

#32981-058, \$125.00/pr) which allow hand entry, the glove box can be equipped with gas-tight neoprene gloves (medium gloves, VWR #32981-105, \$54.00/pr plus glove holders #41-905-320, \$12.00/pr) if desired. The glove box can be modified to insure a negative working pressure by venting it into an evacuation system.

I would like to express my sincere appreciation to Drs. S. Abrahamson and R. Valencia, University of Wisconsin, Madison, Wisconsin, for their assistance in establishing our *D. melanogaster* mutagenicity testing capability. The basic transfer technique described in this technical note was developed by Dr. Abrahamson. The support of LTC J. T. Fruin and MAJ H. G. H. Eisenberg for the *Drosophila* program is also greatly appreciated.

Reference: Ashburner, M. and J.N. Thompson, Jr. 1978, in: *The Genetics and Biology of Drosophila*, Vol. 2a, pp. 1-109 (M. Ashburner and T.R.F. Wright, eds.), Academic Press, NY.

†The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

Wirtz, R. A. and H. G. Semey\*. Toxicology Services Group and \*Division of Cutaneous Hazards, Letterman Army Institute of Research, Presidio of San Francisco, California. The *Drosophila* kitchen - equipment, media preparation, and supplies.†

Tasked with establishing a large *D. melanogaster* insectary for mutagenicity testing we found the information in the articles by Merriam (1973) and Ashburner & Thompson (1978) helpful. The following is an update and expansion of Merriam's article, with emphasis on media preparation and sources of equipment and supplies used in our laboratory or recommended by laboratories visited.

Equipment (Table 1): Media cookers and mixers are available in a variety of sizes, styles and power sources (Table 1). The Agarmatic Bench-top Agar Sterilizer is ideal for small volume

(1-3 l) preparation. This self-contained unit requires only a 110 volt outlet and a suitable tap water supply (40 psi) and drain. Sterilization and mixing capabilities, as well as separately adjustable cooking and dispensing temperatures, make this a versatile unit. However, the accessory peristaltic pump (Model 1062) was not effective in dispensing corn meal-based medium.

Steam-jacketed kettles (Table 1) are used for large volume media preparation in many *Drosophila* kitchens. We, as well as several other laboratories, have used the Groen FT or EE models (20 gal) with excellent dependability and results. These are stainless steel kettles equipped with 1-1/2" draw-off valves and hinged covers (Fig. 1). The EE model is an electrically operated, self-contained unit. The FT model requires an external steam source of 5-20 psi. This model has the capability of being attached to a cold water source, through the steam system, for rapid cooling of media before addition of temperature-sensitive material.

Self-contained mixers are available on some models of kettles; however, many laboratories purchase the mixer separately. This usually requires modification of the kettle cover for installation. Mixing volume and medium consistency determine the horsepower rating of the motor required.

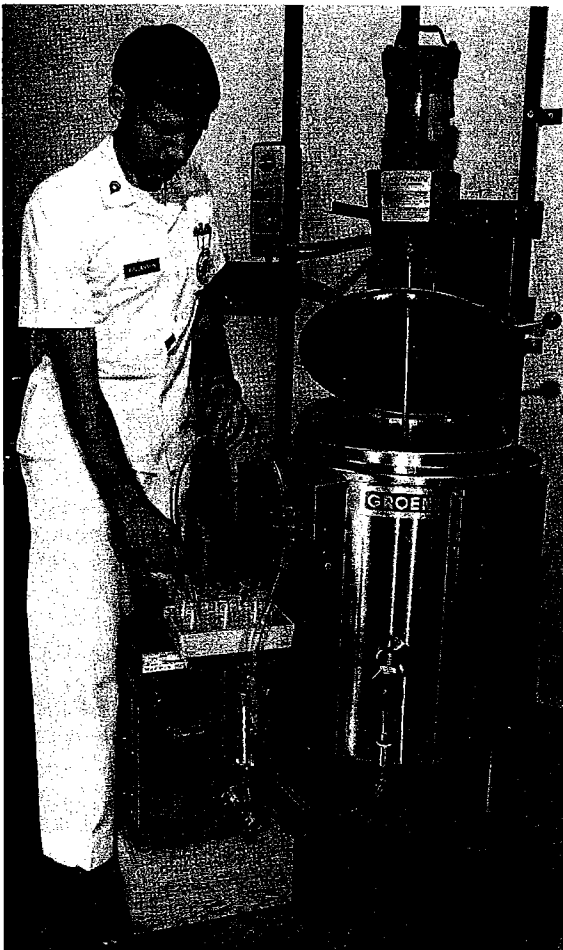


Fig. 1. Steam kettle (20 gal), variable speed mixer and syringe-type dispenser unit used for *Drosophila* media preparation.

Table 1. Equipment and sources (approximate costs are 1980-81 prices).

Item description	Sources	Approximate cost
Agarmatic Bench-top Agar Sterilizer	New Brunswick Scientific, Inc. P.O. Box 986; 44 Talmadge Rd. Edison, NJ 08817 (201) 287-1200	\$2,575.00
Steam Kettles (also check local commercial kitchen supply companies)	Groen Division/Dover Corp. 1900 Pratt Blvd. Elk Grove, IL 60007 (312) 439-2400	EE Model, \$2,945.00 FT Model, \$1,535.00
Mixers (available on many models of steam kettles)	Mixing Equipment Co., Inc. 221 Rochester Street Avon, NY 14414 (716) 226-6136	Model ND-1 SCR, \$1,535.00
Reduction Fittings (also check local dairy equipment suppliers)	Duhig, Inc. 14375 Wicks Blvd. San Leandro, CA 94557 (415) 352-6460	see text
Media Dispensers (prices do not include costs for syringe units)	National Instrument Co. 4119 Fordleigh Road Baltimore, MD 21215 (301) 764-0900	Model AB, \$795.00 Model AB-5, \$870.00
	Brewer Pipetting Machine BBL, Division of BioQuest Cockeysville, MD 21030 (301) 666-0900	Model 40-SS \$630.00
Vacuum Pump, hand operated	Fisher Scientific Co. 711 Forbes Avenue Pittsburgh, PA 15219 (412) 562-8300	Nalge 6131, \$23.50

Table 2. Drosophila medium recipes for use with the steam kettle (5-70 liter capacity).

Ingredients	Volume	or	Weight	Approx. weight percent <sup>1</sup>
Water (ml)	8,000		20,000	80.0
Agar (gm)	100-150		250-375	1.0-1.5
Molasses (ml)	800		2,000	8.0
Corn meal (gm)	700		1,750	7.0
Yeast (gm)	400		1,000	4.0
Propionic acid (ml)	50		125	0.5
20% Methyl p- hydroxybenzoate (ml) <sup>2</sup>	125		315	0.25
Approximate total volume (ml)	10,200		25,490	100.75-101.25%

<sup>1</sup>Recipes for total volumes other than those listed can be computed using the given approximate weight percentages. Changes in the volume/weight of ingredients used may be required because of the age of the ingredients or variation between batches. These changes will be recorded on the "Drosophila Medium Record Sheet". Some variation in the final composition of the medium will occur due to moisture change during cooking. These variations have not affected performance in previous tests.

<sup>2</sup>Stock solution: 20% methyl-p-hydroxybenzoate (100 gm q.s. 500 ml 95% ethanol). Final concentration in the medium is 0.25%.

We use the Lightnin Model ND-1 SCR (1/3 hp) and feel that the convenience of the variable-speed mixer is well worth the additional cost. One laboratory we visited hand-mixes the ingredients during the initial cooking, thus eliminating the need for a mechanical mixer.

Stainless steel reducing adapters, required for the attachment of dispensing equipment to a kettle, are usually carried by dairy equipment suppliers. The following are required to reduce the 1 1/2" sanitary draw-off valve on the Groen kettles to 1/4": 1 1/2x1" taper reducer (#31-19F1 1/2x1", \$72), 1 1/2" hex nut (#13H-1 1/2", \$12), 150 lb screwed reducing coupling (#1x1/4", \$7), and a 1/4x1" nipple (# schedule 40304 nipple, \$1). Order numbers and approximate prices are those of Duhig, Inc. (Table 1). We routinely use Teflon® tape on the connections to prevent leaks and allow easy disassembly for cleaning. A 1" hose can be clamped directly to the taper reducer for rapid draining after cleaning.

Sources of syringe-type media dispensers are given in Table 1. We have found that stainless steel syringe units (#FUS-60ml, \$415; #FUS-130ml, \$480, National Instrument Co.) are excellent for dispensing corn meal based media, especially when used in conjunction with an adjustable suck-back filling unit (#1886 ASB-130ml, \$73) to prevent dripping. An air-actuated spool valve dispensing attachment, recommended by National Instrument Co. for use with corn meal based media, is also available for approximately \$1700; however, none of the laboratories contacted were using this unit.

Media preparation (Table 2): A standard *Drosophila* medium is used for most mutagenicity testing and routine colony rearing. When small volumes of medium are required, or when special larval feeding procedures are needed, Carolina Biological Supply Instant *Drosophila* medium formula 4-24® may be used. The following is the procedure used for preparation of our standard medium: (1) Record the sequential batch number, date and starting time on a standardized "Drosophila Medium Record Sheet" (Fig. 2). (2) Weigh the 10, 100, and 500 gm standard weights on the balance used for weighing the medium ingredients. Record the weight readings to the nearest 10th of a gram on the record sheet. (3) Fill in the record sheet as to the weight/volume of ingredients required for the desired batch size (Table 2). As ingredients are used, check the item off the designated area of the record sheet. (4) Add the designated volume of deionized water, minus 1000 ml, to the kettle. Turn on the mixer and set the temperature on the steam kettle to 95±5°C (setting of 7.0 on the model EE-20). The setting light will go out when the kettle reaches the cooking temperature. (5) Slowly add the following to the water in the order listed: (a) agar, (b) molasses, and (c) premixed corn meal plus yeast. When adding these materials increase the mixer

1. Batch No: _____	2. Date: _____	3. Agarmatic: _____	
	4. Time: _____	5. Steam Kettle: _____	
6. Check Ingredients	Volume/Weight	7. Std. Weight (gm)	Weight (gm)
_____ a. Water	_____ ml	10	_____
_____ b. Agar	_____ gm	100	_____
_____ c. Molasses	_____ ml	500	_____
_____ d. Corn meal	_____ gm		
_____ e. Yeast	_____ gm		
_____ f. Propionic acid	_____ ml		
_____ g. 20% MHB	_____ ml		
8. Cooking Time: _____ minutes (before adding mold inhibitors).			
9. Cooking Temperature: _____ °C/Kettle setting: _____.			
10. Mold inhibitor mixing time: _____ minutes.			
11. Label medium as to batch number, date mixed, and expiration date.			
12. Clean up the equipment and work area.			
13. Notes: _____			
_____			
_____			
Signature: _____			

speed to prevent settling. Wash out the molasses container with 1000 ml of deionized water and add it to the mixture. (6) Cook for 10±3 minutes at 95±5°C. Reduce the temperature to 45±5°C (setting of 3.0) and cook for an additional 10±3 minutes. (7) After approximately 20 minutes of cooking add the propionic acid and/or methyl p-hydroxybenzoate, cook for an additional 5-10 minutes and dispense the medium. (8) Record all pertinent information on the record sheet, sign and place the sheet in the record log notebook. (9) Thoroughly clean the steam kettle and mixer.

Fig. 2. *Drosophila* Medium Record Sheet.

Granular baker's yeast (Table 3) is currently used because of the ease of handling. When we used brewer's yeast the material was sifted, if necessary (using a large tea strainer)

Table 3. Sources and approximate costs of *Drosophila media* ingredients and rearing supplies. (Approximate costs are 1980-81 prices.)

Item Description	Source	Approximate cost
Agar, fine ground powder form "A"	Moorehead & Co. 14801 Oxnard Street Van Nuys, CA 91401 (231) 873-6640	\$8.90/lb (less than 25 lb) \$8.45/lb (more than 25 lb)
Corn meal, ground, yellow (#901411)	ICN Nutritional Biochemical Co. 26201 Miles Road Cleveland, OH 44128 (216) 831-3000	\$24.00/100 lb \$8.00/25 lb
Molasses, unsulfured, "Home Made Molasses"	Saroni Total Foods, Inc. P.O. Box 96 Oakland, CA 94604 (415) 428-2662	\$41.05/5 gal
Yeast, brewer's, powdered (#103312)	ICN Nutritional Biochemical Co.	\$104.00/100 lb \$30.00/25 lb
Yeast, baker's, active dry (#ADY-12/2 lb cans)	Fleischmann 921 - 98th Avenue Oakland, CA 94603 (415) 562-7677	\$48.50/24 lb
Methyl p-hydroxybenzoate (EKC 2844)	American Scientific Products 255 Caspian Drive Sunnyvale, CA 94086 (408) 743-3100	\$25.07/500 gm
Propionic acid (MAL 7179)	American Scientific Products	\$7.80/pt
Instant <i>Drosophila</i> Medium (#67-5003)	Carolina Biological Supply Co. Gladstone, OR 97027 (503) 656-1514	\$28.80/16 liters
Bottle, glass, urine specimen, 6 oz (#B 7925)	American Scientific Products	\$7.76/pkg 12
Bottle, polypropylene, urine specimen, 6 oz (square base, #B7928)	American Scientific Products	\$70.95/cs 500
Bottle, polypropylene, urine specimen, 6 oz (round base, #11500)	Superior Plastics Products Cumberland Industrial Park Cumberland, RI 02864 (401) 333-6061	\$22.60/cs 250
Bottle lids, paper (#B7926)	American Scientific Products	\$5.24/pkg 500
Bottle lids, paper (#11900)	Superior Plastics Products	\$8.40/cs 500
Burco <i>Drosophila</i> anesthetizer (#F14300)	Bell-Art Products Technilab Instruments, Inc. Pequannock, NJ 07440 (201) 694-0500	\$4.00 each (\$50.00 min. order)
Ball, absorbent, cotton, 2" (#1404A)	Chaston Medical & Surgical Products Lake Road Melville, NY 06241 (800) 243-1172	\$35.77/cs 2000
Ball, absorbent, rayon, 2" (#6890)	Kendall Co. 1 Federal Street Boston, MA 02101 (617) 432-2000	\$13.80/cs 2000
Vials, shell, 95x25 mm (#66020-188)	VWR Scientific P.O. Box 3200 San Francisco, CA 94119 (415) 469-0100	\$27.70/cs 144

and added to the dry corn meal. Adding this yeast-corn meal mixture eliminated the necessity of making a yeast slurry to prevent caking.

Molasses is easily transferred to a 1000 ml Fleaker<sup>TM</sup> (Corning) flask using a vacuum system. A 40 x 1.2 cm glass tube, run through a hole of the same size in the lid of the molasses pail, is connected to a #10 2-hole stopper with clear tubing. The second hole in the stopper is attached to a hand-operated vacuum pump (Nalgene Co., Table 1). This allows transfer of the molasses, without opening the container, by placing the stopper into the Fleaker<sup>TM</sup> and pulling a vacuum on the system.

Medium ingredients are purchased from several suppliers (Table 3). Check with the manufacturer as to the shelf life of ingredients before large volume purchases. Yeast, molasses, and agar can deteriorate with age when stored at room temperature. Unless all materials are used rapidly, insure that they are kept in cold storage to maintain viability and reduce the possibility of microbe or arthropod contamination.

Rearing supplies (Table 3): Glass urine specimen bottles or disposable polypropylene urine bottles are excellent replacements for the scarce 1/2-pint milk bottles traditionally used for stock colony rearing. These bottles can be capped with disposable paper closures or reusable foam plugs (27-35mm diameter). Burco anesthetizers fit into the cap seats of the half-pint milk bottles and glass or plastic urine specimen bottles for easy collection and etherization of flies. Glass shell vials (25x95mm) with 2" rayon or cotton plugs may also be used. Medium volumes for the containers are: bottles - 50±10 ml, and vials - 10±2 ml.

Bottle trays (10 1/4 x 12 1/4 x 2 1/2" inside dimensions) were constructed from exterior "A" grade 1/2" plywood. However, these were difficult to clean and invited microbial and mite infestations. Trays constructed of non-porous materials are therefore highly desirable. Rubbermaid® drawer organizers are excellent, inexpensive vial and bottle trays. The 15x6" (#2918, \$1.29 each) hold approximately 85 standard 95x25mm vials, 10 round-bottomed or 12 square-bottomed plastic urine bottles. The 9x6" (#2916, \$0.79 each) hold 50 vials or 6 bottles (Fig. 3). The vial trays are available in several colors for coding of colonies and are easily equipped with sliding dividers cut from 1/8" acrylic plastic or made from 4x6" index cards.

We would like to express our sincere appreciation to Drs. S. Abrahamson and R. Valencia, R. Burns, K. Houtchens and D. White, University of Wisconsin; Dr. G. Schmolesky, Raltech Scientific Services, Inc., Madison, Wisconsin; and Dr. B. Evans and J. White, SRI International, Palo Alto, California, for their assistance in establishing our *Drosophila* insectary and mutagenicity testing capability. We also acknowledge the support and assistance of LTC J.T. Fruin and MAJ H.G.G. Eisenberg of Letterman Army Institute of Research.

References: Ashburner, M. and J.N. Thompson, Jr., 1978, in: *The Genetics & Biology of Drosophila*, Vol. 2a (M. Ashburner and T.R.F. Wright, eds.), pp. 1-109, Academic Press, NY; Merriam, J.R. 1973, DIS 50:196-197.

†The opinions or assertions contained herein are the private views of the authors and are

not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Citation of a commercial or proprietary product in this paper does not constitute an official endorsement of the product by the Dept. of the Army or the Dept. of Defense.



Fig. 3. Bottle and vial trays and ether anesthetizer used for *Drosophila* rearing.