

## 16.16.05

### AOAC Official Method 972.41 Urine on Grain

#### Urease-Bromothymol Blue-Agar Test First Action 1972

(Applicable to grains, seeds, and packaging materials that do not show fluorescent residues or where background fluorescence is present.)

#### A. Apparatus

(a) *Disposable trays*.—Microtiter<sup>®</sup> plates, flexible vinyl, flat-bottom (Dynatech Laboratories, Inc., 14340 Sullyfield Circle, Chantilly, VA 22021, USA), or equivalent.

(b) *Culture tubes*.—6 (od) 50 mm.

#### B. Reagents

(a) *Bromothymol blue (BTB) indicator solution*.—Soluble form (Fisher Scientific Co., B-100, or equivalent). Transfer 50 mg BTB to 20 mL glass-stoppered test tube and add 10 mL H<sub>2</sub>O. Add 1 drop H<sub>3</sub>PO<sub>4</sub> (1 + 9) and dissolve completely by stirring. Add ca 0.1M NaOH dropwise to dark green (pH 5.8–6.0). (This preparation is enough for 1 batch of test agar.)

(b) *Urease suspension*.—Grind 0.30 g urease in small mortar, add few mL H<sub>2</sub>O, and continue grinding. Slowly dilute to 15 mL with stirring.

(c) *Test agar*.—Add 0.75 g bacteriological grade agar and 0.30 g sodium benzoate to 300 mL cold water with vigorous stirring, and place on magnetic stirrer-hot plate, **945.75B(n)** (see 16.1.01); heat with stirring and boil gently 1 min. Cool to 45–48°C, add BTB solution, (a), and adjust to pH 5.5 (yellow-green), using 0.9–1.1M NaOH or 0.5M H<sub>2</sub>SO<sub>4</sub>. Add urease suspension to pH-adjusted, 45–48°C agar and readjust pH, if necessary. Divide agar into 2 equal portions in beakers. To one portion add 0.5 mL 0.1M AgNO<sub>3</sub>,

To other portion add 0.05M H<sub>2</sub>SO<sub>4</sub> (ca 0.2 mL) to adjust to match color shade or pH of first portion. Mix each portion thoroughly and let stand ca 5 min.

*Agar sensitivity test*.—Prepare known urea-contaminated seeds by spotting each seed with 0.5 L 0.25% aqueous solution of urea and let dry. Test these known samples as in C. If no color response is obtained with known grains, either obtain new lot of urease or increase urease content after checking activity as in **941.04A(b)** and **B** (see 4.3.01) so that suspension contains enough urease to convert 0.80 g urea, and repeat sensitivity test.

#### C. Test

Initially check test product (grain) for free alkali, using test agar containing AgNO<sub>3</sub> solution (45–48°C), by (a) or (b) below:

(a) *For immediate use*.—Add grain, or unknown test sample, to wells of disposable tray, **A(a)**, and, using dropping pipet, add agar until object is covered.

(b) *For storage and/or intermittent use*.—Add agar to culture tubes, **A(b)**, to  $\frac{1}{2}$  height. Transfer tubes to heat-sealable plastic bags, seal, and store at 4–1°C. Agar can be stored 120 days. To use, let stand 1 h at room temperature. Use small glass rod to force test object below surface of agar. Remove rod and shake down agar. Observe frequently for color change near surface of object.

If test for free alkali is negative (no color change of indicator), proceed with AgNO<sub>3</sub>-free test agar, analyzing test grain in similar manner. (Grains must be totally immersed during test.) Color change of indicator is yellow green blue, depending on concentration of NH<sub>3</sub> produced. Reaction usually requires 1–3 min to give detectable color. Time varies inversely with urea concentration. Spots from higher levels of urea continue to develop and enlarge for 10–12 min and then fade gradually.

Reference: *JAOAC* **55**, 76(1972).