

# Answering your questions

### Must an ESR be completed within two hours?

**Q** What is the longest stability on an EDTA tube on which one can process an erythrocyte sedimentation rate (ESR)? Our policy is to perform all ESRs within two hours of collection. Is there any documentation as to the longest one can wait after collection to perform an ESR on the sample?

**A** The most important consideration initially would be the labeling or instructions provided by the manufacturer of the test and collection tube. If the test is FDA-approved or cleared, then the test must be performed according to manufacturer's instructions for sample stability. Most waived or moderate-complexity tests have instructions relating to specimen stability. If you find that the manufacturer's instructions do not provide enough time to complete testing, then it is possible to validate a longer processing time for the test. Be aware, however, that this may change the complexity of the test from waived or moderate-complexity to high-complexity. This has implications for the personnel eligible to perform the test as well as the extent of in-house validation required prior to testing.

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In many cases, the requirements for validation become similar to the validation of a lab-developed test, which requires much more documentation than verifying the performance characteristics of an FDA-approved assay.

If neither the manufacturer of the test or collection tube dictates that the test be completed within two hours, but your lab policy states this limitation, then you do have options to expand your acceptable

processing times. Good lab practice would dictate some type of stability study in this instance, though it would not be strictly required as long as you are using the test within manufacturer specifications. If the FDA labeling allows longer transport or storage times but some staff are not comfortable with that, then a small internal study would be warranted. In our institution, we are using an ESR method that allows up to eight hours before testing if samples are stored correctly; and we have validated internally that the test is stable over that time.

—Brad S. Karon, MD, PhD

### Is it necessary to protect all bilirubin specimens from light?

**Q** The usual practice for neonatal bilirubin is to protect the specimen from light, but no such practice is used for specimens obtained from adults. Since bilirubin is degraded by light, should we protect all specimens from light? What should be the practice on jaundiced adults?

**A** In the clinical laboratory, it is well recognized that bilirubin concentrations decrease when specimens are exposed to light. The phenomenon of light decreasing bilirubin concentrations in blood, identified in the 1950s,<sup>1</sup> is the basis of today's current treatment for hyperbilirubinemia in infants — phototherapy. While protecting specimens from light is well documented in hyperbilirubinemic samples,<sup>2</sup> it has also been documented in more recent literature where the photodegradation of bilirubin was examined in human serum with “normal” bilirubin concentrations.<sup>3</sup> Whether the photodegradation of bilirubin will result in a clinically significant change in concentration depends on the clinical circumstance in which the testing was requested.

The mechanism of phototherapy uses light (blue light works particularly well) to create different isomers of unconjugated bilirubin. Configurational

changes of unconjugated bilirubin, specifically bilirubin IX alpha, involve the rotation of carbon-carbon bonds on the bilirubin carbon atoms designated 4 and 15. These changes result in decreased hydrophobicity of the molecule, resulting in excretion from the body.

Thus, the measurement of bilirubin is subjected to photodegradation both *in vitro* and *in vivo*. The clinical laboratory can only affect the *in vitro* formation of bilirubin photoisomers by protecting samples from light, from sample collection to analysis. In addition, samples being collected for direct bilirubin analysis should be “promptly cooled to 4°C” and minimally kept at room temperature to prevent their degradation from artificially inflating the unconjugated bilirubin concentration.<sup>4</sup> Protecting all bilirubin specimens from light degradation can be impractical, though not impossible. Each clinical laboratory's circumstances are different; thus, each laboratory's ability to minimize bilirubin samples to room light may be different. □

—Stanley F. Lo, PhD

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#### References

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