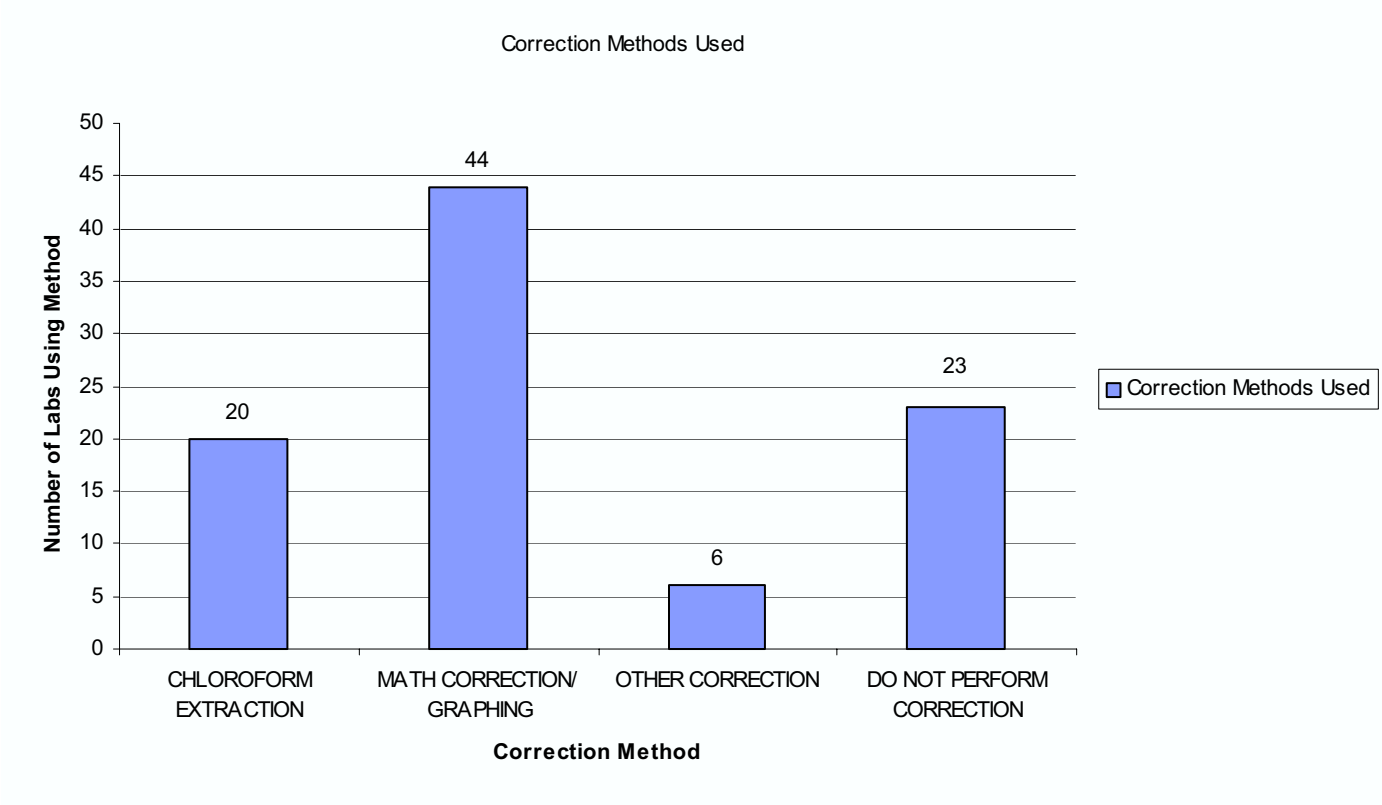
## Supplemental Question Discussion for 2005 LM-A.

This mailing included the following supplemental question regarding the ΔOD450 test.

When performing analysis for amniotic fluid bilirubin, how does your laboratory correct for potential blood interference?

A. Chloroform extraction
B. Mathematical (e.g., Allen) correction/graphing
C. Other correction; please specify
D. We do not perform a correction

The distribution of responses is shown in the following graph.




These results indicate that there is no clear consensus on the method to correct for blood interference, or even that a correction is necessary. Since critical patient management decisions may be based on the ΔOD450 test, laboratories must have a consistent, clinically relevant procedure to deal with this problem.

Liley described the basis for the ΔOD450 test.[1] One can assess the degree of intrauterine hemolysis in hemolytic disease of the newborn (HDN) by measuring the absorbance of bilirubin pigments in amniotic fluid (ΔOD450) and classifying the results based on gestational age into low risk, intermediate risk, and high risk zones. Queenan proposed an alternative risk categorization scheme based on the ΔOD450 test.[2] With both systems, the severity of HDN is more accurately predicted with serial ΔOD450 measurements. A typical procedure for ΔOD450 is described by Ashwood.[3] Because amniocentesis is an invasive procedure, there is a risk that the amniotic fluid specimen may be contaminated with hemoglobin, which

has an absorbance peak at 410 nm. The amniotic fluid specimen should be centrifuged before spectrophotometric scanning to remove red blood cells or other particulate matter. If there is an oxyhemoglobin peak at 410 nm, one can correct the ΔOD450 by subtracting 5% of the ΔOD410.

An alternative correction for the presence of hemoglobin in the amniocentesis specimen is extraction of the bilirubin fraction into chloroform before performing the scanning spectrophotometry.[4,5] However, chloroform extraction reduces the ΔOD450, even when no hemoglobin is present.[6] If chloroform extraction is used, all specimens should be subjected to chloroform extraction, not just the specimens that are visibly contaminated with blood. Otherwise, ΔOD450 results obtained on two serial amniotic fluid specimens from the same patient, one of which was extracted and the other of which was not extracted, may lead to an incorrect interpretation about the trend of the ΔOD450 measurements and the clinical course of the HDN. Furthermore, since the Liley graph that is used to interpret the ΔOD450 result was created using unextracted material, some authors have suggested that a new Liley graph should be constructed using chloroform-extracted material.[6]

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**References:**

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2. Queenan JT, Tomai TP, Ural SH, King JC. Deviation in amniotic fluid optical density at a wavelength of 450 nm in Rh-immunized pregnancies from 14 to 40 weeks' gestation: a proposal for clinical management. *Am J Obstet Gynecol*. 1993;168:1370-6.
3. Ashwood ER. Determnation of ΔA450 in amniotic fluid. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry, 3rd ed. Philadelphia: WB Saunders, 1999:1768-70.
4. Hochberg CJ, Witheiler AP, Cook H. Accurate amniotic fluid bilirubin analysis from the "bloody tap". A preliminary report. *Am J Obstet Gynecol*. 1976;126:531-4.
5. Spinnato JA, Ralston KK, Greenwell ER, Marcell CA, Spinnato JA 3rd. Amniotic fluid bilirubin and fetal hemolytic disease. *Am J Obstet Gynecol*. 1991;165:1030-5.
6. Foster K, Moore J, Hankins K, Parvin CA and Gronowski. Effect of blood contamination on delta 450 bilirubin measurement: an in vitro comparison of two corrective methods. *Clin Chem*. 2004;50:1420-2.