Clot Signature Curves and the ACL Advance™
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What is a Clot Signature Curve?

**A Clot Signature Curve is a visual representation of the clot formation in the test cuvette during a coagulometric test performed on the ACL Advance™.**

The Clot Signature Curve, also known as a result curve or clot curve, is the product of data readings taken by the Analytical Module of the ACL Advance after the introduction of the “start” or “trigger” reagent for the test. The data readings are collected, sent to the Control Module, processed through predefined mathematical algorithms, and the final data is displayed as a graph plotting Raw Counts versus Time. Raw Counts are the light measurements made by the detector of the reading unit in the Analytical Module. Decreasing Raw Counts correspond to increased optical density.

The Clot Signature Curve is a valuable tool for the investigation of abnormal or unexpected test results, or for the investigation of warnings or errors related to sample analysis.

The Clot Curve is accessed by selecting the test result on the **Loadlist** or **Validated** screen, pressing **F9 More Detail**, and then pressing **F9 Show Curve**. The Clot Curve for a test result may be viewed on the ACL Advance screen or printed.

**Note:** The Clot Curve must have been archived prior to validation to be viewed through the Validated screen.

The Clot Curve is comprised of five main phases: Delay, Baseline, Acceleration, Deceleration and Endpoint. (See Figure 1).

The Delay period precedes the Baseline and begins at time zero. It is not an actual part of the clot signature curve as no data from the delay period is displayed; hence there is a gap between time zero and the first actual point on the curve. During this phase sample and reagent are mixing and Autoranging takes place. Autoranging is a feature of the ACL Advance which adjusts the light...
source intensity to provide optimal measuring sensitivity. This lamp adjustment also helps overcome the effects of lipemia. Autoranging occurs only with the coagulometric channel (880 nm).

The Baseline is the portion of the curve which depicts the data collected after all reagents have been added and the time allowed for mixing (Delay Time) has passed, to the time that clot formation begins. Generally no significant change in optical density occurs during a normal Baseline phase. No data from the Delay period is displayed, so the Baseline does not begin at Time = 0. Slow initiation of clot formation is seen as a prolonged baseline phase.

The Acceleration phase represents the formation of the fibrin clot. Typically the Clot Curve shows a steep upward slope, indicating a rapid decrease in light reaching the detector (rapid increase in optical density). This change in optical density (delta OD) is due to the formation of fibrin and its light scattering properties. Slow clot formation is seen as an acceleration phase with a shallower slope.

The Deceleration phase immediately follows Acceleration and represents the decreasing rate of clot formation as all available fibrinogen is converted to fibrin. The Endpoint is the final phase of clot formation in which the acquisition time has ended. Typically all the available fibrinogen has been converted to fibrin and the clot curve flattens and becomes horizontal as no further change in optical density occurs. Weak clots, as seen in samples with low fibrinogen, may dissociate and can be seen on a clot signature curve as an endpoint which drops down toward the baseline. An endpoint which continues to climb upward at the end of the acquisition time indicates the continued progression of clot formation.

Figure 2 shows the APTT test Clot Signature Curve for a normal patient. It demonstrates the typical “lazy S” shape with the initial Baseline phase, steep Acceleration, and then the flat, horizontal Endpoint. The X-axis shows the time span over which raw data readings were taken and the Y-axis shows the raw counts. The clotting point was determined by the ACL Advance to be 22.90 seconds, as denoted by the vertical line from the Clot Curve to the X-axis (at 22.90 seconds). This basic shape for a Clot Curve holds true for APTT-based assays as well as PT tests and PT-based assays.
How is Clot Signature Curve derived from the raw data?

The raw optical readings are measured, converted to a usable form, processed and analyzed according to the parameters defined in the Test Definition for the specific test.

Each test that can be performed on the ACL Advance has a Test Definition. This Test Definition outlines how the physical aspects of testing are carried out (such as dispensing of reagents) as well as how the data is processed and the algorithm used for clot point determination.

The Test Definition for a test may be accessed by entering the Setup Menu (Alt S), selecting Test Definitions and pressing Enter. The specific test can then be selected and its Test Definition viewed by pressing Enter.

A Test Definition consists of parameters defined on up to 8 separate screens. Two of these screens contain the parameters which most affect the raw optical data and therefore the appearance of the Clot Curve: F3 Test Definitions and F7 Test Parameter Definition.

### F3 Test Definition

This screen contains information on how the test is defined, such as name; it also contains more important parameters such as liquid aspiration and dispensation and incubation. Additional parameters which relate to the raw data and Clot Curve are:

- **Mode:** this describes the algorithm being used for clot point determination:
  - Clotting (first derivative)  
    time at which maximum velocity of clot formation is reached.
  - Clotting (second derivative)  
    time at which maximum change in velocity (maximum acceleration) of clot formation is reached.
  - Clotting (delta)  
    Total change in optical density, referenced against a calibration curve (used for PT-based Fibrinogen).
  - Clotting (threshold)  
    time at which a pre-defined optical density value is reached.
  - Clotting (threshold - second derivative)  
    time at which a pre-defined optical density value is reached. If the threshold algorithm fails, the data is processed using the second derivative algorithm.
  - Clotting (maximum slope)  
    not currently used.
  - Clotting (final-initial)  
    The change in absorbance between the endpoint mean value and the baseline mean value (used for D-Dimer).

- **Photometer Linear Kinetics**  
  Rate of change in absorbance between the endpoint mean value and the baseline mean value (used for chromogenic assays).
Wavelength
880 nm (Coagulation) or 405 nm (Chromogenic/Immunological/Fibrinogen-C, Thrombin Time 2 mL).

Acquisition Time
Time period (in seconds) during which data acquisition of the sample takes place.

Delay Time
Time period (in seconds) between the start of the test and the start of acquisition time.

F7 Test Parameter Definition
This screen shows the key parameters affecting data processing and clot point determination.

Continuity
A pre-defined percent of the total signal of the curve. The raw data points must fall within this range. If two adjacent points exceed this range, a failure occurs. This would indicate that the curve is not continuous (Data Error 4).

Normalization
Method used to convert the raw data to a real number.

Baseline
Number of data points used to calculate the baseline value.

Smoothing Offsets
Defines how many points to use to smooth the data curve. This reduces the effects of “noisy” raw data and smooths the appearance of the clot curve.

Threshold Criteria
Determines if the threshold is looked at from the end or beginning of the curve, how the time span between the threshold is checked, or if threshold is related to the curve delta.

Threshold Values
Values of threshold that the curve must cross. If the curve fails to meet/exceed the value(s), the curve will fail.

Error 11 (Coag Error)
Error 12 (Coag Error)
Error 18 (Coag Error)

First/Second Derivative
Method to use for determining the derivative, number of points used to calculate the derivative (Offset), and the criteria for how the peaks that determine the derivative are selected.

ROI (Region of Interest)
Region used around the maxima value of the 1st or 2nd derivative.

Endpoint Stability
Used to determine the stability of the endpoint, and therefore the stability of the clot.
What affects the appearance of a Clot Signature Curve?

The Clot Curve is the final product of the whole testing process. Any influencing factor introduced at any stage may affect the clotting process and affect the appearance of the clot signature curve. The appendix contains examples of actual curves. Influencing factors may include:

- **Sample quality and collection**
  - clotted
  - improperly stored
  - over-anticoagulated (high hematocrit)
  - too old

- **Reagents**
  - old reagent
  - inappropriately reconstituted
  - improper placement of reagent on the ACL Advance
  - lack of stir bar, if required
  - cross-contamination

- **Mechanical**
  - damaged probe (sample or reagent)
  - spillage in reading area
  - debris in cuvette
  - damage to optics

- **Clinical Condition of Patient**
  - factor deficiency
  - disseminated intravascular coagulation (DIC)
  - excessive lipemia
  - liver disease
  - anticoagulant therapy.

How can I use the Clot Signature Curves?

The Clot Signature Curve can indicate why a test result incurred an error or warning, whether a result is valid, or can suggest clinical conditions. The appendix contains examples of a variety of abnormal curves and what could be determined from them.

A user can:

- **Confirm Results**
  - high/low results
  - identify samples that have not clotted
  - identify samples that are atypical and may require additional investigation

- **Save Time and Reagents**
  - eliminate the need for multiple tests on the same sample

- **Collect Useful Patient Information**
  - identify biphasic curves indicative of DIC
  - identify low fibrinogen samples
  - identify low factor activity

- **Troubleshoot**
  - sample problems
  - instrument problems.
How do I interpret a Clot Signature Curve?

The interpretation of clot signature curves can be simple or complex depending on the factors influencing the curve. Normal test results typically show the characteristic “S” (sigmoidal) shaped curve. The factors which can influence a clot curve are innumerable. However, it is not difficult to become adept at interpreting common curve characteristics seen in clinical conditions or errors and warnings reported by the ACL Advance.

In interpreting clot signature curves, please note that the ACL Advance will alter the size of a clot curve plot to fill the screen. A plot showing a small change in optical density will fill the screen as much as a plot showing a large change. **Always note the range of optical readings on the Y-axis.** Additionally, this scale reflects the raw counts (amount of light hitting the detector of the reading unit in the ACL Advance Analytical Module) and are displayed in reverse order; decreasing values going up the y-axis.

<table>
<thead>
<tr>
<th>Curve Characteristic</th>
<th>Possible Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat Curve (no indication of clotting)</td>
<td>• lipemia or other interfering substance masking clotting activity</td>
</tr>
<tr>
<td></td>
<td>• low fibrinogen</td>
</tr>
<tr>
<td></td>
<td>• excessive anticoagulant</td>
</tr>
<tr>
<td></td>
<td>• reagent problem</td>
</tr>
<tr>
<td></td>
<td>• sample problem</td>
</tr>
<tr>
<td></td>
<td>• instrument liquid handling problem</td>
</tr>
<tr>
<td>Shallow Acceleration Phase</td>
<td>• slow clot progression due to slow conversion of fibrinogen to fibrin (similar to “Flat Curve“)</td>
</tr>
<tr>
<td>Small Change in Raw Counts from Baseline to Endpoint</td>
<td>• If extremely small OD change and very short clot time, noisy baseline causing false result.</td>
</tr>
<tr>
<td></td>
<td>• Low Fibrinogen concentration</td>
</tr>
<tr>
<td>Large Change in Raw Counts from Baseline to Endpoint</td>
<td>• High Fibrinogen concentration</td>
</tr>
<tr>
<td>Endpoint drops towards Baseline</td>
<td>• Unstable clot</td>
</tr>
<tr>
<td>Endpoint rises</td>
<td>• Clot formation is still progressing</td>
</tr>
<tr>
<td>Acceleration phase seen but no Endpoint</td>
<td>• Clot completion did not occur within the Acquisition Time</td>
</tr>
<tr>
<td>Long Baseline</td>
<td>• Prolonged/impaired clotting mechanism</td>
</tr>
</tbody>
</table>
How do I investigate Errors and Warnings using Clot Signature Curves?

Errors and warnings related to sample analysis are derived from the raw data and checks on this data carried out by the ACL Advance. Since this is the same raw data used to plot the clot curve, many errors and warning conditions can be seen on the clot curve. The limits for errors and warning are defined in the **Test Definition; F8 Definition of Legal Coag Limits and Check Limits**.

<table>
<thead>
<tr>
<th>Limit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start Point Lamp Low</td>
<td>Result will fail if first point of Baseline is below the defined raw count value: Error 2 (Optics).</td>
</tr>
<tr>
<td>Start Point Lamp High</td>
<td>Result will fail if first point of Baseline is above the defined raw count value: Error 1 (Optics).</td>
</tr>
<tr>
<td>Minimum Delta</td>
<td>Result will fail if this minimum change in optical density is not met: Error 8 (Coag Error).</td>
</tr>
<tr>
<td>Maxima 1st Derivative</td>
<td>Value determines when a peak will be considered a maxima peak. Failure produces Error 17 (Coag Error).</td>
</tr>
<tr>
<td>Maxima 2nd Derivative</td>
<td>Value determines when a peak will be considered a maxima peak. Failure produces Error 17 (Coag Error).</td>
</tr>
<tr>
<td>Minima 2nd Derivative</td>
<td>Value determines when a peak will be considered a minima peak. Failure produces Error 17 (Coag Error).</td>
</tr>
<tr>
<td>ROI Limit of Maxima Peak</td>
<td>Time (seconds) of the maximum time allowed for the calculated region of interest (ROI) around the maxima peak. Failure produces Error 14 (Coag Error).</td>
</tr>
<tr>
<td>ROI Limit of Minima Peak</td>
<td>Time (seconds) of the maximum time allowed for the calculated region of interest (ROI) around the minima peak. Failure produces Error 15 (Coag Error).</td>
</tr>
<tr>
<td>Delta of Minima and Maxima D2 Peak</td>
<td>Maximum delta allowed between the normalized curve values located at the minima peak and maxima peak of the 2nd derivative curve. This value helps eliminate false 2nd derivative peaks. Failure produces Error 17 (Coag Error).</td>
</tr>
<tr>
<td>Max SD Limit</td>
<td>Maximum SD allowed for either the endpoint stability check or linear regression check (chromogenic tests). Failure produces Error 9 (Coag Error) for clotting assays or Error 10 (Coag Error) for tests using the Photometric Linear Kinetic algorithm.</td>
</tr>
</tbody>
</table>
Additional Checks

**Get Min and Max of Raw Curve**
The raw data curve is searched for the minimum and maximum raw values. The minimum value must follow the maximum value. If the maximum value follows the minimum value the curve is invalid and an error is produced:

Error 6 (Coag Error) - Curve minima and maxima are not in correct sequence.

**Check Curve Basics**
Checks for basic curve integrity. These checks include verifying that the curve has data points and that data points were collected past the end of the delay period (during the acquisition time). If this check fails, an error is produced:

Error 5 (Data) Missing data points.

**Check Last Point**
This function checks the validity of the last point in the data curve. The minimum and maximum values of the raw data curve are found (the minima of the curve must follow the maxima). The difference between these points represents the total signal or change in optical density of the data curve. The last point in the data curve is checked to ensure that it is not less than 50% of the total signal of the data curve. If it is, the Endpoint is drifting downwards and the curve fails. The failure produces an error:

Error 7 (Coag Error) Endpoint drift.

**Check Test Range (Measured Result)**
This function checks the measured result against a test range for validity. If the measured result is outside of the test range, the measured result will be considered “failed”.

Error 21 (Range) Measured Result out of test range low.
Error 22 (Range) Measured Result out of test range high.
<table>
<thead>
<tr>
<th>Common Errors</th>
<th>Description</th>
<th>Possible Clot Curve Observation</th>
<th>Possible Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error 1 (Optics)</td>
<td>Starting point out of range high</td>
<td>Curve starts at very high raw value</td>
<td>Interfering substances, Optics Autoranging not functioning properly</td>
</tr>
<tr>
<td>Error 2 (Optics)</td>
<td>Starting point out of range low</td>
<td>Curve starts at very low raw value</td>
<td>Sample may be lipemic, Optics Autoranging not functioning properly</td>
</tr>
<tr>
<td>Error 4 (Data)</td>
<td>Curve does not maintain continuity between points</td>
<td>One or more data points are located off of the data curve</td>
<td>Noisy data, Movement of cuvette during reading</td>
</tr>
<tr>
<td>Error 6 (Coag Error)</td>
<td>Curve minima and maxima not in correct sequence</td>
<td>The Baseline is higher than the Endpoint</td>
<td>Low fibrinogen, Acquisition Time ended before clot formation</td>
</tr>
<tr>
<td>Error 7 (Coag Error)</td>
<td>End point drift</td>
<td>Endpoint drifts downward excessively</td>
<td>Low fibrinogen, Acquisition Time ended before clot formation, Improper reagent placement</td>
</tr>
<tr>
<td>Error 8 (Coag Error)</td>
<td>Minima delta not found</td>
<td>Small change in optical density</td>
<td>Low fibrinogen, Acquisition Time ended before clot formation, Improper reagent placement</td>
</tr>
<tr>
<td>Error 9 (Coag Error)</td>
<td>Endpoint SD exceeded</td>
<td>Endpoint drifts excessively</td>
<td>Acquisition Time ended before clot formation, Low Fibrinogen</td>
</tr>
<tr>
<td>Error 11 (Coag Error)</td>
<td>Threshold value not found in data curve</td>
<td>Flat curve, Curve does not pass Threshold value within Acquisition Time</td>
<td>Acquisition Time ended before clot formation, Low fibrinogen</td>
</tr>
<tr>
<td>Error 17 (Coag Error)</td>
<td>Min/Max peak criteria not met</td>
<td>No Endpoint, Small change in optical density, Biphasic curve</td>
<td>Acquisition Time ended before clot formation, Low Fibrinogen</td>
</tr>
<tr>
<td>Error 18 (Coag Error)</td>
<td>Threshold check not found in data curve</td>
<td>No Endpoint, Small change in optical density, Biphasic curve</td>
<td>Acquisition Time ended before clot formation, Low fibrinogen</td>
</tr>
<tr>
<td>Error 21 (Range)</td>
<td>Measured result out of test range low</td>
<td>Clot point below defined Test Range lower limit (see Test Definition)</td>
<td>Improper reagent placement, Sample problem</td>
</tr>
<tr>
<td>Error 22 (Range)</td>
<td>Measured result out of test range high</td>
<td>Clot point above defined Test Range upper limit (see Test Definition)</td>
<td>Acquisition Time ended before clot formation, Improper Test Range defined in Test Definition, Sample problem</td>
</tr>
<tr>
<td>Error 23 (Parent)</td>
<td>Parent test failed</td>
<td>Parent Test for Shadow Test (e.g. PT for PT-based Fib) failed</td>
<td>Acquisition Time ended before clot formation, Sample problem</td>
</tr>
</tbody>
</table>
Appendix A

Sample Curves
Figure 3 - Short PT

This clot signature curve depicts the clot formation for a Prothrombin Time test with a short result. The curve has a short baseline, rapid acceleration, and a stable, flat endpoint. This shape is typical for the PT test and assays based on this test. The algorithm used for the PT test determined the clot time to be 10.75 seconds, the point where the change in rate of clot formation was the greatest (second derivative). Longer PT test curves usually show the same shape but have a longer baseline which shifts the rest of the curve further along the time scale on the x-axis.
Figure 4 - PT, Prolonged

This PT was run in duplicate and produced prolonged results; Measured 1 = 41.40, Measured 2 = 42.35. This prolongation can result from oral anticoagulant therapy, factor deficiency, or the presence of inhibitors. This curve resulted from excessive warfarin.
Figure 5 - PT-Based Fibrinogen, low

These curves describe a sample tested for fibrinogen using the PT-based fibrinogen assay. This assay is based on the change in raw counts (delta raw) for the PT. The sample delta raw is related to a calibration curve for the PT-based fibrinogen assay to provide a quantitative, measured fibrinogen value. The horizontal lines extending from the clot curves to y-axis indicate where the raw readings were taken. In this case the delta raw was low, so the fibrinogen result will also be low.

These curves also show “shift”. The curves have very similar characteristics however they start at different raw count values and are not overlaid. This occurs due to Autoranging and though visually apparent it has no significance regarding the results.
Figure 6 - PT-Based Fibrinogen, high

This PT-Based fibrinogen curve shows a large raw delta indicative of a high fibrinogen concentration.
Figure 7 - Prolonged APTT, Duplicate testing

Figure 7 shows the clot curves for a prolonged APTT analyzed in duplicate using an extended Acquisition Time (240 seconds). When viewing the curve for the sample both curves of the duplicate determination are plotted for comparison. The overlapping lines drawn from the clot curves to the x-axis denote the clot time; in this case 110.5 seconds and 111.1 seconds. The shape of the curves is typical for the APTT and APTT-based tests. Prolongation of the APTT test as seen in this example may be caused by heparin therapy, factor deficiency, or inhibitors.
Figure 8 - Prolonged APTT, Duplicate testing

This curve shows the plots for duplicate APTT determinations which have prolonged results. This example differs from the previous example (Figure 7) in that the acceleration phase is not a near vertical line and the endpoint is not horizontal. This indicates slower clot formation during acceleration and residual clot formation during the endpoint.
Figure 9 - Prolonged APTT, Single determination

This curve shows a prolonged APTT test result run in single determination using the standard acquisition time (120 seconds).
Figure 10 - APTT, Coag Error 8

This curve is very abnormal in appearance and generated a Coag Error 8. The key to determining what caused the error and why the curve has this appearance is the y-axis and the change in raw counts (delta raw). The delta for this test is quite small, approximately 225 raw counts. Coag Error 8 is “Minima delta not found”. The minima delta is defined in the F8 Definition of Legal Coag Limits and Check Limits section of the Test Definition for APTT as 250. Since the test delta raw (225) was less than the defined minima (250), the ACL Advance determined that a clot had not formed within the defined Acquisition Time (120 seconds). The sample should be rerun using extended acquisition time as this curve may represent only the baseline and beginning of the acceleration phase of clot formation.
The Coag Error 8 was generated because the APTT test requires a delta greater than 250 raw counts for clot determination but only a delta of 225 was produced. The typical APTT curve shape is still depicted with the baseline, acceleration, and endpoint. In this case it was determined the sample had an abnormally low fibrinogen concentration. Since the delta is dependent on the conversion of fibrinogen to fibrin, the low fibrinogen level resulted in a low delta.
The error “Coag Error 8” was generated on this sample. Coag Error 8 relates to the minima delta not being met or exceeded for the APTT test. The limited delta shown by both determinations was due to a low fibrinogen content in the sample. Another abnormality shown by the clot curve plot is biphasic curves; the curves show clot formation (significant change in optical density) during both the baseline phase and acceleration phase of clot formation. Biphasic curves are often indicative of Disseminated Intravascular Coagulation, as was the case with this patient. DIC results in clotting protein consumption, including the reduction of available fibrinogen.
Figure 13 a & b - APTT, Duplicate testing - Problematic Lyophilized Sample

These curves were produced by the analysis of a lyophilized proficiency survey sample. The survey sample was analyzed as part of a larger run which included patient samples. All other samples in the run showed normal clot curves with acceptable results. This indicates a sample related problem rather than an instrument or reagent related cause.
Figure 14 - APTT, Coag Error 9

The Coag Error 9 generated for this sample was due to the Endpoint SD being exceeded. If the Endpoint climbs and exceeds the pre-defined Endpoint SD value, the curve fails. In this case the acceleration phase continues at the end of the Acquisition time (start of endpoint); therefore, the optical density is still changing rapidly and exceeds the Endpoint SD value. The sample should be reanalyzed using extended acquisition time.
Figure 15 - PT, Flat Curve (Water)

This curve was the result of running a PT test on water. The curve is extremely flat and shows little change in optical density. There is no clot formation.
This APTT test showed almost no change in optical density and generated a Coag Error 6. The Coag Error 6 “Curve minima and maxima not in correct sequence” was reported because a point in the baseline is higher than the endpoint. The cause of the problem was that the APTT Cephalin and PT reagent vials were reversed in the reagent block.

Figure 16 - APTT Test, APTT Cephalin and PT reagent reversed
Figure 17 - PT Test, PT-Fib reagent and APTT Cephalin reversed

This curve demonstrates the other possible occurrence to the reagent reversal noted in Figure 16. The PT was run on the sample, accidentally using APTT Cephalin instead of PT-Fib reagent. Clot formation did not occur as can be seen by the insignificant change in raw counts. Coag Error 6 “Curve minima and maxima not in correct sequence” was reported because a point in the baseline is higher than the endpoint.
Figure 18 - PT-Fib, Error 7 Unstable Endpoint

This curve failed and produced an Error 7 “Endpoint Drift”. This error was the result of the Endpoint of the curve dropping down towards the baseline after clot formation. This drop exceeded 50% of the delta of the entire curve. This indicates a very unstable endpoint and that the clot is possibly dissociating. The limited delta shown by the curve coupled with an unstable endpoint is indicative of a low fibrinogen concentration.
Figure 19 - PT-Fib, Error 8 Minima Delta not found

This curve appears very similar to Figure 18. In this case the unstable endpoint was not as severe as that shown in Figure 18 and was not the cause of the curve failure. The Coag Error 8 “Minima delta not found” was generated due to insufficient change in raw counts caused by a low fibrinogen concentration.
Figure 20 - PT-Fibrin Calibration, Coag Error 6

This curve resulted during a PT-Fibrinogen HS calibration. The Coag Error 6 was generated because the baseline value was higher on the curve (lower raw reading) than the endpoint. The problem was resolved by using a fresh aliquot of Factor Diluent.
Figure 21 - APTT Test, Only Cephalin added

This APTT test curve resulted from the addition of APTT Cephalin to the sample, but no calcium chloride was added to initiate clotting. A possible aspiration problem or bubble in the bottle on top of the calcium chloride could lead to no or insufficient calcium chloride being added to the test cuvette. Clotting does not occur or occurs very slowly without the addition of calcium chloride, so the curve shows a gradually climbing acceleration phase, with little overall change in raw delta. Coag Error 17 “Minimum/Maximum peak criteria not met” resulted from a poor acceleration phase giving an indistinct clotting point.
Figure 22 - APTT, Data 4 Error

The data for this APTT curve generated a Data 4 error “Curve does not maintain continuity between points”. At approximately 122 seconds the curve suddenly climbs again rapidly (5 data points). The probable cause for this error was a centrifuge located on the bench next to the analyzer. The problem was experienced when the centrifuge was running and samples were being analyzed at the same time.
Figure 23 - APTT, Duplicate testing - Coag Error 8, Heparin

This sample was the first sample collected following the administration of a heparin bolus (porcine). The method and site of collection is unknown but the APTT test showed very little clot formation even when using the extended acquisition time. Coag Error 8 was generated due to insufficient delta raw for the curve.
Figure 24a - PT, Duplicate testing - High Hematocrit, uncorrected

This curve was produced from a sample collected from a patient with a hematocrit of 65%.
No correction was made to the amount of anticoagulant used for collection.
**Figure 24b - PT, Duplicate testing - High Hematocrit, corrected**

This sample was collected for repeat PT testing on the patient from Figure 24a. This sample was collected using a corrected amount of anticoagulant to compensate for the patient’s high hematocrit.

![Graph showing PT test results with measured times]
Figure 25 - PT, Optics Error 2

This flat PT curve shows virtually no clot formation. A clue to the problem is the raw optical reading at the start of the curve. This low reading generated the Optics 2 error “Starting point out of range low”. The Start Point Lamp Low value pre-defined in the PT Test Definition is 4500 raw counts; therefore, this curve starts at a raw count value that is too low for accurate readings to be collected. The extremely low raw count value indicates the contents of the cuvette is very optically dense. The most frequent cause for this error is extreme lipemia.
Figure 26 - PT-Fibrinogen, Lipemia

This is another example of a lipemic sample and the resulting flat PT curves. The low initial raw optical density generated the Optics 2 error range.
This curve was the result of accidentally running an APTT test with the PT reagent and calcium chloride positions reversed. The error generated, Range 21 “Measured result out of test range low”, occurred because the clot point for the sample was determined to be less than the lower end of the Test Range defined in the Test Definition for the APTT. The default Test Range for APTT is 20.0 - 240.0 seconds. This rapid clot formation resulted because PT reagent was added to the test cuvette rather than calcium chloride and resulted in a “PT-like” test being run instead of an APTT.
Figure 28 a & b - APTT, Range 22

Figure 28a resulted from testing a sample for APTT. The clot curve appears normal but the error Range 22 "Measured result out of test range high" was generated. The Test Range as defined in the Test Definition was checked and found to be incorrect (default APTT Test Range is 20.0-240.0 seconds). The Test Range was corrected and the sample reanalyzed, resulting in Figure 28b and a result of 83.60 seconds. Figure 28b is almost identical to 28a but the error was not generated.
Figure 29 - PT-Fibrinogen, Poor Sample Handling, Optics 2

This plasma sample was frozen improperly, thawed improperly, and then not mixed prior to analysis. The result was an erratic clot curve and an Optics 2 error. The Start Point for this curve is approximately 3425 raw counts, well below the Start Point defined for the PT assay (Start Point 4500).
Figure 30 - APTT, Improper storage and thaw

The sample which produced this curve was poorly frozen and thawed. This treatment probably had a detrimental effect on the clotting proteins and therefore the clotting mechanism. The curve (particularly the baseline) is slightly erratic but acceptable and the data did not generate any errors.
• ACL Advance Operator's Manual, Instrumentation Laboratory.
A series of Monographs devoted to relevant topics in the field of Hemostasis.


5. The Anticoagulants: Protein C and Protein S. M. R. Ledford.


10. D-Dimer. J. Serra (available also in German).


12. Clot Signature Curves and the ACL ADVANCE. K. Doubleday, S. Kumnick (available also in German).
