Technical Briefs (~300 words text) summarize findings that are of interest to a relatively limited audience. Readers desiring fuller details may obtain them by writing directly to the author(s) at the address given. Briefs dealing with procedure or instrumentation intercomparisons, evaluations, or improvements (including kit applications) should be sent to Clinical Chemistry News, 2029 K Street, Washington, DC 20006.


The "Synchron CX3 System" (Beckman Instruments, Inc., Brea, CA 92821) measures Cl with a two-phase Ag/AgCl ion-selective electrode (ISE). The Cl result is calculated from the difference, SR, between the ADC (digital form of analog signal) numbers for electrolyte reference reagent (Cl^- = 100 mmol/L), IR, and for a sample, S; i.e., SR = S - IR. According to the manufacturer, Br in concentrations of 1.0 and 5.0 mmol/L increases the results for apparent Cl by 3% and 6%, respectively. We determined that repeat analysis of "Gilford Normal" (GN) control serum (Ciba Corning Diagnostic Corp., Irving, CA 92714), subsequently found to contain 1.1 mmol of Br per liter, adversely affected the precision of Cl measurements and caused intermittent "excessive reference drift" (ERD) error codes. Therefore, we measured Cl, sequentially, in aliquots (n =15) of GN control and Br-free control sample supplemented with Br (0.2–2.0 mmol/L). We monitored the ISE response by recording the ADC numbers.

The cumulative interference pattern for GN control and Br samples was the same (Figure 1). In general, the repeat analysis of aliquots caused gradual increases in both ADC numbers, the extent of which depended on the concentration of Br, and which, when large enough, caused ERD error. Except for the first aliquot of each sequence, the stepwise increments tended to be greater for S; i.e., the SR values and, therefore, the results for Cl were affected, the changes being significant when Br was ≥0.8 mmol/L, and there was also a significant carryover effect on Br-free samples (Figure 1). The ISE was normalized only by a repeat analysis of a Br-free sample. There was no interference or carryover effect when, in sequence, Br-containing aliquots were separated by at least one Br-free aliquot. However, analysis of a random sample with Br ≥2.5 mmol/L caused ERD error.

We conclude that Br ≤2.0 mmol/L does not affect Cl measurements unless repeated analysis is attempted, as would be in case in troubleshooting the method. We also suggest that all control materials intended for use with the CX3 should be tested for such cumulative interference.

Enzymic Measurement of Total Bile Acids Adapted to the Cobas Fara Centrifugal Analyzer, Naomi Quast Hanson and Esther F. Freier (Dept. of Lab. Med. and Pathol., Univ. of Minnesota Med. School, Box 198, Harvard St. at East River Rd., Minneapolis, MN 55455-9980)

Measurement of total bile acids can be simplified without loss of sensitivity or precision by use of an assay kit: EnzabiLe® (Nycomed, Oslo 4, Norway; distributed by Accurate Chemicals, Westbury, NY 11590). This direct spectrophotometric method makes use of the enzyme 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50), with linkage of the product NADH to nitroblue tetrazolium to generate a chromophore (1, 2). We have adapted this kit to the Cobas Fara (Roche Analytical Instruments, Inc., Nutley, NJ 07110) and found it easy to perform, accurate, and precise, giving results that correlated well with those by an enzymic–fluorometric method (3, 4).

To perform the assay, dilute sample and blank reagent with 8.0 mL of the kit's reconstituting buffer. Do not use the stop reagent. Run a test and a blank run on the Cobas Fara at 37.0 °C, using the reaction mode "R1-I1-S-T-AO."
Pipet 125 μL of test or blank reagent and, during the 60-s incubation, measure the absorbance (M1) at 50 s at 540 nm vs each respective reagent blank. Add 50 μL of sample and 40 μL of diluent and measure the change in absorbance (ΔA) at 540 nm for 360 s (A0, first 5 s, A1, interval 360 s). The ΔA values of each run are calculated as the difference of the A0 reading at 360 s and the M1 reading at 50 s. The difference in ΔA of the test and blank runs (net ΔA) is then manually compared with the net ΔA for Enzable standards (Nycomed). We used a reaction time of 6 min rather than 15 min as described for the kit assay, because bile acid concentrations of serum specimens changed <1% with this decrease in reaction time.

As Figure 1 shows, standards must be of the same matrix as samples for accurate results. When cholic acid standards were prepared in de-ionized water or in 60 g/L bovine serum albumin reagent, the slope of the standard curve (sensitivity) was decreased from that for the Enzable standards, which are lyophilized in a bovine serum matrix. Preparing the standards in a normal serum pool with negligible bile acid not only increased the sensitivity but also produced a line essentially the same as that obtained with Enzable standards. This is consistent with the findings of Qureshi et al. (2), who noted that bovine and human sera contain a factor that enhances the reaction. This factor is probably not protein, as evidenced by the lack of enhancement of the standard line prepared in bovine serum albumin. Contrary to the manufacturer's suggestion of making dilutions (up to four times) with isotonic saline, we dilute serum and bile fluid (regardless of dilution) with a fourfold dilution in saline of normal serum selected to contain little or no bile acid. This dilution solution maintains the serum enhancement factor at an optimal concentration. Bile fluids may also be diluted with saline, if aqueous standards are used.

Within-run precision (CV) for determinations of serum pools with normal and above normal bile acid concentrations were 4.3% (mean 5.6 μmol/L, SD 0.24 μmol/L, n = 24) and 1.2% (mean 35.6 μmol/L, SD 0.44 μmol/L, n = 25). The CV for 21 replicate inter-run determinations performed over 11 days was 5.1% (mean 37.5 μmol/L, SD 1.91 μmol/L).

Bile acid values determined with the kit (y) for 44 serum and bile specimens ranging in concentration from 2 to 320 μmol/L, with Enzable standards in bovine serum matrix, correlated well with those obtained by an enzymic-fluorometric method (x) with aqueous cholic acid standards (3, 4):

$$y = 0.992x - 1.62 \mu \text{mol/L}, S_{xy} = 5.45 \mu \text{mol/L}, r = 0.998.$$  

**References**


**Improved Staining Method for Thin-Layer Chromatograms of Amniotic Fluid Phospholipids**, B. Tamagno, V. Gatti, and F. Setal (Dept. de Patología, Facultad de Bioquímica y Farmacia, Universidad Nacional de Rosario. Address correspondence to this author, at: Rodríguez 827, 2000 Rosario, Argentina)

We have developed a simple, rapid, and convenient staining method for detecting phospholipids on thin-layer chromatograms of amniotic fluid (AF) by modifying Zinza-de’s reagent, originally used in the determination of phosphorus (1, 2).

After centrifuging 5-mL aliquots of AF for 5 min at 600 × g to remove cellular debris, we extract the lipids from the supernatant fluid and precipitate the phospholipids with cold acetone.

Extracts and standards in chloroform are streaked onto thin-layer chromatographic plates (plastic sheets, silicone gel 60, no. 5748; Merck, Darmstadt, F.R.G.), previously activated (100 °C, 30 min) and allowed to cool in a desiccator. We use the solvent system chloroform/methanol/ammonium hydroxide (580 mL), 5/30/3 by vol, for one-dimensional chromatography (3).

After developing the plates, we dry them in hot air until the odor of ammonia is no longer detectable, then leave them at 37 °C for 5 min, let them cool, and immerse them for about 5 s in staining solution. The staining solution (2) is prepared just before use by diluting fourfold with water the following reagent: To a solution of 10 g of sodium molybdate in 60 mL of 4 mol/L HCl, add 14 mL of 4 mol/L HCl containing 400 mg of hydrazinium chloride. Heat in a boiling water-bath for 20 min, cool, then add 14 mL of concentrated sulfuric acid and mix, with cooling. Adjust the volume to 100 mL with water, and store in a dark bottle at room temperature.

After the staining, we dry the chromatogram in a current of cold air (to prevent development of background color) until blue spots become visible.

We quantitate the phospholipids either densitometrically or by visual comparison with the spots produced by solutions of phospholipids of known concentration.

We have successfully used this procedure in assessing normal and high-risk pregnancies.

Advantages of this method as compared with commonly used methods for staining phospholipids in AF are: high reagent stability (the stock reagent shows no deterioration for more than six months); lower acid content; good sensitivity; and high contrast between spots and background.

![Fig. 1. Bile acid standard curves obtained with solutions of cholic acid diluted in water (Δ—Δ), in bovine serum albumin (Δ—Δ), and in serum (Δ—Δ) compared with that for Enzable standards (Δ—Δ)](image-url)

CLINICAL CHEMISTRY, Vol. 35, No. 7, 1989 1539