

14.1.05

AOAC Official Method 991.10 Cholinesterase Activity in Whole Blood Enzymatic–Spectroscopic Method First Action 1991 Final Action 1995

(Applicable to determination of cholinesterase activity in whole blood [0.1–2.0 mole/mL/min].)

Results of the interlaboratory study supporting acceptance of the method:

$s_r = 0.04\text{--}0.15$; $s_R = 0.05\text{--}0.20$; $RSD_r = 4.30\text{--}14.2\%$; $RSD_R = 6.99\text{--}19.3\%$

A. Principle

Whole blood is diluted with pH 8.0 phosphate buffer and analyzed by an enzymatic method with acetylthiocholine as substrate. In presence of enzyme, acetylthiocholine yields thiocholine + acetate. Thiocholine is reacted with dithiobisnitrobenzoate to form yellow reaction product, thionitrobenzoic acid, which is monitored at 412 nm. Activity is calculated as moles of substrate hydrolyzed per mL of whole blood per min, mole/mL/min.

B. Apparatus

(a) *Spectrophotometer*.—Set at 412 nm for assay (410 nm for evaluation of spectrophotometer performance). Single or double (preferred) beam, readable to 0.001 absorbance unit. With recording device to monitor absorbance change vs time.

(b) *Cuvets*.—1 cm, optical glass or disposable polystyrene.

(c) *pH meter*.—Readability 0.01 pH unit. Calibrate according to manufacturer's instructions.

C. Reagents

(a) *External control*.—SeraChem® Level 1 Clinical Chemistry Control Serum (Human) (Fisher Scientific), or equivalent normal serum control. Perform 10 or more replicate determinations to establish mean value. Acceptable range is 10% of that mean.

(b) *Potassium hydroxide solution*.—0.05M KOH. Dissolve 3.3 g reagent grade (85%) KOH in sufficient water to make 1 L.

(c) *Chromate solution*.—0.0400 g K_2CrO_4/L 0.05M KOH.

(d) *Phosphate buffer solutions*.—All solutions must be at room temperature prior to pH adjustment. (1) *Dibasic sodium phosphate solution*.—0.1M. Dissolve 26.808 g $Na_2HPO_4 \cdot 7H_2O$ in water and dilute to 1 L. (2) *Monobasic potassium phosphate solution*.—0.1M. Dissolve 13.609 g KH_2PO_4 in water and dilute to 1 L. (3) *Phosphate buffer, pH 7.0*.—To 100 mL reagent 1, add sufficient reagent 2 (ca 150 mL) to adjust to pH 7.00. Keep solution refrigerated. (4) *Phosphate buffer, pH 8.0*.—To ca 450 mL reagent 1, add sufficient reagent 2 (ca 50 mL) to adjust to pH 8.00. Keep refrigerated for long term storage. Warm to room temperature before use.

(e) *Acetylthiocholine iodide (ATCI) solution*.—0.075M. Dissolve 0.10835 g ATCI (Sigma Chemical Co. No. A 5751) in water and dilute to 5 mL. Keep frozen when not in use.

(f) *Dithiobisnitrobenzoic acid (DTNB) solution*.—0.01M. Dissolve 0.0396 g DTNB (Sigma Chemical Co. No. D 8130) in

ca 9 mL pH 7.0 phosphate buffer, add ca 15 mg $NaHCO_3$, and dilute to 10 mL with same buffer. Keep frozen when not in use.

D. Spectrophotometer Evaluation

Set wavelength to 410 nm. Adjust absorbance to zero with water in both sample and reference cuvetts. Remove water from sample cuvet, rinse cuvet 3 times with ca 0.5 mL portions of K_2CrO_4 solution, and then fill cuvet with same solution. Absorbance reading at 410 nm should be 0.186–0.210. If reading is not within this range, recheck instrument performance before proceeding and, if necessary, recalibrate instrument according to manufacturer's specifications.

E. Preparation of Control and Test Solutions

Prepare human serum control according to manufacturer's instructions. Dilute well-mixed whole blood 1:1000 with pH 8.0 phosphate buffer (buffer should be 22.0–25.5°C): Dilute 10 L whole blood to 10 mL or 100 L to 100 mL if 10 L pipettor is not known to be accurate for blood. (Note: If air-displacement pipet is used, follow procedure for viscous blood.) Pipet 3 mL well-mixed, diluted blood into cuvet. Prepare second cuvet identical to first to serve as blank.

F. Determination

All reagents must be allowed to reach ambient laboratory temperature before performing assay; acceptable limits are 22.0–25.5°C.

Add 50 L DTNB to each cuvet and mix well. Place one cuvet in sample position and one in blank position. Set to 0 absorbance at 412 nm.

Add 20 L ATCI reagent to sample cuvet only and mix well. Follow absorbance increase over time. Allow 60-s delay prior to first reading to ensure linearity of reaction. Let reaction proceed minimum of 5 min and record A every 60 s. Calculate change in A for each time interval. A/min should be constant to ensure linear kinetics. Correlation coefficient (r^2) for plots of A vs time should be 0.95–1.00.

Run external control in similar manner. Repeat assay if control value is not within acceptable range.

G. Calculations

Use linear portion of reaction curve to calculate activity.

Calculate A change/min as follows:

$$A/\text{min} = \frac{\text{Final } A - \text{initial } A}{\text{min}}$$

Calculate ChE activity as moles/mL/min = $A/\text{min} \cdot 73.53$.

$$73.53 = \frac{1000}{1.36 \cdot 10^4 \cdot 1} \cdot DF$$

where $1.36 \cdot 10^4 M^{-1} cm^{-1}$ = extinction coefficient of yellow anion; 1000 = conversion from mmoles/mL to moles/mL; 1 = pathlength, cm; and DF = dilution factor = 1000.

Reference: *JAOAC* 73, 616(1990).