Use of Clot Curve Analysis with PT and APTT on the ACL TOP® Family Systems

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Introduction

The ACL TOP Family of analyzers, designed by Instrumentation Laboratory, provide high quality results from both an accuracy and precision perspective. The analyzers incorporate optical-based reading systems with the means to precisely monitor clotting, chromogenic, and immuno-turbidimetric reactions within the cuvette during the data acquisition cycle. The optical data is processed by means of sophisticated algorithms as well as data checks designed to ensure that minimum acceptance criteria are met, and also ensuring that results are not reported from significantly abnormal reaction curves.

In the case of clotting reactions, significantly abnormal conditions may be found, for example, in samples from patients with liver function impairments. These pathologic conditions are known to cause coagulation factor deficiencies as well as low fibrinogen levels. Samples from patients with these conditions can be difficult to analyze. They may result in non-optimal readings especially from mechanical based clotting analyzers which do not allow the clot to form undisturbed. Samples of this nature should be flagged on all systems and the reaction visually evaluated. This evaluation can be performed on the ACL TOP systems to establish a clot time.

Clot Curve Analysis

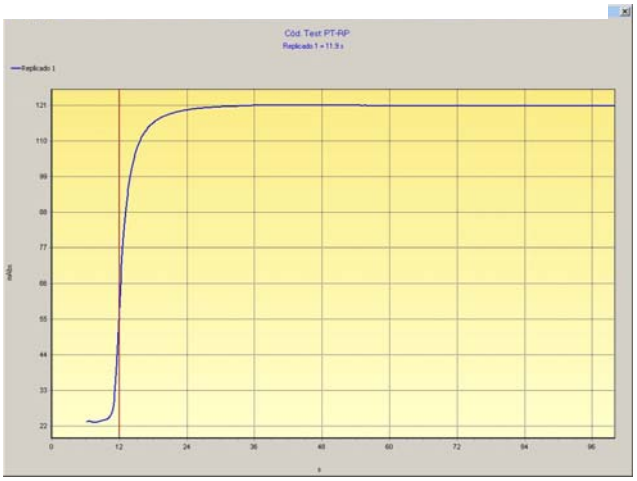
On ACL TOP Family of analyzers, the monitored clotting reaction is known as the clot curve and is available for display for all processed samples. Samples with low fibrinogen will often produce a normal looking clotting reaction.

However, if the reaction does not meet the minimum delta specification, the system does not provide a result.

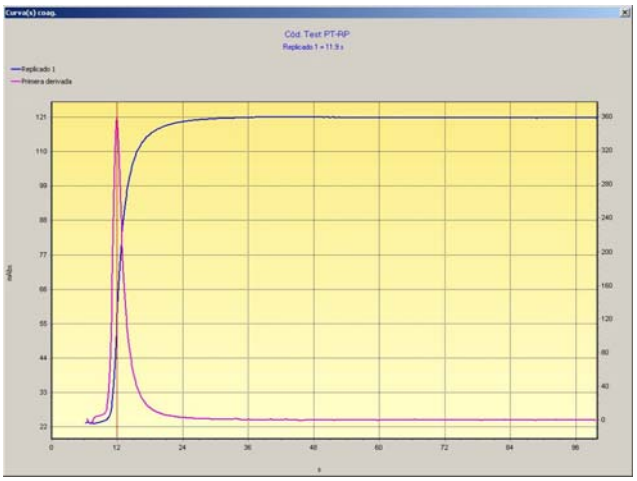
On the ACL TOP systems the clot curve display may be reviewed in two ways:

1. Clot Curve reaction solely (no curves)
2. Clot Curve reaction plus derivative curves.

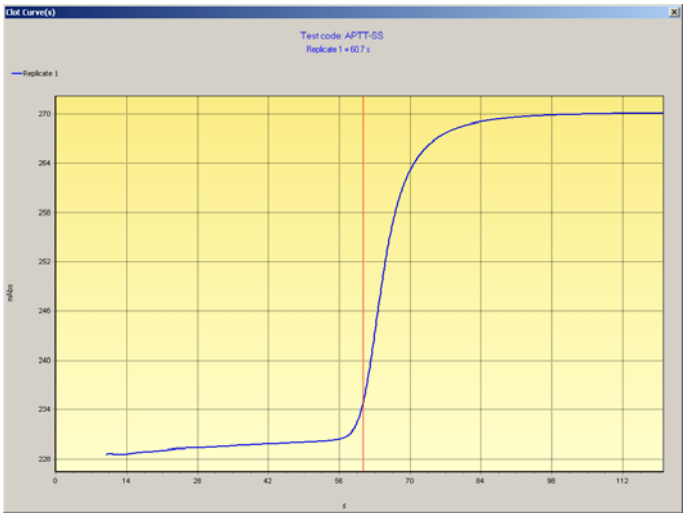
An example of each of these is shown below for a PT and APTT reaction:



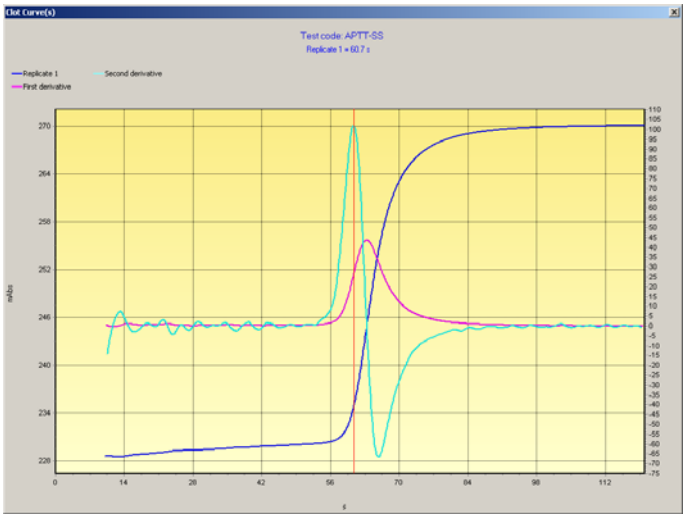
PT Curve without Derivative Curves



PT Curve with Derivative Curves



APTT Curve without Derivative Curves



APTT Curve with Derivative Curves

The PT and APTT appear different due to the algorithm used to identify the clot point of the sample:

- The PT using HemosIL™ RecombiPlasTin 2G reagent is resulted using the First Derivative algorithm; therefore a single derivative curve is overlayed on the clot curve.
- The APTT using HemosIL SynthASil or APTT SP is resulted using a Second Derivative algorithm; therefore two Derivative curves are overlayed on the APTT clot curve.

The display of derivative curves is enabled/disabled on the TOP systems under the Setup → Global Definitions → "Enable Derivative Curves".

When viewing the reactions with the derivative curves you will notice the First Derivative is plotted as a pink line and the Second Derivative is plotted in light blue. The dark blue line is the standard sample reaction plotted.

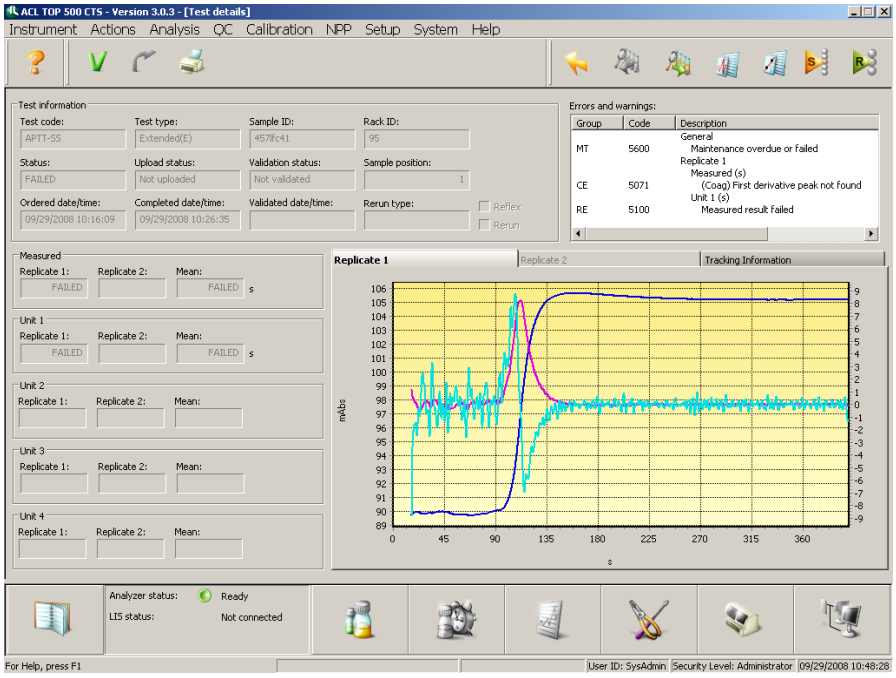
For the PT reaction the clot time is noted at the peak of the First Derivative and for the APTT the clot time is noted at the peak of the Second Derivative. These selections correspond to the settings found in the test definition for each individual test. The settings can be found by viewing the DR Parameters→ Primary Wavelength →Primary Algorithm for the test.

When a sample does not meet the criteria defined within the test definition, no result value will be displayed. For these samples the clot curve, with the derivatives curves enabled, can be a useful tool to establish a clot time for the sample visually.

The display of a clotting reaction is a dynamic display. You can utilize the mouse cursor to view the time by placing the cursor anywhere along the entire acquisition interval for the standard curve, First Derivative (pink line), or Second Derivative (light blue line). Positioning the cursor at the peak of the respective curve (i.e. Second Derivative for APTT) would provide the clot time for that sample, had it met all of the data check criteria.

Please view the APTT sample on the following page as an example on how to read the curve value.

Example1: Low Fibrinogen APTT Sample



This reaction resulted in a “Failed” result with the error 5071 “(Coag) First derivative peak not found”.

If you double click on the reaction graph, it will expand the display to fill the whole screen as shown below:

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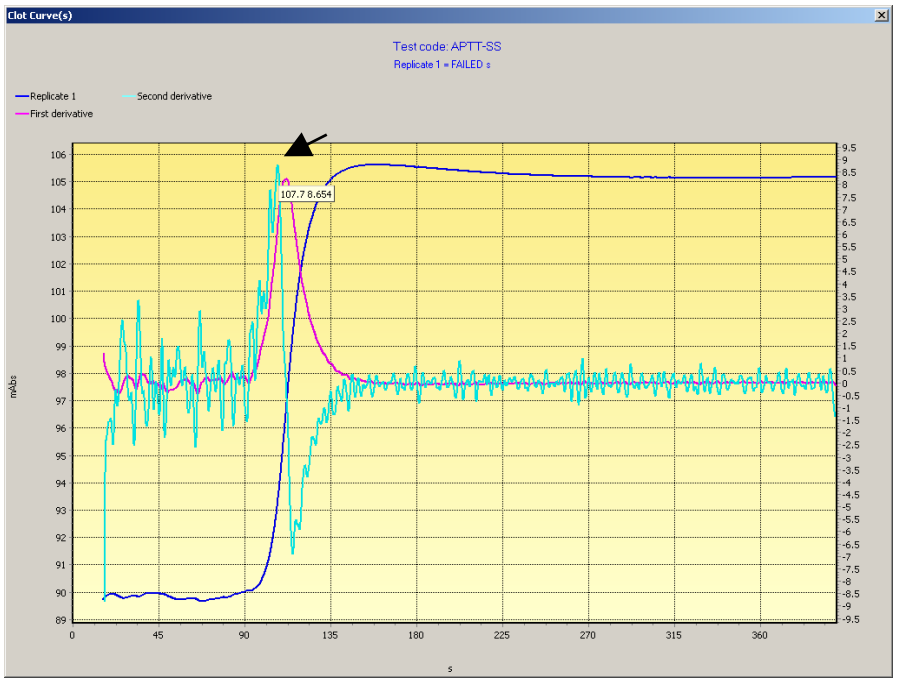

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This is a reaction for an APTT processed using HemosIL SynthASil. This assay uses the Second Derivative to establish a clotting time. On this reaction you will notice a standard looking clot curve with the characteristic "S" shape. The "S" shape indicates the reaction has a baseline, acceleration, and plateau phase. You can also notice a distinct peak for the Second Derivative (light blue) plot. The "Y" axis for the graph however has a limited scale. The readings range from 89 to 106 (curve delta of 17) which is much smaller than what you would normally obtain processing a sample with a normal fibrinogen value.

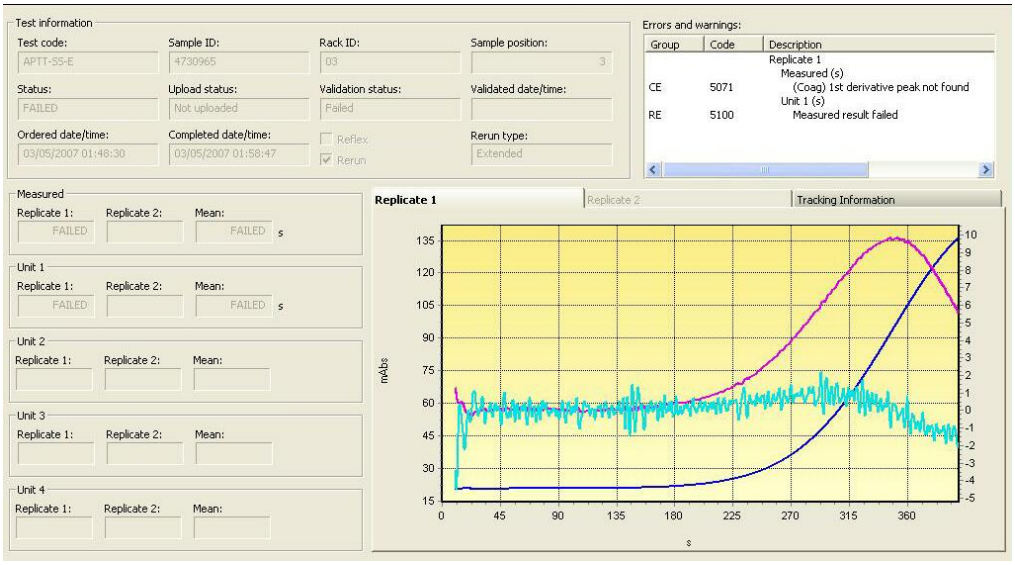
For a sample such as this, if you place the cursor at the peak of the Second Derivative you will note a reading of 107.7 seconds. This would correspond to the reading obtained if all data check criteria had passed.

The clot curve can be a useful tool to establish what a clot time would be for a sample if the quality data checks were not enabled in the test definition.

Things to keep in mind when using this tool:

1. The standard clot curve (dark blue line) should have the characteristic "S" shape
2. A distinct peak for the First Derivative (pink line) should be present for assays using this algorithm.
3. A distinct peak for both the First Derivative (pink line) and Second Derivative (light blue line) should be present for assays using the Second Derivative algorithm.

Example2: Extended APTT Sample – Failed Result



This reaction resulted in a "Failed" result with the error 5071 "(Coag) First derivative peak not found".

If you double click on the reaction graph it will expand the display to fill the whole screen as shown below:

For this example you will note the standard clot curve (dark blue line) does not present the classic "S" shape. The curve has a baseline and acceleration phase but lacks the plateau phase. In addition you will note there is no distinct peak for the Second Derivative (light blue line). This particular clot curve cannot be used to manually establish a clotting time.

This example presents a sample with an extremely long clotting time. This type of sample would need to be analyzed on a test/system that does not limit the data acquisition phase in order to establish the APTT clot time.

Conclusion

As you can see, the clot curve can be an extremely valuable tool for evaluating results from compromised samples. In many situations, samples that presented a "failed" value, for example due to very low fibrinogen content (fragile clot), can be assessed via the clot curve and a visual result obtained. It is always important to first ensure a clot has formed by reviewing the clot curve for a baseline, acceleration and plateau phase, as demonstrated in example1 on page 3, before reading the result off the appropriate derivative curve for the particular assay.