

14.1.06

AOAC Official Method 992.20 Cholinesterase in Blood

pH Method

First Action 1992

Final Action 1995

(Applicable for determining cholinesterase activity in whole bovine blood, < 0.1–0.60 pH/h.)

Results of the interlaboratory study supporting acceptance of the method:

$s_r = 0.035\text{--}0.04$; $s_R = 0.064\text{--}0.069$; $RSD_r = 6.6\text{--}8.6\%$; $RSD_R = 10.7\text{--}17.3\%$

A. Principle

Whole blood is lysed with saponin to release cholinesterase and pseudocholinesterase, and diluted in barbital buffer. Lysed blood is incubated with acetylcholine bromide, which is hydrolyzed by cholinesterase and pseudocholinesterase, yielding acetic acid and choline bromide. Acetic acid produced is measured by monitoring change in pH over 1 h. Cholinesterase activity is reported as pH/h.

B. Apparatus and Reagents

(a) *pH Meter*.—Reading 0.01 pH unit, with single-junction pH combination electrode.

(b) *Certified pH buffers*.—Commercial certified calibration buffers, pH 4.0 and pH 7.0.

(c) *Acetylcholine substrate*.—0.11M (2.0%). Dissolve 2.00 g acetylcholine bromide in 100 mL H₂O. Store, in 0.4 mL portions, at 4°C (stable 1 week) or at 10°C (stable 1 year).

(d) *Saponin solution*.—0.01%. Dissolve 0.1 mL saponin in 1.00 L H₂O.

(e) *Enzyme buffer*.—0.02M sodium barbital, 0.004M KH₂PO₄, 0.6M KCl. Dissolve 4.12 g sodium barbital (controlled substance in the United States, see *U.S. Code of Federal Regulations* 160.107), 0.55 g KH₂PO₄, 44.73 g KCl, and 28 mL 0.1M HCl in H₂O and dilute to 1 L.

(f) *External control specimen*.—Obtain several hundred mL cattle whole blood in heparin from animal(s) known not exposed to inhibitors. Store at 10°C in 2 mL aliquots. Ensure blood is not inhibited by 986.34 (see 17.4.04). Establish mean cholinesterase activity using following method on 20 replicates. Acceptable external control specimen has results within 2 standard deviations of mean.

C. Calibration of pH Meter

Calibrate pH meter, according to manufacturer's instructions, using certified pH 4.0 and 7.0 buffers prior to pH measurements. Buffers must be room temperature for calibration.

D. Procedure

Dilute 1.0 mL heparinized blood to 10 mL with saponin solution in 17 × 100 mm tube, and mix 10 s on Vortex mixer. Transfer 2.0 mL diluted test sample to 17 × 100 mm tube containing 2.0 mL enzyme buffer and mix on a Vortex mixer 10 s.

Prepare blanks by adding 2.0 mL saponin solution to 2.0 mL enzyme buffer. Analyze one blank and one external control specimen/each 10 test samples.

Calibrate pH meter, measure pH of blanks, external controls, and test samples. Record as pH₁ for each, respectively. Rinse electrode with water and pat dry between tests. Add 0.4 mL acetylcholine substrate to blanks, external controls, and lysed blood. Immediately record time and mix on a Vortex mixer each tube 10 s. Leave at room temperature. After ca 50 min, recalibrate pH meter. At 1 h, measure pH of blanks, external controls, and lysed blood. Record as pH₂ for each, respectively.

E. Calculations

Cholinesterase, $\text{pH/h} = \text{pH}_1 - \text{pH}_2$

If pH/h for blank is not zero, subtract average blank value from external controls and test samples. For lysed blood <0.1 pH/h, report as <0.1.

Reference: *J. AOAC Int.* **76**, 899(1993).