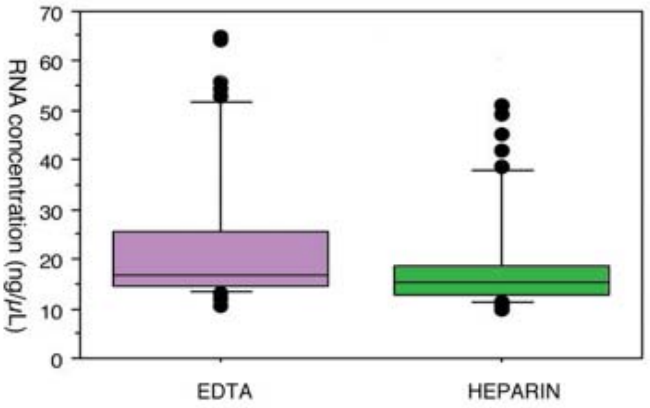
Specimen Collection for Quantitative PCR Assays

(P BCR ABL and P PML RARA)

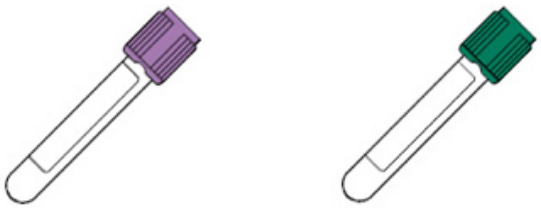
Optimal sample quality is a prerequisite to generate valid results for detecting minimal residual disease (MRD) by quantitative PCR in Chronic Myelogenous Leukemia (CML) and Acute Promyelocytic Leukemia (APL). This bulletin provides suggestions to prevent receiving a "quantity not sufficient" (QNS) report. Some sample submission factors that may affect sensitivity and validity are discussed below.

What Is the Appropriate Specimen Type?

EDTA (purple top tube) is the preferred anticoagulant for either peripheral blood or bone marrow aspirate. Sodium heparin (green top tube) may be used, but recovery of RNA may be adversely affected.



RNA concentrations isolated from the different anticoagulants



EDTA vs. sodium heparin

What Is the Appropriate Amount?

- The minimum volume of peripheral blood is 10 mL.
- The minimum volume for bone marrow aspirate is 1 mL.

The patient's peripheral blood WBC is needed to determine if a larger volume should be submitted. For leukopenic patients additional tubes (15 – 20 mL peripheral blood in EDTA) are required. Note the patient's most recent WBC result on the requisition.

What Are Appropriate Transport Conditions and Timing?

For optimal recovery of RNA, specimens should be sent with cold packs, but cannot be allowed to freeze. The sample should be sent the same day of collection to be received within 24 hours. Avoid shipping on Friday. Samples must be rejected if received in the laboratory greater than 48 hours from time of collection.

Why Is So Much Blood Needed So Quickly?

Following patients for minimal residual disease (CML and APL) relies on accurate isolation and quantitation of RNA, not DNA. RNA is extremely labile (degrades rapidly with time and increased temperature). As you can see from the graph, RNA concentration drops off quickly over time. The test requires approximately 9 µg of isolated RNA and the yield is only about 1 µg RNA per mL blood submitted. Therefore, the required volume is 10 mL of blood to recover this amount.