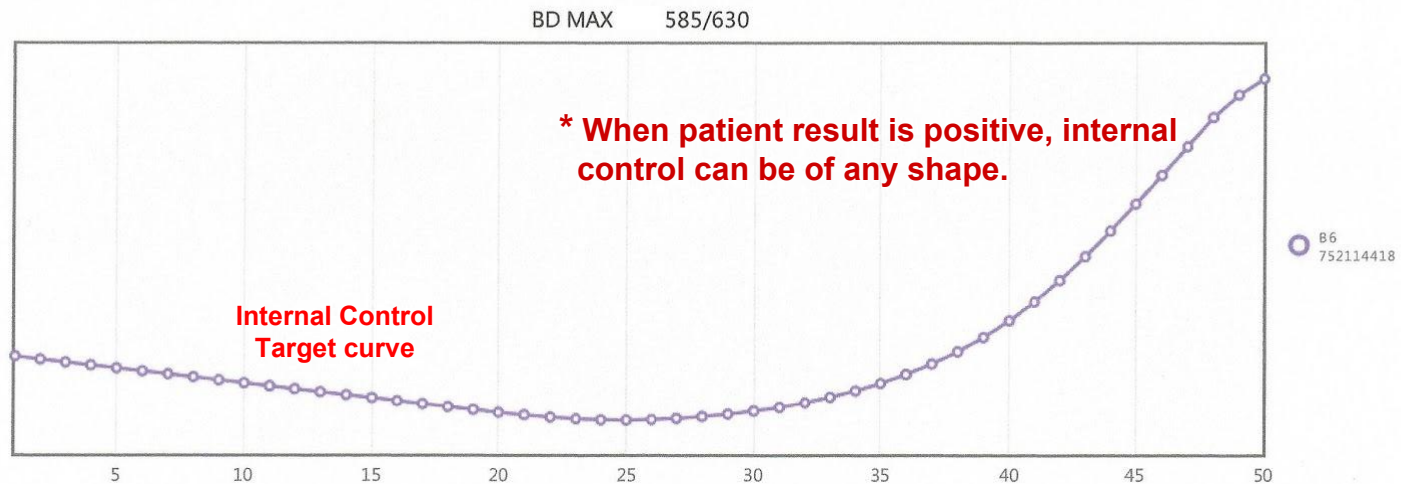
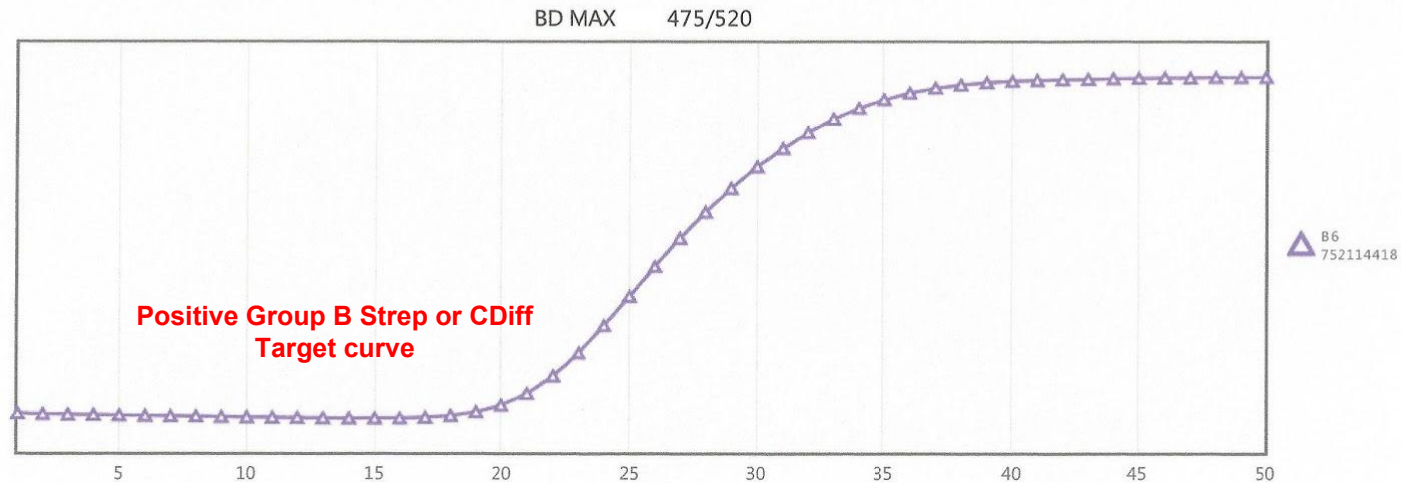
Positive Result. Example 2: Positive Patient with Odd Internal Control*

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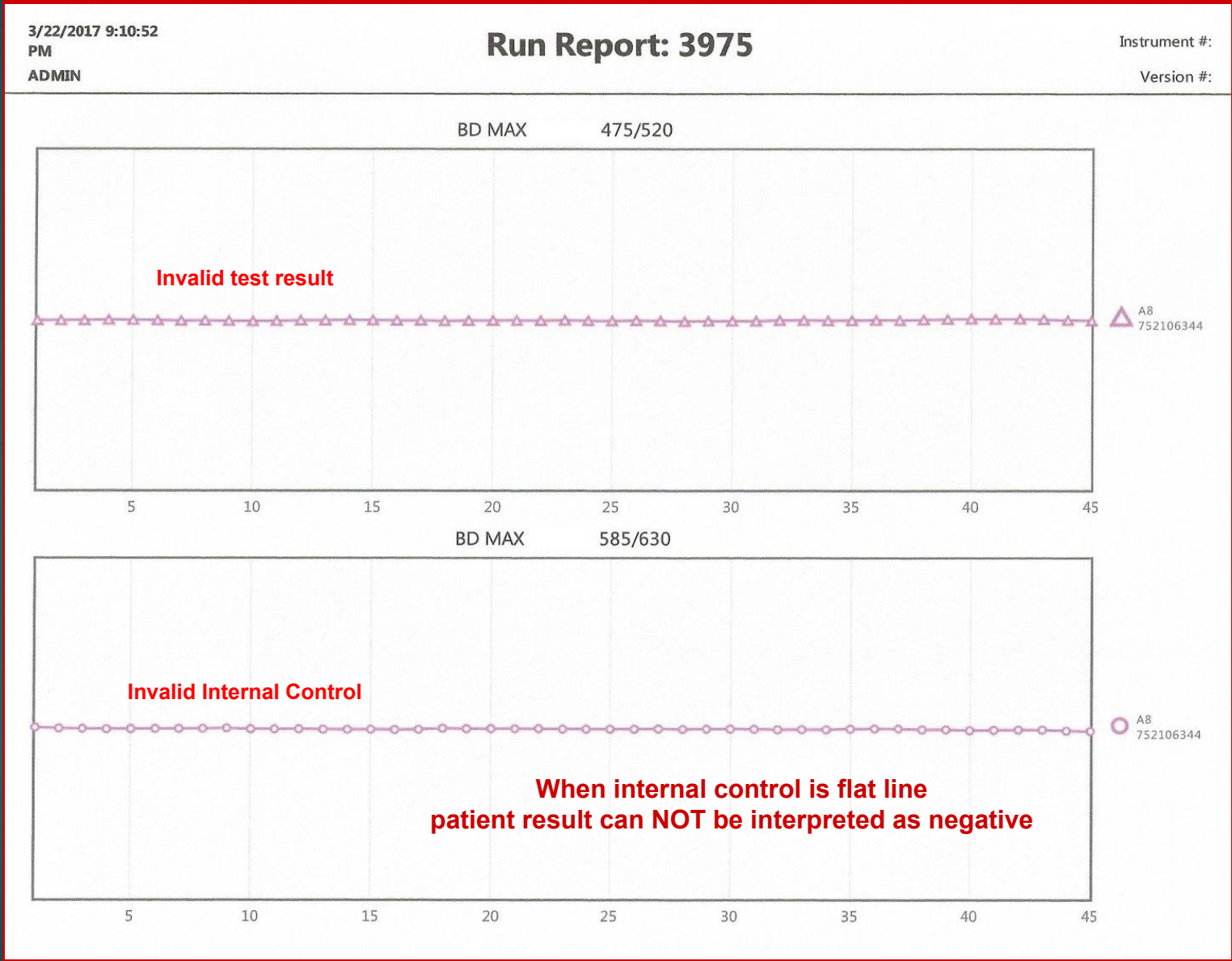
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Example of Indeterminate Test Result



Indeterminate Results Procedure

In the case of Indeterminate test result on the BD MAX, follow the procedure outlined below:

Group B Streptococcus

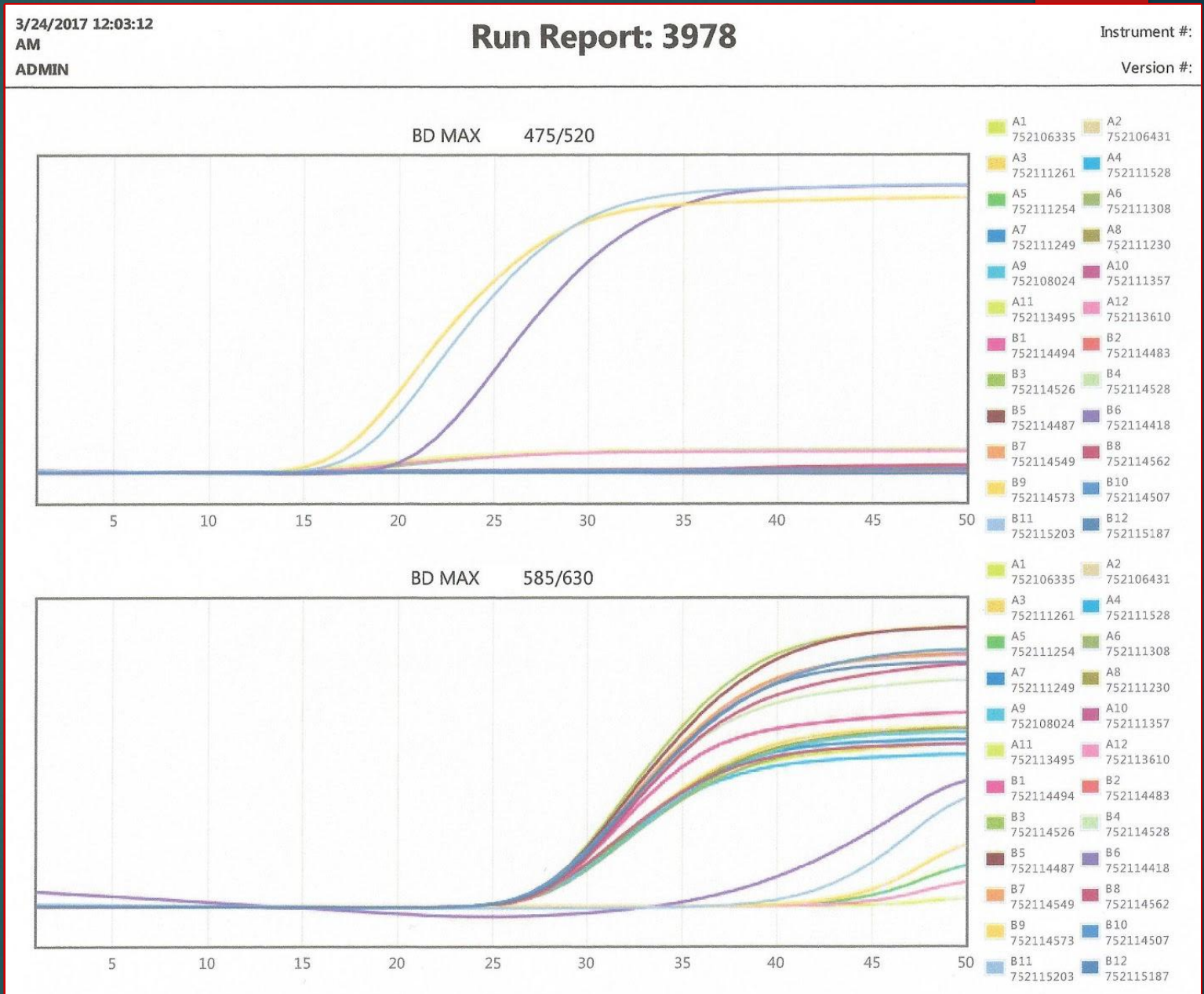
Repeat once, If specimen remains indeterminate, result as such in a preliminary report then do the culture.

C. difficile Toxin

Repeat once using the original stool specimen, if still indeterminate– send to Troy for alternate method.

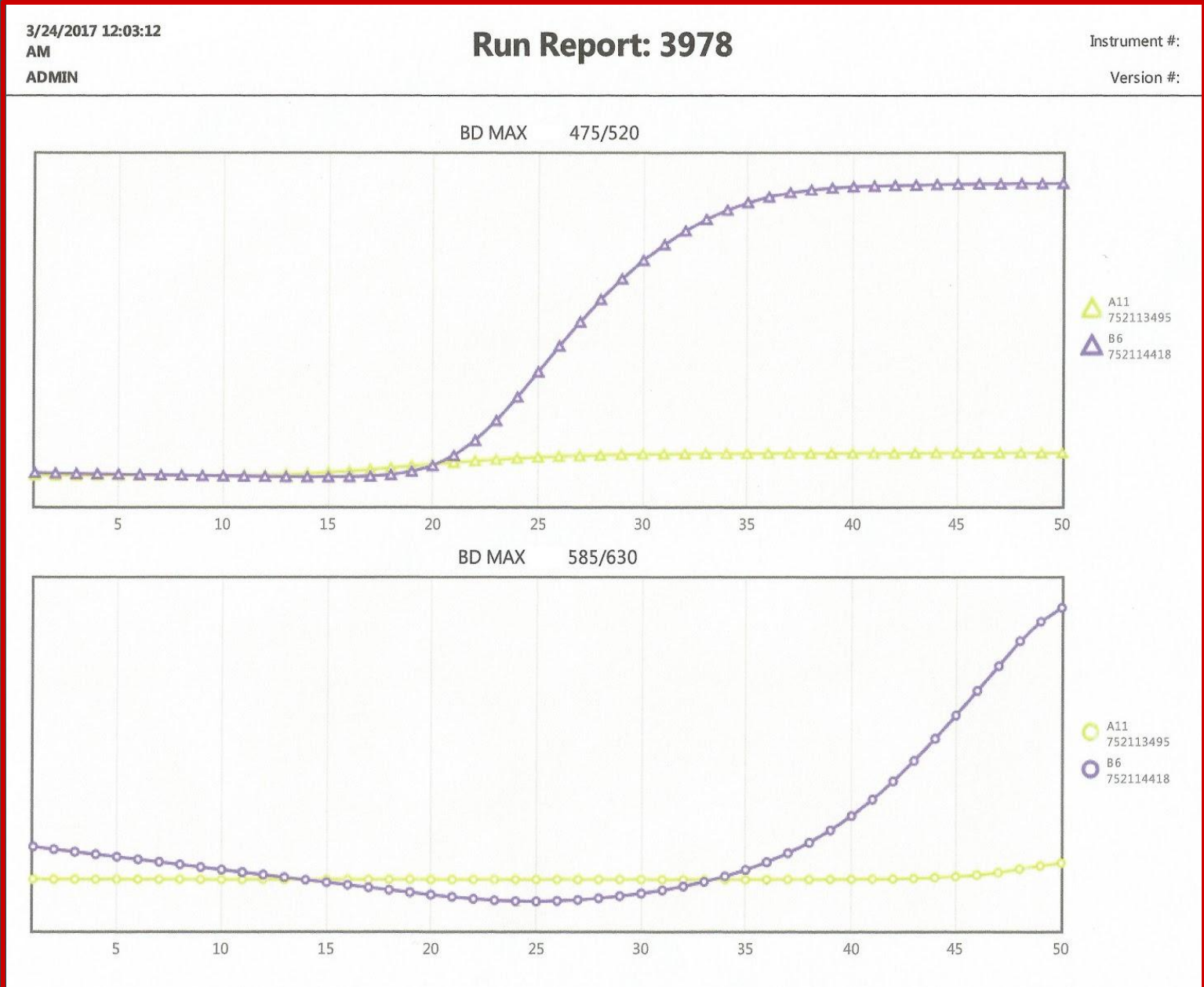
Proper interpretation of test results should always be performed on each test curve individually. It is not recommended to look at the whole test run results at the same time. BD MAX software does not offer scale for the Y axis which makes the interpretation of results inaccurate and misleading.

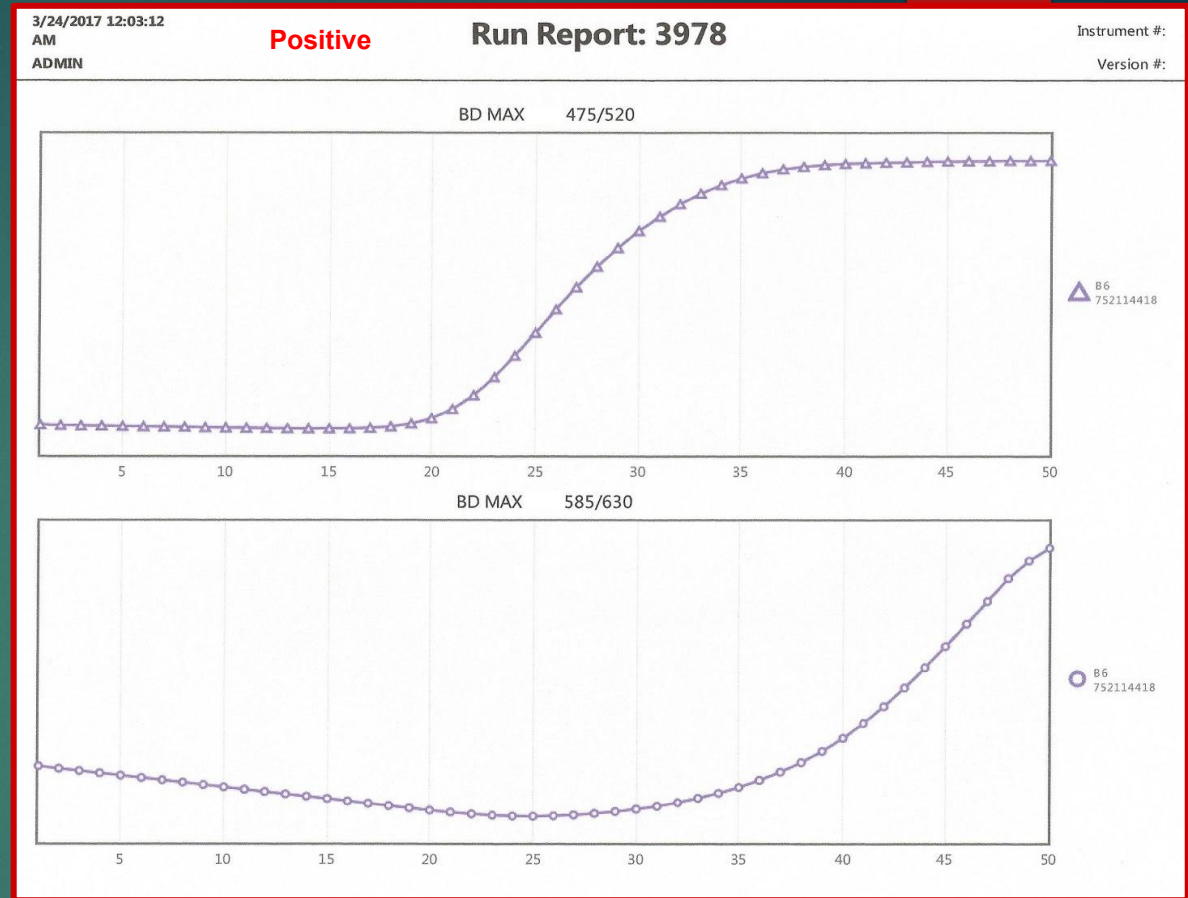
The report to the right shows only three tests to be positive while actually a total of six tests were positive.



Upon observing the report shown to the right. It will be assumed that one test (B6) is positive and the other test (A11) is Indeterminate.

In the next slide, the results of each test will be shown in an individual report.



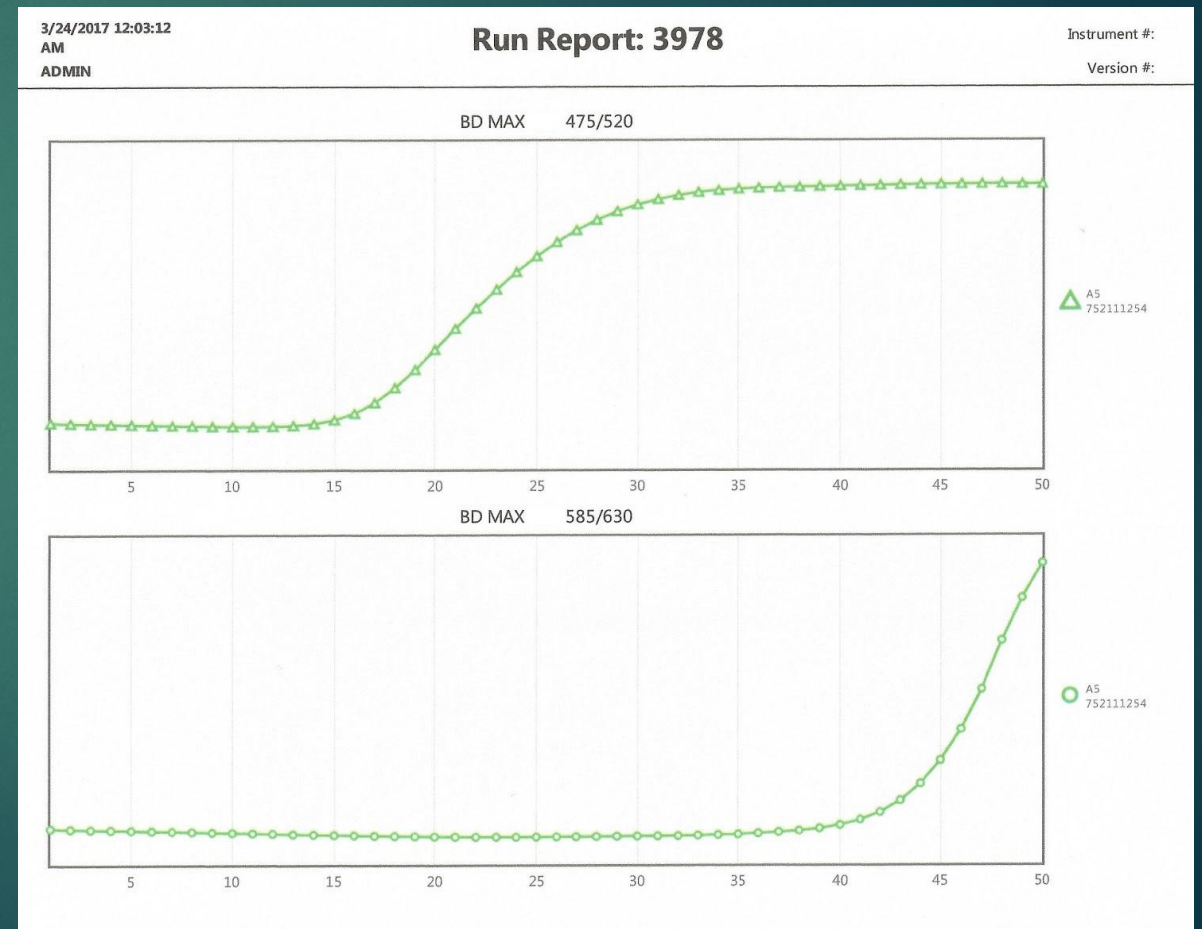



Both B6 and A11 are positive. Separating each test into an individual curve is recommended for accurate interpretation of results. Inconsistencies between the instrument results and the laboratory technologist interpretation will occur when reading multiple curves at once.

Training Test

1. Based on the run report shown below, select the appropriate choice that represents the exponential phase of specimen reaction.

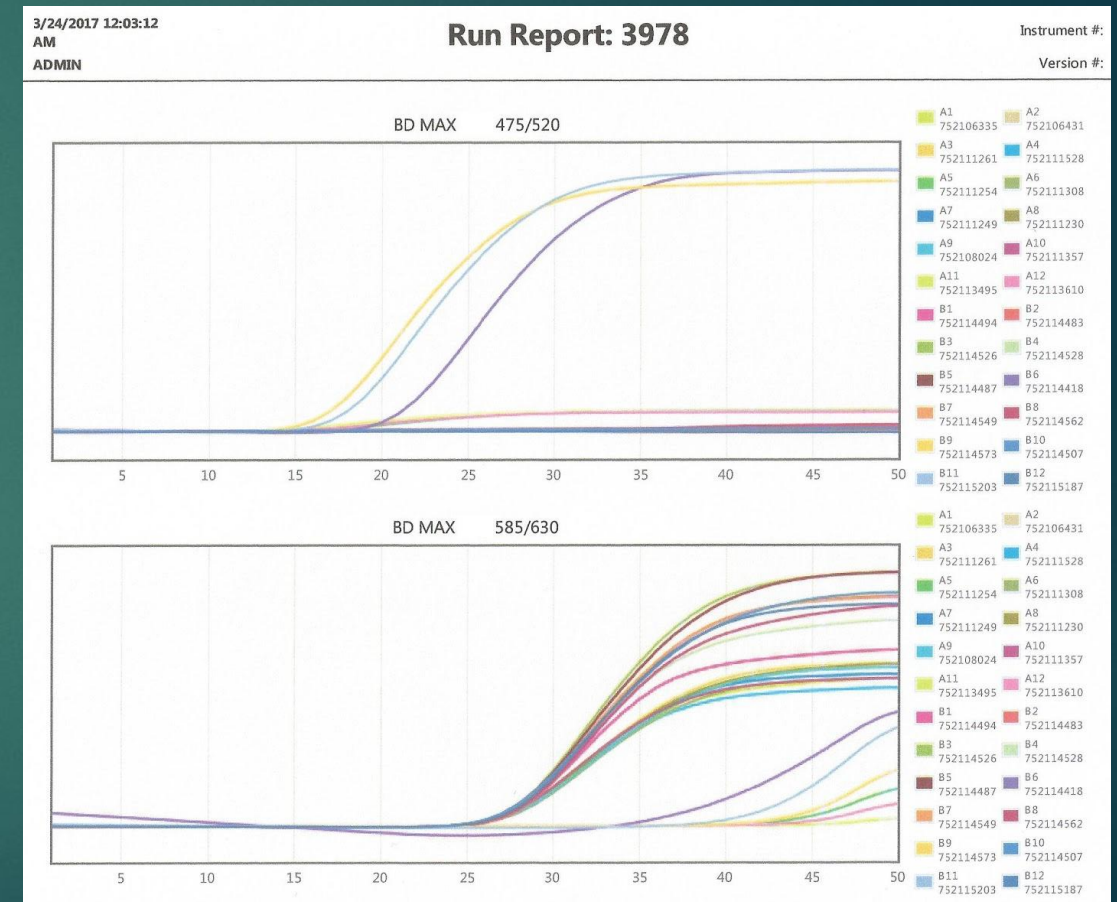
- a. Cycles 0-15
- b. Cycles 15-30
- c. Cycles 30-50
- d. Cycles 15-50



- 
2. An internal control is used in each BD MAX Assay and run with the patient sample. The Internal control is used to:
 - a. Identify specimen handling and/or a contamination problems
 - b. Check for reagent or BD MAX System error
 - c. Monitor the efficacy of the DNA extraction and PCR amplification processes
 - d. A and C

3. You ran 20 patients samples for CDiff B toxin gene on the BD MAX instrument. Upon completion of the run, the software shows the following screen. Your best course of action is:

- Report three positive specimens.
- Change the report view to show each result on an individual curve.
- Repeat the run
- Check the reagents



4. The run report shown below is for two specimens tested for Group B Strep on the BD MAX. What is the correct interpretation of the results in the curve plot shown?

- a. B6 Positive, A11 negative
- b. B6 Negative, A11 Negative
- c. Each specimen should be looked at individually in a separate graph
- d. Indeterminate



5. The run report shown below is for Group B Strep test on the BD MAX instrument. What is the correct interpretation of the result shown in the curve plot?

- a. Positive result
- b. Negative result
- c. Indeterminate

