

APC RESISTANCE TESTING – TECH TIP

RATIOS AND CLOTTING TIMES

- When performing Coatest® APC Resistance and Coatest® APC Resistance V/V_S, pay attention to the clotting times as well as your ratio.
- The baseline APTT time (only CaCl₂), which is the bottom number in your ratio, should be within the range of APTT times you observed when you established when determining your cut-off.
- Typical values are about 30 - 45 seconds, and will vary between labs and instrumentation.

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BASELINE CLOTTING TIMES

It's important to flag any results from patient plasmas with a baseline APTT time that lies outside of your normal distribution to avoid misleading APC ratio results

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DILUTION IN FACTOR V DEF PLASMA

Although the 1:4 pre-dilution with Factor V Deficient Plasma strongly decreases interferences, prolonged baseline APTT may occur in patient plasmas with high inhibitor activity (e.g. phospholipid antibodies).

- In that case, increasing the dilution (e.g. 1:9 or 1:19) may correct the result. If the additional dilution with Factor V Def Plasma does not correct the clotting time, then the calculated APC ratio is not valid, and it is recommended to use PCR results to determine APC resistance.

Use only the Factor V Deficient Plasma supplied in the kit

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CUT-OFF DETERMINATION

Calculating the Cut-off:

- A cut-off should be established with each new lot
- Use at least 30 patient plasma samples from healthy individuals
- Include Control Plasma Level 1 (normal) and Level 2 (heterozygous) for QC
- Determine the MEDIAN ratio...not the average...you may have an outlier in that supposedly normal population
- Multiply the median ratio by 0.8 if the ratio is below 2.8 OR by 0.75 times the median ratio when 2.8 or higher

APC R – TROUBLESHOOTING

RECALCULATION OF CUT-OFF

Recalculation of cut-off may be needed periodically

- How do you know if you have to?
 - Look back at last 30 normal patients, find the median ratio, and recalculate the cut-off as described in the package insert
 - Compare this cut-off to your current cut-off, and decide whether it needs to be changed using new normal plasma

APC R – TROUBLESHOOTING CONTROLS

Quality Control:

- Chromogenix Control Plasma Level 1: Normal
 - Ratio should be in normal range
- Chromogenix Control Plasma Level 2: Heterozygous for FV Leiden
 - Ratio should be BELOW the cut-off
- Chromogenix Controls should be used

APC R – TROUBLESHOOTING LOT-TO-LOT VARIATION

Some lot-to-lot variation is expected due to the nature of the assay

- APC is added to activator to adjust the APC/CaCl₂ clotting time to keep ratio within about +/- 5% of prior lots

APC R – TROUBLESHOOTING CONTROLS

What if my controls don't come in range?

- Are you using the Chromogenix controls (normal and heterozygous)?
- Chromogenix controls are tested to have specific clotting times and ratios
- If using a different brand of controls, test the kit with the Chromogenix controls and see if they are within range

APC R – TROUBLESHOOTING INSTRUMENT EFFECT

What role does the coagulation analyzer play?

- Kits are validated on the ACL line of instruments
- Clotting times can vary depending on the instrument, especially with optical vs. electromechanical clot detection principles
 - Sometimes baseline clotting times are more prolonged on electromechanical vs. optical instruments
- Cut-offs should be calculated for each analyzer

Changing analyzers = validate the assay

APC R – TROUBLESHOOTING REAGENTS

Reagents are carefully standardized

Use only the Factor V deficient plasma supplied in the kit

- This is not a reagent to be used in FV mixing studies, but is specifically formulated with concentrations of FV and FVIII to work with the aPTT reagent in the Coatest APC R-V kit

Don't mix reagents from different lots

Proper storage matters

- Don't freeze the aPTT
- Mix the aPTT well; don't let it settle
- APC/CaCl₂, FV-def plasma, control plasmas can be frozen, but thaw rapidly at 37°C and don't refreeze (only 1 freeze/thaw cycle)

QUESTIONS?

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Questions about any product in our line-up? We're just a click or phone call away.

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