

16.1.01

AOAC Official Method 945.75 Extraneous Materials (Foreign Matter) in Products

Isolation Techniques

First Action 1945

Final Action 1996

A. Definition of Terms

Extraneous materials.—Any foreign matter in product associated with objectionable conditions or practices in production, storage, or distribution; included are filth [see 970.66A(a)–(d) (see 16.1.02)], decomposed material (decayed tissues due to parasitic or nonparasitic causes), and miscellaneous matter such as sand and soil, glass, rust, or other foreign substances. Excluded are bacterial counts.

B. Apparatus

(Avoid use of polyethylene beakers, funnels, containers, etc., as insect fragments and rodent hairs adhere to apparatus made from this material.)

(a) **Aerator, water.**—Modify to produce fine, even spray of water by removing screen(s). If aerator has more than 1 disk, use only the one with small (ca 1 mm) holes. (No. 00200, Faucet-Queens, Inc., 550 Palwaukee Dr, Wheeling, IL 60090, USA, or equivalent.) Unit may be securely attached to faucet with heavy rubber tubing (wall thickness ca 3.2 mm) to provide suitable working distance from sieve and to allow some movement of sprayer head.

(b) **Autoclave.**—(1) *Slow exhaust type.*—Set “slow exhaust” to lower pressure from 15 to 0 in 15–20 min. (2) *Nonslow exhaust.*—Let cool to 0 psi before opening or venting.

(c) **Blenders.**—(1) *High-speed.*—Use 1 L, 4-lobe jar fitted with 4-blade assembly, 2 blades tilted upward ca 30° with diameter 60 mm and 2 blades tilted downward ca 25° with diameter 55 mm. Operate at speed specified in method, using variable transformer. Use tachometer to measure speed of blending jar blades or blade shaft, after removing blades. (2) *High-speed overhead.*—Alternative to high-speed blender: Mixer with 6 canted, sharp-edge stainless steel blades rotating on shaft of suspended motor and speed control. Blades rotate at bottom of stainless steel cup having 4 indentations, forming lobes. Sorvall Omni-Mixer, or equivalent, meets these requirements.

(d) **Bolting cloth.**—Silk cloth woven to standard size opening and thickness used in flour mills. Number of silk specifies number of mesh/linear in. “X,” “XX,” or “XXX” after number refers to thickness of thread from which cloth is woven; this also affects size of opening in cloth. Therefore, follow designation exactly as to both number and “X” of bolting cloth. (Available from Tetko, Inc., 420 Saw Mill Rd, Elmsford, NY 10523, USA.)

Prepare disks by boiling large squares of silk before cutting them into circles. Circles cut from unboiled silk shrink and become misshapen. Make rulings ca 5–7 mm apart with India ink or other permanent marking material, using fine pen, on boiled and pressed cloth marked off in circles ca 85 mm diameter.

When needed, dye ruled cloth by placing in hot (80–85 C) solution of 50 mg FD&C Blue No. 1 in 1 L H₂O containing 2.5 mL CH₃COOH, and holding at this temperature ca 15 min with frequent stirring. Rinse well and store in dark.

(e) **Butter stirrer.**—See Figure 945.75A.

(f) **Centrifuge.**—International type EXD centrifuge (International Equipment Co.) with 8-place No. 240 head, No. 320 shield,

No. 325 trunion ring, and No. 571 cushion, or other centrifuge giving equivalent maximum relative centrifugal force and with a swing-out rotor. The following formula may be used to determine equivalent centrifuge: $N_1^2 r_1 = N_2^2 r_2$, where $N_1 = 2200$ rpm and $r_1 = 19.6$ cm (distance from center of centrifuge head to bottom of horizontal centrifuge tube).

(g) **Cyclone.**—Laboratory cyclone or pulper consists of cylindrical perforated metal screen in which revolves paddle which forces soft material from food product out through openings in screen. Tough materials such as seeds, skins, and stems are moved along and out opening in end of cylinder. Use as power source 1/4 horsepower, 110 V, 1725 rpm electric motor. Screen is 22 gage material, 400 holes/sq. in., each 0.027 in. (0.7 mm) diameter. Screen is 2.5 in. (6.35 cm) id and length of effective screen is 3 in. (7.6 cm). Paddle has 2 fins, each 25/32 in. (1.98 cm) wide, set alternately and extending 1 3/16 in. (2.01 cm) from center of shaft. Pulper is fed through hopper which leads into basin 3.5 in. (8.9 cm) long and 2.5 in. (6.35 cm) id. Portion of paddle with fins inserted at 30° angle forces material from basin into screening compartment. Cyclone is so constructed that waste opening may be closed, as needed. Sieved material is caught in shield and delivered through spout to container. Machine may be readily disassembled for washing. (Blueprints available from Microanalytical Branch [HFS-315], U.S. Food and Drug Administration, 200 C St, SW, Washington, DC 20204, USA.)

(h) **Extraction vessels.**—(1) *Kilborn funnel.*—1 L, 3.5 in. (8.9 cm) od by 9.5 in. (24.1 cm) high, 8 mm opening at tip. Rubber

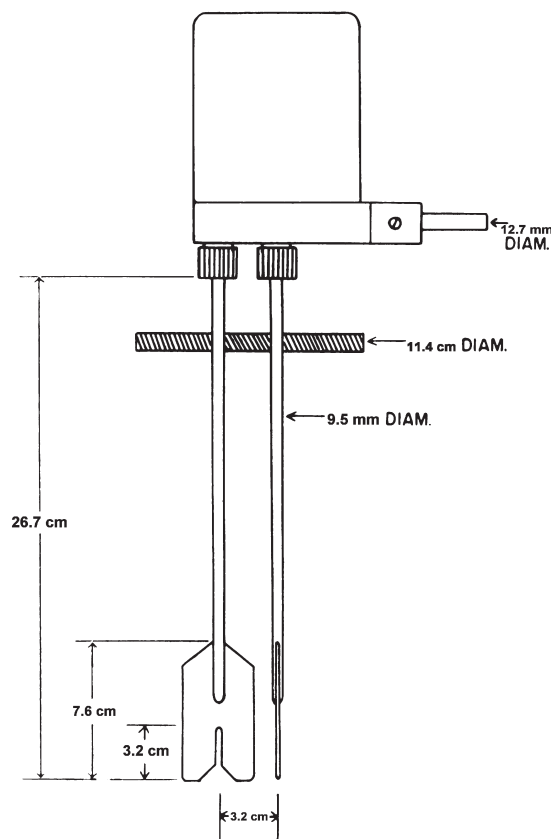


Figure 945.75A. Mechanical butter stirrer.

tubing 3/8 in. (0.95 cm) id and pinch clamp provides convenient cut-off.

(2) *Percolator*.—2 L, Corning Glass Works No. 7040, or equivalent, conforming to following general size and shape: 115 mm id 400 mm long, ca 90 mm id at 200 mm down from top, with 8–9 mm bore tip, with cut-off as in (1). Use stirring rod 370 10 mm diameter, when specified, to prevent compacting of sample in drain opening.

(3) *Percolator with oversize bore tip*.—Use standard percolator as in (2) but replace standard bore tip (8–9 mm id) with 17–18 mm id bore tip and appropriate size rubber tubing and pinch clamp. Use stirring rod described in (2).

(4) *Trap flask*.—*Wildman*.—Consists of 1 or 2 L Erlenmeyer into which is inserted close-fitting rubber stopper or wafer stopper (Entomological Supply Co., Inc., 2411 S. Harbor City Blvd, Melbourne, FL 32901, USA, Tel: +1-321-723-0135, Fax: +1-321-723-0151) supported on stiff metal rod 5 mm diameter and ca 10 cm longer than height of flask. (Rod of greater diameter is not desirable because of its greater displacement of liquid.) Rod is threaded (No. 10–32) at lower end and furnished with nuts and washers to hold it in place on stopper. Countersink lower nut and washer in the rubber to prevent striking flask. See 970.66B(b) (see 16.1.02) and Figure 945.75B.

(i) *Filter paper*.—Use smooth, high wet-strength, rapid-acting filter paper ruled with oil-, alcohol-, and water-proof lines 5 mm apart. S&S No. 8 is satisfactory.

(j) *Filter paper defatting cup*.—Center S&S 588 folded filter paper, or equivalent, over bottom of smaller beaker specified in method. Partially shape paper over bottom of beaker and gently insert beaker and paper into larger specified beaker. Remove smaller beaker and transfer weighed test portion into formed paper cup.

(k) *Funnels for filtration with suction*.—Use funnels with filter papers or bolting cloth cupped up on sides to eliminate loss of solids. Use rapid filter paper for filtration through Hirsch funnel.

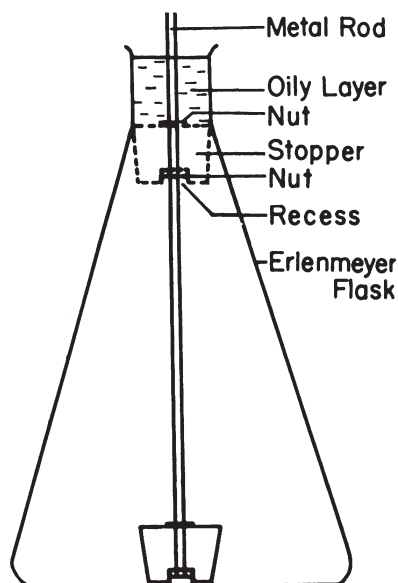


Figure 945.75B. Wildman trap flask.

Use of wire screen or bolting cloth between perforated funnel plate and filter paper accelerates filtration and gives more uniform distribution of solids.

(l) *Illuminators for widefield stereoscopic microscopes*.—*Filth examination*.—Illuminator for this purpose should have: compactness and flexibility; transformer or resistor to vary light intensity; focusing adjustment to give uniformly lighted field of view; blue-white color from cool low-voltage source.

(m) *Howard mold-counting apparatus*.—(1) *Howard mold-counting slide*.—Glass slide of one-piece construction with flat plane circle ca 19 mm diameter or rectangle 20 15 mm surrounded by moat and flanked on each side by shoulders 0.1 mm higher than plane surface. Cover glass is supported on shoulders and leaves depth of 0.1 mm between underside of cover glass and plane surface. Central plane, shoulders, and cover glass have optically worked surfaces. To facilitate calibration of microscope, newer slides are engraved with circle 1.382 mm diameter or with 2 fine parallel lines 1.382 mm apart. Available on special order from Ace Glass (www.aceglass.com).

(2) *Reticle (accessory disk) for Huygenian eyepiece*.—Glass disk that fits into microscope eyepiece, ruled into squares each side of which is equal to 1/6 of diameter of field. Since limiting diaphragm is eyepiece field stop, rulings equal 1/6 of this diaphragm opening. Field viewed on slide with mold-counting microscope has diameter of 1.382 mm at magnification of 90–125 . Reticles (accessory disks) available for widefield oculars may have field of view >1.382 mm in diameter. Center portion of field of view is used for mold counting and is delineated by inscribed circle having 1.382 mm specimen field diameter. Inscribed circle is ruled into squares each having side equal to 1/6 diameter of circle.

(n) *Magnetic stirring bar and stirrer-hot plate*.—Teflon-covered bars ca 47 mm long 9 mm od; use with hot plate having independent, continuously variable heat and speed controls. See also 970.66B(c) (see 16.1.02).

(o) *Microscopes*.—(1) *Compound microscope*.—For mold counting and other filth and decomposition work, the microscope should have following minimum specifications: binocular body with inclined oculars; 4 parfocal achromatic objectives of ca 4, 10, 20, and 40 ; revolving 4-place nosepiece; Abbe condensers with N.A. of 0.90; 10 Huygenian or widefield eyepieces; fine adjustment; mechanical stage.

(2) *Widefield stereoscopic microscope recommended for filth examination*.—Microscope should have following minimum specifications: binocular body with inclined oculars; sliding or revolving nosepiece to accommodate 3 objectives; 3 parfocal objectives 1, 3, and 6 or 7.5 ; paired 10 and paired 15 widefield oculars; mounted on base and capable of illumination by transmitted or reflected light. 30 is ordinarily used for routine examination of filter papers. Verification at higher magnification may be required.

(p) *Pipet for tissue transfer*.—Use 1 mL measuring pipet with bore 3.0 0.5 mm and tip cut off at 1.0 mL mark. In pipetting, draw material slightly above 0.5 mL mark and let it drop slowly to mark.

(q) *Rot fragment counting plate and cover preparation*.—Glass plate; 55 100 mm, 1.5–4.0 mm thick with cover 50 85 mm, ca 1.5 mm thick. Carefully paint on coat of resist over the entire surface, avoiding pinholes. Asphaltum varnish makes excellent resist; paraffin wax may also be used. Carefully scribe crosswise parallel lines, 4.5 mm apart with 15 mm space at each end, through

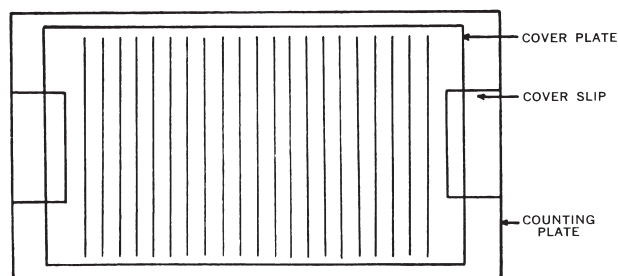


Figure 945.75C. Rot fragment counting slide.

resist. If asphaltum varnish is used, lines may be scribed with new steel-wheel glass cutter.

Place coated scribed slides face down over HF in polyethylene container. Determine proper acid fume exposure by trial and error. Following etching, remove resist by placing slide in H₂O containing detergent. If resist is not easily scrubbed off, use toluene for cleanup.

Alternatively, use clear plastic plate; 55 × 100 mm, 4–6 mm thick with glass cover 50 × 85 mm, ca 2 mm thick. With sharp needle, carefully scribe crosswise parallel lines, 4.5 mm apart with 15 mm spaces at each end. Several slides can be made at one time by using strip of plastic 100 mm wide and any multiple of 55 mm long, allowing extra length to compensate for each cut of 2–3 mm thickness.

Fasten half of ca 11 × 50 mm of rectangular No. 2 cover slip, ca 22 × 50 mm, at each end of counting plate to raise cover plate above ruled plate. See Figure 945.75C. Glass slides are available on special order from Ace Glass, Inc. (www.aceglass.com).

(r) *Sieves*.—See “Definitions of Terms and Explanatory Notes.” Sieves of No. 100 or finer should be “plain (not twill) weave” of stainless steel. Plain weave is woven with one wire alternately over and under next.

(s) *Sieve handle*.—For holding 8 in. (20.3 cm) diameter sieve during wet sieving. See Figure 945.75D. Constructed from 1 in. (25 mm) od hard aluminum tube, 14 in. (35.5 cm) long; ¼ in. (6.35 mm)-28, 2 in. (5.0 cm) brass bolt; ¼ in. (6.35 mm)-28 brass wingnut; and two ¼ in. (6.35 mm) brass washers. Cross drill ¼ in. (6.35 mm) hole. Saw tube lengthwise, perpendicular to ¼ in. (6.35 mm) hole. Cut off 2 in. (5.0 cm) of upper half of slit tube. Spread split tube ca 2 in. (5.0 cm) and flatten ca 2 in. (5.0 cm) of top and bottom halves. Bend ¼ in. (6.35 mm) right-angle lip on each flattened end.

(t) *Thin-layer chromatographic apparatus*.

(1) *Desaga/Brinkmann standard model applicator* (no longer available), or equivalent.

(2) *Desaga/Brinkmann standard mounting board*, or equivalent.

(3) *Desaga/Brinkmann drying rack*, or equivalent.—Accommodates ten 8 × 8 in. (20 × 20 cm) plates.

(4) *Desaga/Brinkmann Model 51 stainless steel desiccating cabinet*, or equivalent.

(5) *Window glass*.—8 × 8 in. (20 × 20 cm), double strength window glass plates of uniform width and thickness; smooth off corners and edges with file or other tool.

(6) *Chromatographic tank and accessories*.—Metal instead of glass troughs.

(7) *Dipping tank and accessories*.—Stainless steel, 8½ × 8½ × ¼ in. (21.6 cm × 21.6 cm × 6.25 mm × 4.7 mm) inside width with metal supports and close-fitting U-shaped cover ca 9 × ½ in. (23 cm × 12.7 mm). Capacity ca 300 mL.

(8) *Spotting pipets*.—1 L.

(9) *Spray bottle*.—8 oz. (Thomas Scientific No. 2753-J10 or Lurex Scientific, Inc., No. 131-0514, 250 mL).

(10) *Chromatography spray flask*.—250 mL (Microchemical Specialties Co., 1825 Eastshore Hwy, Berkeley, CA 94710, USA, No. S-4530-D).

(11) *Tank liner*.—Cut 2 pieces, 12¼ × 8¾ in. (31 × 22 cm), from desk blotter, white or colored, and bend into L-shape to fit tank.

(12) *Strong ultraviolet light source*.—Such as germicidal lamps (General Electric Co., Nela Park, Cleveland, OH 44112, USA; www.gelighting.com), either (1) two 30 watt, 36 in. tubes, No. G30T8, mounted in standard 30 watt reflector fixture ca 20 cm above papers; or (2) two 15 watt, 18 in. tubes, No. G15T8, mounted in standard 15 watt desk lamp fixture placed ca 10 cm above papers. Shield to protect eyes and skin at all times.

C. Reagents

(a) *Acid-alcohol solution*.—HCl and 60% alcohol (1 + 9, v/v) or HCl and 40% isopropanol (1 + 9, v/v).

(b) *Alcohol*.—95% commercial ethanol (not denatured) unless otherwise specified. Make all dilutions by volume.

(c) *60% Alcohol-calcium chloride solution*.—To each 3 L 60% alcohol (amount for one analysis), add 200 g anhydrous CaCl₂. Stir until salt dissolves. Cloudiness from traces of CaCO₃ will clear up during analysis when solution is acidified.

(d) *Allantoin standard solution*.—Prepare aqueous solution containing 2 mg/mL. Pipet 1 mL aliquot into 10 mL volumetric flask and dilute to volume with acetone. Stable ca 3 months.

(e) *Antifoam solution*.—1 g Dow Corning Antifoam A compound diluted with 20 mL ethyl acetate. Use supernate and keep tightly closed.

(f) *Carob bean solution*.—Blend 0.75% carob bean gum in H₂O. Boil 2 min and cool to 20–25 °C. Add 2 mL formaldehyde/100 mL and stir gently. Let settle and use clear supernate.

(g) *Cellulose powder*.—TLC grade, MN 300 (Brinkmann Instruments, Inc., Cat. No. 66 00 100-8, or equivalent).

(h) *Crystal violet solution*.—Dissolve 10 g dye (Colour Index 42555) in 100 mL alcohol and filter.

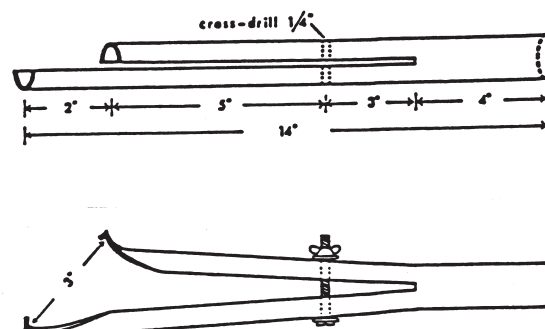


Figure 945.75D. Sieve handle (1 in. = 2.54 cm).

(i) *Detergent solution*.—Prepare aqueous sodium lauryl sulfate solution as required.

(j) *Emulsifiers*.—Nonionic, water-soluble surfactants.
(1) *Nonyl Phenol 15 mole ethoxylate*.—Igepal 730 (Stephan Co.-United States, 22 W. Fontage Rd, Northfield, IL 60093, USA; Tel: +1-847-446-7500; Fax: +1-847-501-2100; www.stephan.com).
(2) *Nonyl Phenol 10.5 mole ethoxylate*.—Igepal 710. (3) *Nonyl Phenol 9 mole ethoxylate*.—Igepal 630.

(k) *Flotation liquid*.—Mineral oil, (p), and heptane, (l), (85 + 15).

(l) *Heptane*.—Commercial *n*-heptane containing <8% toluene.

(m) *Indoxyl sulfate (urinary indican) standard solution*.—Approximately 0.1 mg/mL. Available from Sigma Chemical Co. Stored in light-resistant container in refrigerator, solution is stable ca 1 month.

(n) *Isopropanol saturated with heptane*.—To 600 mL isopropanol add 45 mL heptane and 430 mL H₂O, mix, and let stand overnight. Siphon from below interface.

(o) *Kerosene, deodorized*.

(p) *Mineral oil*.—Paraffin oil, white, light, 125/135 Saybolt Universal Viscosity (38), specific gravity 0.840–0.860 (24). Request supplier to select lot meeting specifications and provide certificate of analysis.

(q) *Pancreatin solution*.—Use USP or soluble pancreatin kept refrigerated at 10 °C. Use fresh solution. Mix at rate of 5 g/100 mL H₂O at 40 °C. Use special solution for cheese, 10 g/100 mL. Stir with malted milk unit or blender 10 min, or let stand 30 min with frequent shaking. Centrifuge at 1500 rpm and filter supernate through S&S No. 8 paper, or equivalent. Alternatively, filter through cotton pads 10–13 cm thick and then through rapid No. 8 paper in Hirsch funnel with suction.

(r) *p*-Dimethylaminobenzaldehyde (pDMAB) spray.—Mix 30 mL methanol and 10 mL HCl and let cool. Dissolve with stirring 0.5 g pDMAB in 25 mL cooled mixture. Stable ca 1 month. (Caution: Spray reagent is toxic and corrosive.)

(s) *Sodium acetate spray*.—Saturated aqueous solution.

(t) *Sodium oleate*.—Technical grade.

(u) *Sodium phosphate solution*.—Technical grade Na₃PO₄. Prepare 5% solution.

(v) *Stabilizer solutions*.—0.5% sodium carboxymethylcellulose preferred (Hercules Inc., Cellulose and Protein Products Department, 1313 Market St, Wilmington, DE 19894, USA; www.herc.com). Place 500 mL boiling water in high-speed blender. With blender running, add 2.5 g Cellulose Gum and 10 mL ca 37% HCHO solution weight/weight, and blend ca 1 min. Alternatives: 3–5% pectin or 1% algin. Add required amount of stabilizer directly to H₂O while agitating in high-speed blender. Treat solution with vacuum or heat to remove air bubbles. Add 2 mL HCHO solution/100 mL solution as preservative. (If blender is not available, mix dry stabilizer with alcohol to facilitate incorporation with H₂O.) Adjust to pH 7.0–7.5. Filter solution through 8 µm membrane filter (Millipore No. SCWP-047-00, or equivalent) using suitable vacuum filtration apparatus (Millipore No. XX15-047-00, or equivalent).

(w) *Tween 80–60% alcohol solution*.—To 40 mL polysorbate 80 NF, add 210 mL 60% alcohol, mix, and filter. (Proportionate volumes may be prepared.)

(x) *Tween 80–40% isopropanol solution*.—To 40 mL polysorbate 80 NF, add 210 mL 40% isopropanol, mix, and filter. (Proportionate volumes may be prepared.)

(y) *Tetrasodium EDTA–alcohol solution*.—Dissolve 5 g Na₄EDTA in 100 mL H₂O, add 150 mL alcohol, mix, and filter. (Proportionate volumes may be prepared.)

(z) *Tetrasodium EDTA–40% isopropanol solution*.—Dissolve 5 g Na₄EDTA in 150 mL H₂O, add 100 mL isopropanol, mix, and filter. (Proportionate volumes may be prepared.)

(aa) *Urea standard solution*.—20 mg/mL H₂O. Stable ca 3 months.

(bb) *Wetting agents*.—(1) *Tergitol Anionic 7*.—Sodium heptadecyl sulfate (Sigma Chemical Co.). (2) *Triton X-114*.—Octylphenol ethoxylate (The Dow Chemical Co.; www.dow.com).