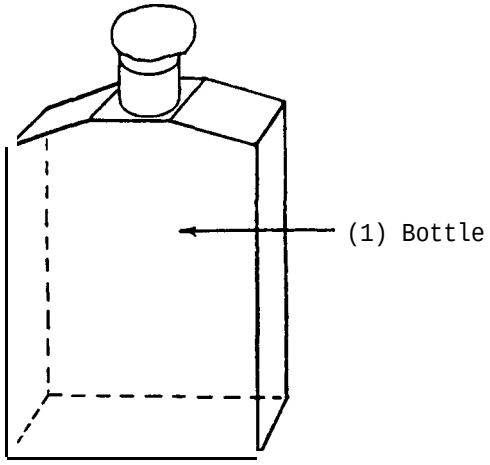
VII. MICROBIAL GROWTH APPARATUS

A. BASIC APPARATUS

Included here are improvised versions of the equipment necessary to perform elementary investigations in microbiology. Information on culturing microorganisms should be obtained from standard texts on the subject.

A. BASIC APPARATUS

A1. Culture Flask

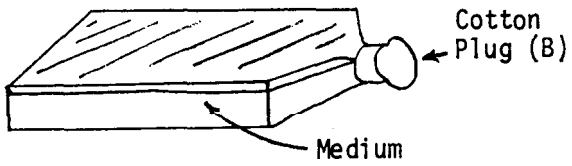


a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Bottle	1	Medicine Bottle (A)	About 100 ml or larger
	1	Cotton Plug (B)	--

b. Construction

(1) Bottle



Sterilize the bottle (A) and fill it 1/6 full of either liquid or gelatin culture medium. Stopper it with the cotton plug (B). If gelatin medium is used, lay the bottle on its side and allow the medium to set. Store the flask with the medium on the upper side of the flask so that no moisture film will form on the medium.

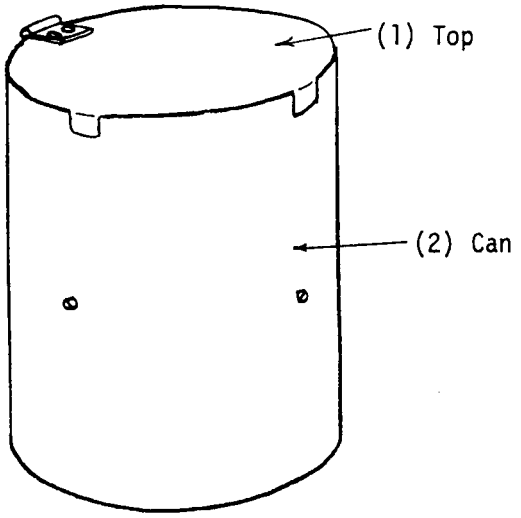
c. Notes

(i) Consult a standard microbiological text or source book for detail in working with bacteria and other microorganisms.

(ii) Use glass medicine bottles with flat sides if these are available.

(iii) Petri dishes are invaluable in working with microbes. See CHEM/V/A6 for instructions in making petri dishes.

A2. Sterilizer



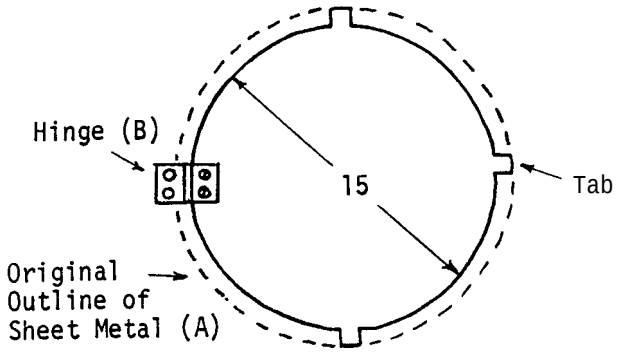
(3) Rack (Not visible)

a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Top	1	Sheet Metal (A)	17 cm diameter, 0.075 cm thick
	1	Hinge (B)	3 cm x 2 cm
(2) Can	1	Tin Can (C)	15 cm diameter, 18 cm high
	4	Screws (D)	1.5 cm long
(3) Rack	1	Sheet Metal (E)	14.5 cm diameter, 0.075 cm thick

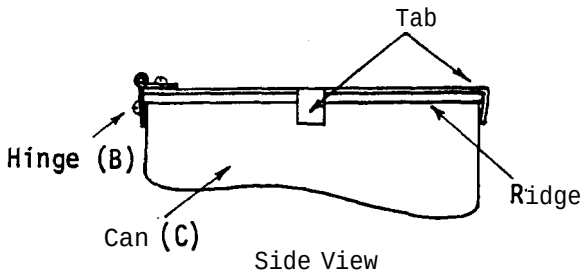
b. Construction

(1) Top



Cut the top from a circular piece of sheet metal (A). Leave three tabs to be bent down at right (90°) angles. The tabs are 1.0 cm long. Screw the small hinge (B) to the top directly opposite the middle tab.

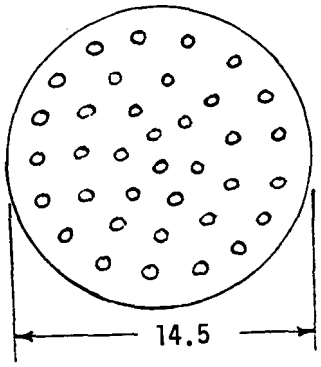
(2) Can



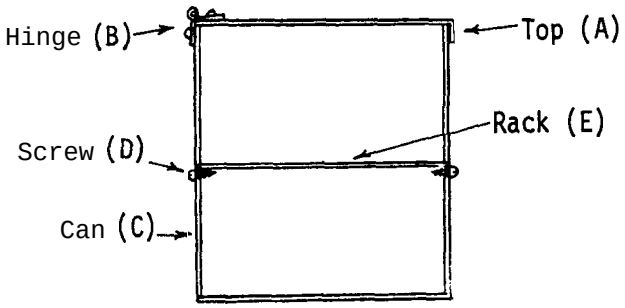
Side View

Remove one end from the tin can (C). Attach the top to the can by means of the hinge. Most tin cans have a ridge around the edge so that when the top is closed, the three tabs should catch on this ridge and hold the top down rather firmly. Finally, screw the four screws (D) through the outside into the inside of the can, 9 cm from the bottom and spaced about 12 cm apart.

(3) Rack



Punch a number of holes into the sheet metal disc (E). Set this disc inside the can so that it is supported by the four screws extending into the can.



Side View

(Cross-section)

C. Notes

(i) To use the sterilizer, simply put 3 - 4 cm of water in the can and place the items to be sterilized on the rack. After the water has begun to boil, leave

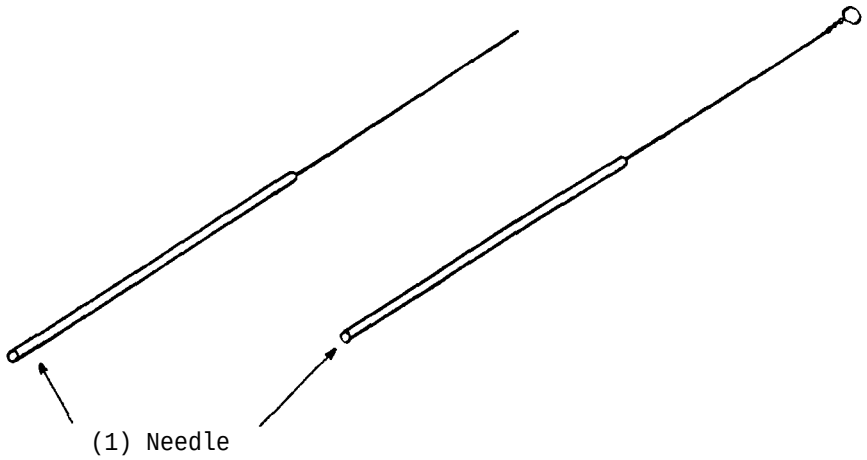
the items in the steam for about 90 minutes.

(ii) If the can used is large enough, two or more racks can be made for it to allow a larger number of articles to be sterilized at the same time.

(iii) This sterilizer will kill most, but not all, common bacterial contaminants. If pure sterility is desired, an autoclave or ordinary pressure cooker is needed. Place the articles on a rack and autoclave or pressure cook them for about 20 minutes.

(iv) An alternate rack can be made by fastening a circular piece of wire mesh to a frame of stiff wire.

A3. Inoculating Needles

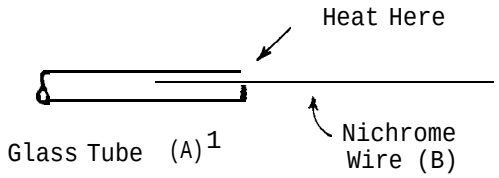


a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Needle	1	Glass Tube (A)	0.3 cm diameter, 12 cm long
	1	Nichrome Wire (B)	10 cm long, #24 gauge

b. Construction

(1) Needle



Side View

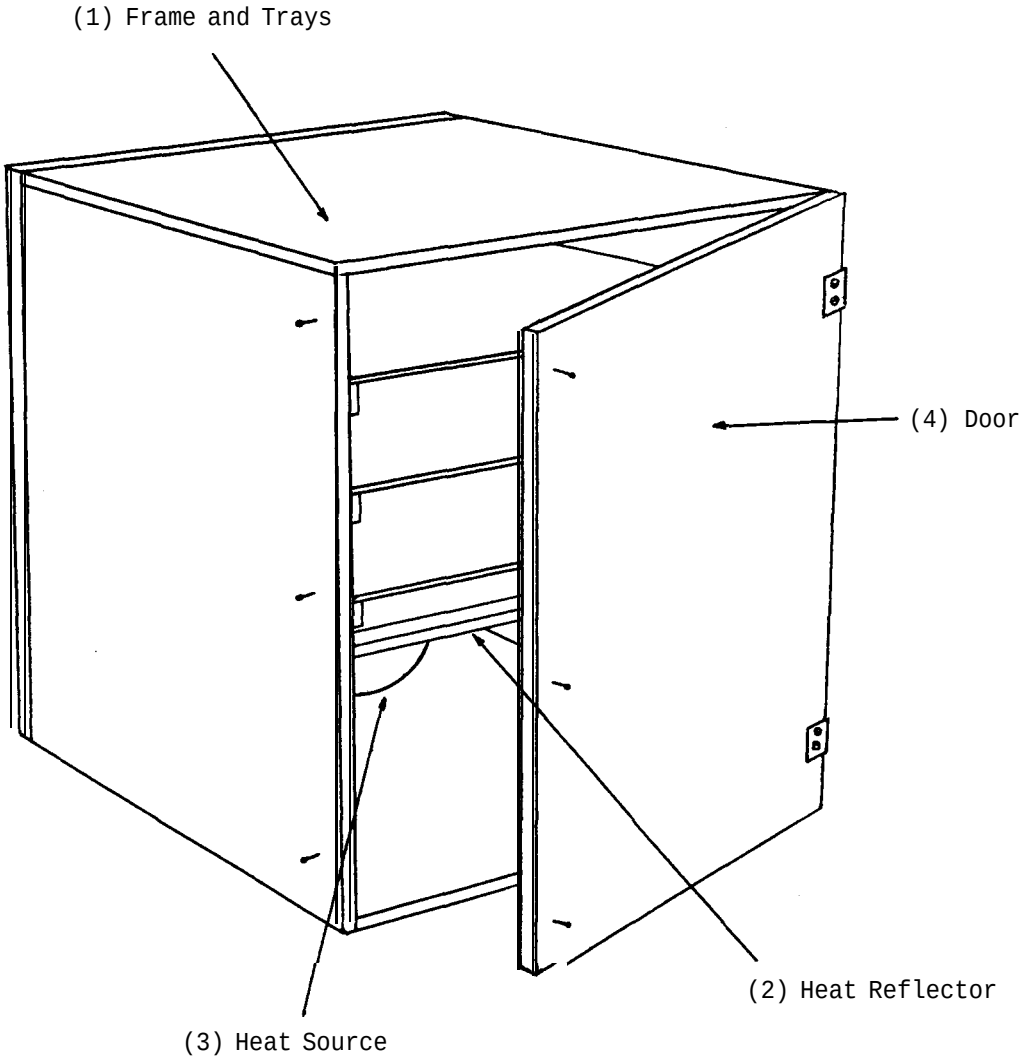
Use soft glass tubing (A) with a small diameter bore. Insert about 2 cm of the wire (B) in one end of the tube and heat this end in a hot flame until the end of the glass constricts and holds the wire fast.

c. Notes

(i) The nichrome wire may be left straight or a 0.3 cm loop may be made in the end by twisting the wire around a 0.3 cm round object with pliers.

(ii) Use inoculating needles for transferring small amounts of bacterial cultures from one medium to another.

A4. Microorganism Incubator



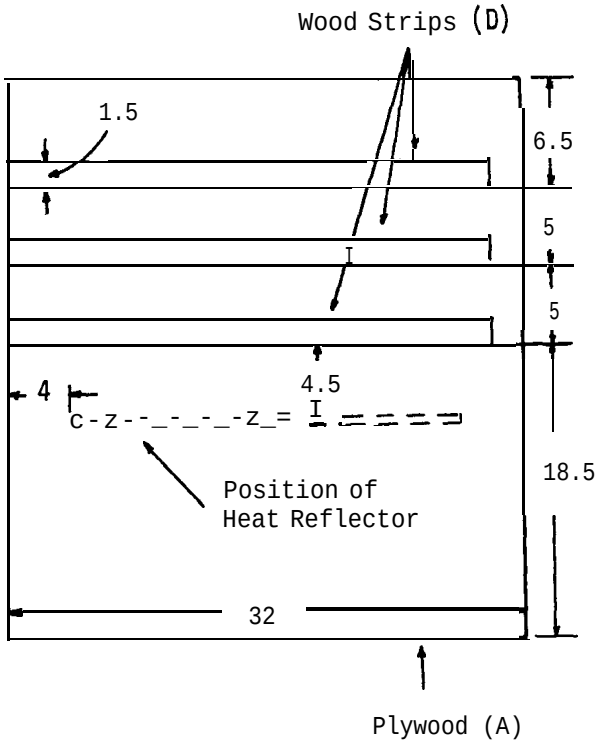
a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Frame and Trays	3	Plywood (A)	35 cm x 32 cm x 1.0 cm
	1	Plywood (B)	33 cm x 32 cm x 1.0 cm
	1	Plywood (C)	35 cm x 36 cm x 1.0 cm
	6	Wood Strips (D)	30 cm x 1.5 cm x 1.0 cm
	3	Perforated Hardboard (E)	32.5 cm x 31 cm x 0.3 cm
(2) Heat Reflector	1	Plywood (F)	33 cm x 24 cm x 0.5 cm
	1	Aluminum Foil (G)	37 cm x 28 cm

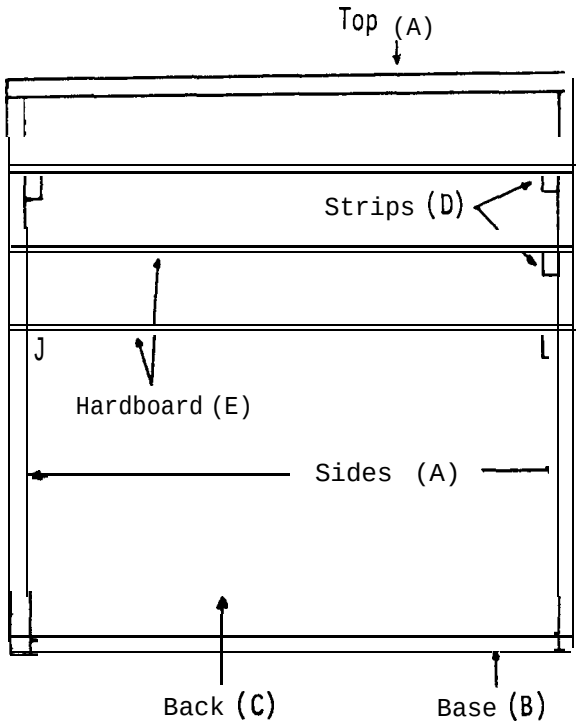
(3) Heat Source	1	Egg Incubator, Heat Source (H)	VI/C2, Component (5)
(4) Door	1	Plywood (I)	35 cm x 36 cm x 1.0 cm
	2	Hinges (J)	Approximately 4 cm long
	8	Screws (K)	0.7 cm long
	6	Nails (L)	1 cm long
	3	Rubber Bands (M)	--

b. Construction

(1) Frame and Trays



Nail three of the wood strips (D) to each of two of the pieces of plywood (A) as illustrated to make the two side pieces of the frame. Nail the bottom edges of the completed side pieces to the wood (B) used as the base. Nail the back piece (C) into position as well as the top piece (A). When the frame is finished, the pieces of perforated cardboard (E) which serve as the trays should slide easily into the frame on the wood strips (D).



Frame and Trays

(Front view)

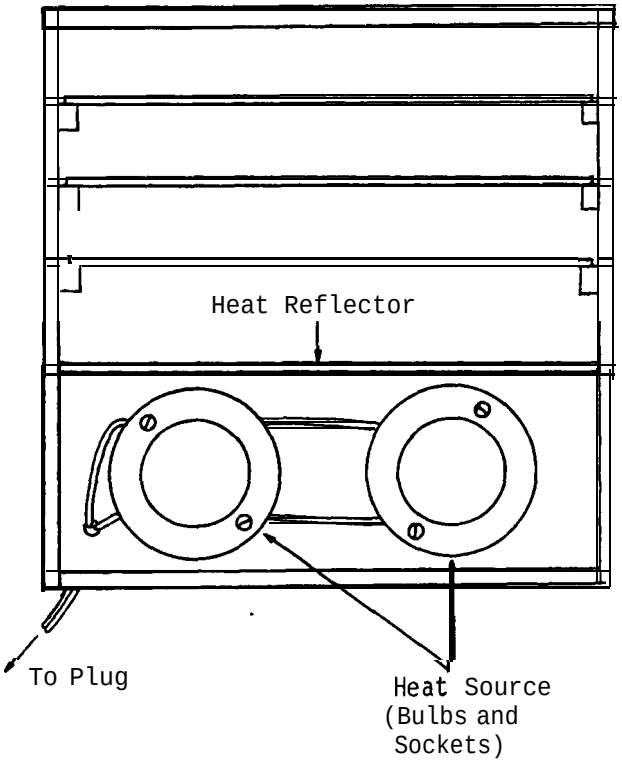
(2) Heat Reflector

Cover one side of the plywood (F) with aluminum foil (G) to make the heat reflector. Nail the reflector into place 13 cm above the base (B) of the frame with a 4 cm gap between the rear edge of the reflector and the back (C) of the frame.

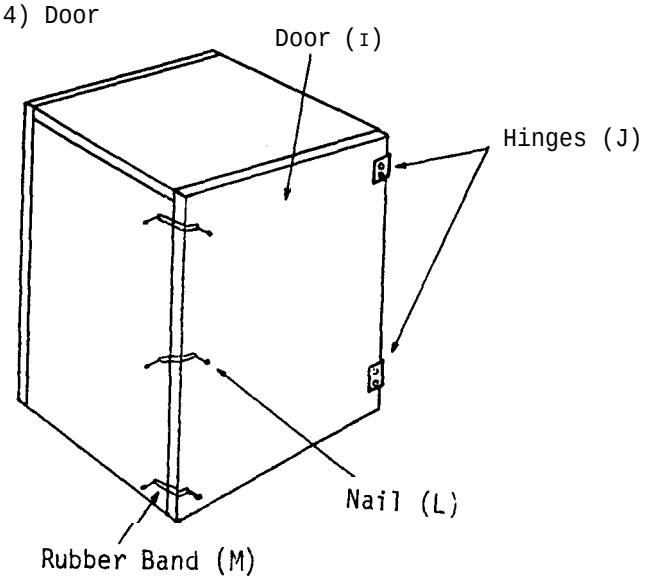
(3) Heat Source

Use two light bulbs as the heat source (H) exactly as described for item VI/C2, Component (5).

Front View



(4) Door



Fasten one edge of the plywood (I) to the side of the frame with the hinges (J) and screws (K) making certain the door shuts as closely to the frame as possible. Felt strips may be used as insulation between the door and frame if necessary, both to conserve heat loss and prevent the introduction of airborne contaminants. The door may be held closed by using rubber bands (M) which are stretched

between adjacent pairs of nails (M) in the frame and door.

c. Notes

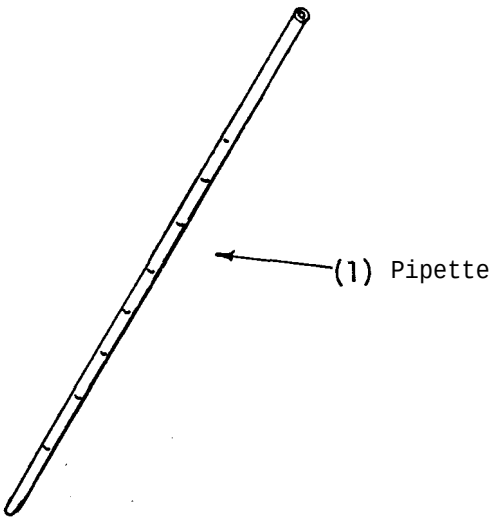
(i) Use the microorganism incubator to provide a proper environment for growing bacteria, mold, and other cultures. The dimensions of the incubator as given are to enable each tray to hold nine (three rows of three) standard petri dishes (9 cm diameter).

(ii) If the incubator is used in a constant temperature room, the temperature in the incubator can be held relatively constant. Using the correct combination of bulbs will yeild an internal temperature close to that desired. Rather than drilling ventilation holes to cool the incubator if it is too hot, it might be better to paint part of the light bulbs with black paint to cut down their heat, Ventilation holes would allow contaminants into the incubator. The following gives a few examples of temperatures which can be maintained in the incubator.

Wattage	Incubator Temperature (°C)	Room Temperature (°C)
40	35.0	23.5
60	40.5	22.0
80	48.0	23.5

(iii) The thermostat (VI/C3) should be used with the incubator to insure that the internal temperature maintains itself at the correct level. Mount it in the top of the incubator, protected by a wire screen which will prevent persons from touching the live wires, In fact, if the incubator is definitely to be used with the thermostat, increase the height of the top above the uppermost tray in order to insure that people placing cultures in the incubator have less chance of touching the thermostat,

A5. Transfer Pipette

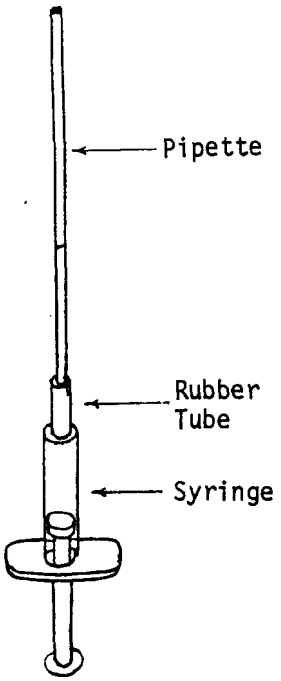


a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Pipette	1	Glass Tube (A)	35 cm long, 0.4 cm inside diameter

b. Construction

(1) Pipette



Hold one end of the glass tube (A) in a flame until the opening begins to constrict slightly. Remove it from the flame and let it cool when the opening is about 0.1 cm wide. To calibrate the pipette, a 10 cc (ml) syringe and short piece of rubber or plastic tubing is needed. Connect the ends of the pipette and syringe with the short (4 - 5 cm) piece of tubing. Fill the syringe and pipette with 7 or 8 cc of water and eliminate air bubbles by gently tapping the pipette. Hold the pipette vertically (syringe at the bottom) and withdraw the

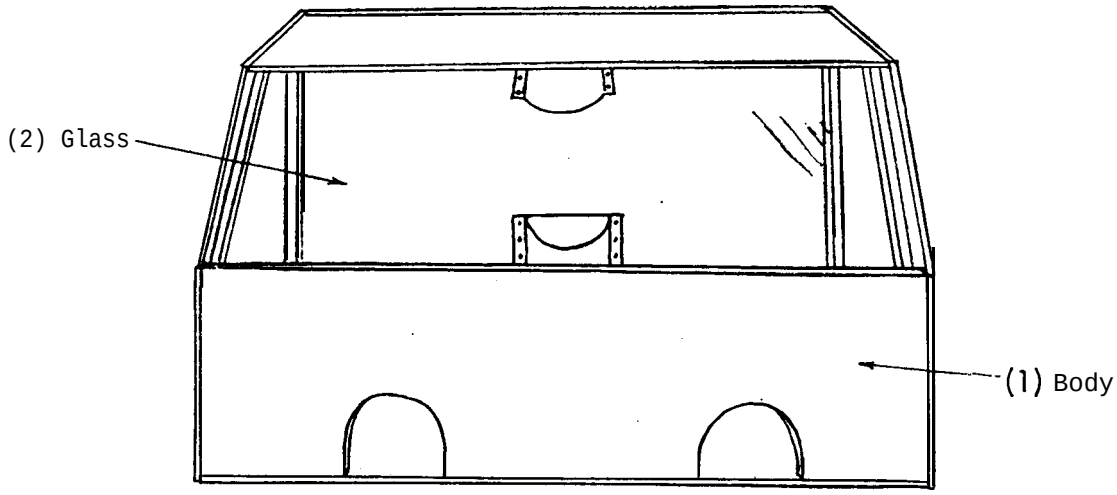
syringe plunger until the water empties from the pipette. Note the position of the syringe plunger on the scale and reinject water into the pipette 0.5 ml at a time until a total of 5 ml is reached. At each injection, mark the position of the water meniscus with a triangular file to form a permanent scale.

C. Notes

(i) This pipette is used in transferring exact amounts of culture broth from one container to another. Draw broth into the pipette with mouth suction and force the liquid out by gently blowing through the tube.

(ii) If desired, numbers may be written by the filed marks to indicate the capacity at that point. These numbers will last longest if they are drawn with waxed crayons or other types of pencils designed for writing on glass.

A6. Transfer Chamber *



a. Materials Required

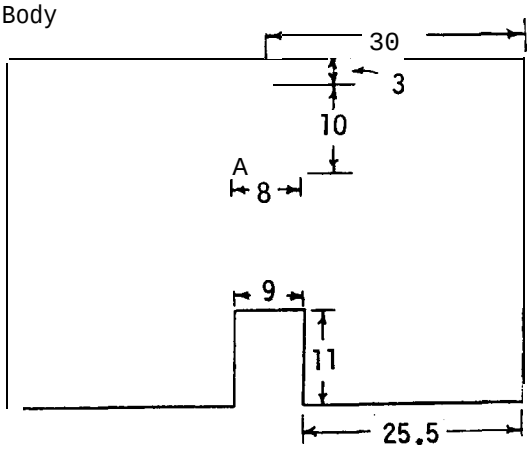
Components	Qu	Items Required	Dimensions
(1) Body	2	Plywood (A)	60 cm x 40 cm x 0.75 cm
	1	Plywood (B)	60 cm x 20 cm x 0.75 cm
	2	Plywood (C)	40.75 cm x 40 cm x 0.75 cm
	1	Plywood (D)	61.5 cm x 18 cm x 0.75 cm
	2	Wood (E)	60 cm x 2 cm x 2 cm
	2	Wood (F)	24 cm x 2 cm x 2 cm
	2	Wood (G)	36 cm x 2 cm x 2 cm
	2	Wood (H)	16 cm x 2 cm x 2 cm
	2	Wood (I)	37.25 cm x 2 cm x 2cm
	2	Wood (J)	28 cm x 2 cm x 2 cm
	2	Wood (K)	15 cm x 2 cm x 2 cm
	2	Wood (L)	6 cm x 2 cm x 2 cm
	1	Wood (M)	20 cm x 2 cm x 2 cm
	1	Aluminum Sheet (N)	25cmxll cmx 0.05 cm
	1	Aluminum Sheet (0)	20 cm x 14 cm x 0.05 cm

*Adapted from Richard E. Barthelemy, et. al., Innovations in Equipment and Techniques for the Biology Teaching Laboratory, (Boston: D. C. Heath, 1964), pp 12-14.

(2) Glass	1	Window Glass (P)	28.5 cm x 58.5 cm x 0.3 cm

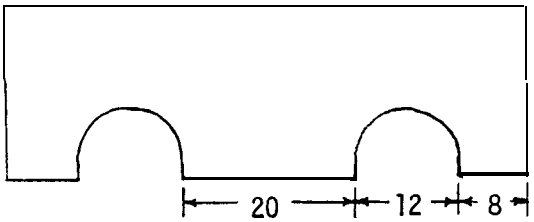
b. Construction

(1) Body



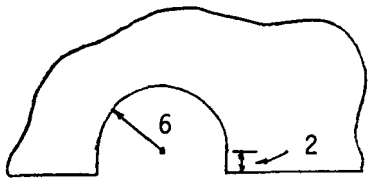
Plywood (A)

Begin the transfer chamber by cutting two holes into one of the large pieces of plywood (A). These will serve as ventilating holes when the chamber is enclosed.

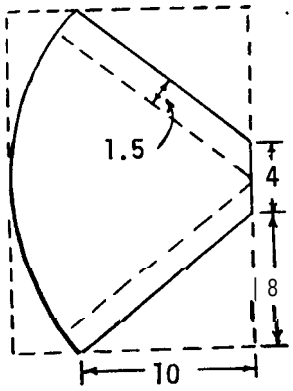


Plywood (B)

Cut two holes in the piece of plywood (B) to serve as arm-holes. The size and distance apart of these holes may be varied to suit personal preferences.

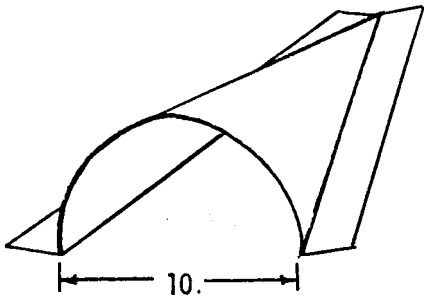


Detail of Hole



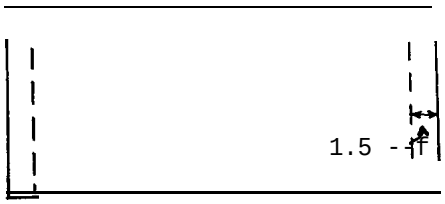
Pattern

Cut the piece of aluminum sheeting (0) (other metal sheeting may be substituted) to the given pattern. Bend up the straight sides along the dotted lines to form two flanges, each 1.5 cm wide.



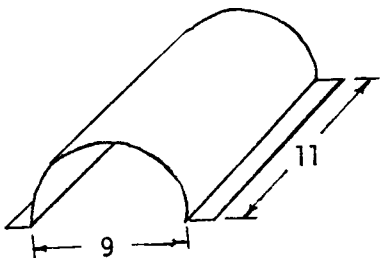
Half Cone

Poll the sheet metal (0) around a round object (e.g., a broom handle) until it takes the shape of a half cone.

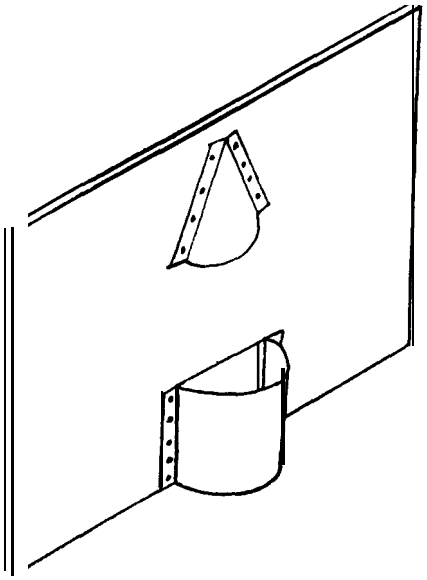


Pattern

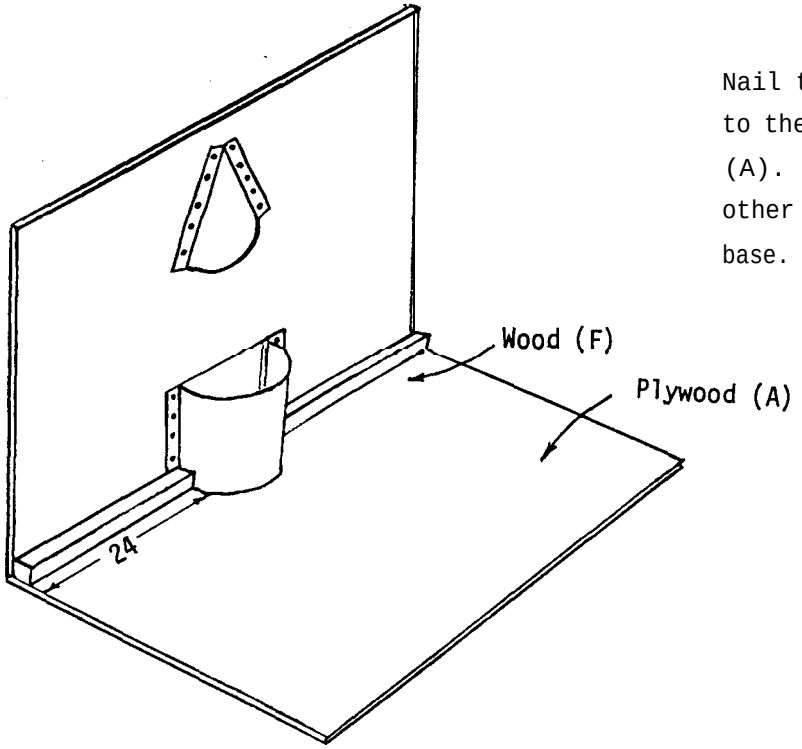
Similarly, bend up the two 11 cm sides of the other piece of aluminum (N), and roll it into a half-cylinder shape.



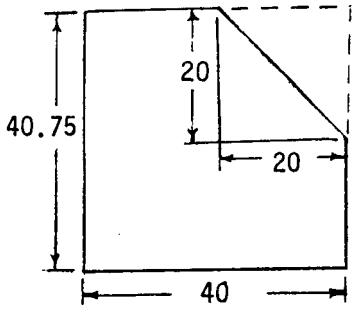
Half Cylinder



Nail the two aluminum pieces into position on the piece of plywood (A) in which the holes have been cut. Position the half cone directly over the triangular hole. Position the half cylinder so that its edges are even with the edges of the rectangular hole.

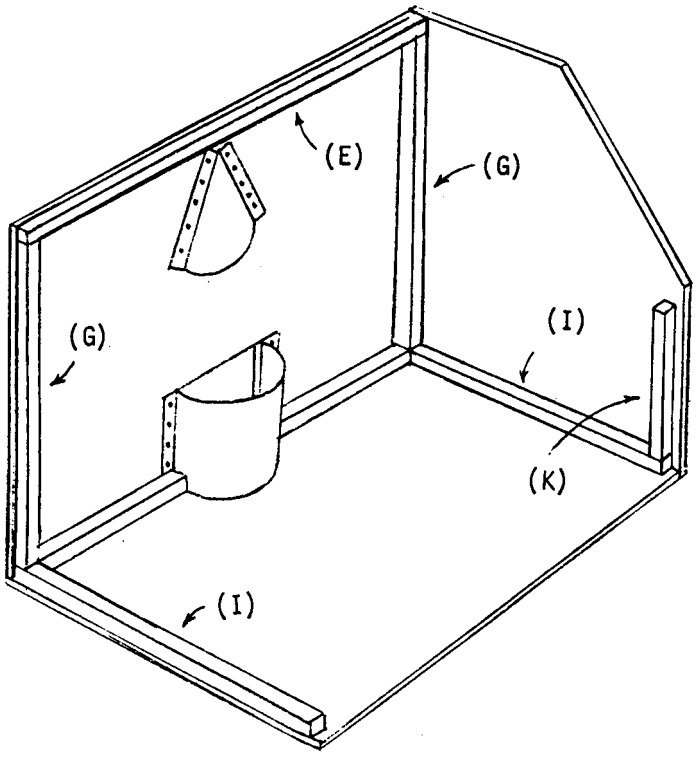


Nail the two wood strips **(F)** to the bottom edge of the back (A). Nail this in turn to the other plywood (A) used as the base.



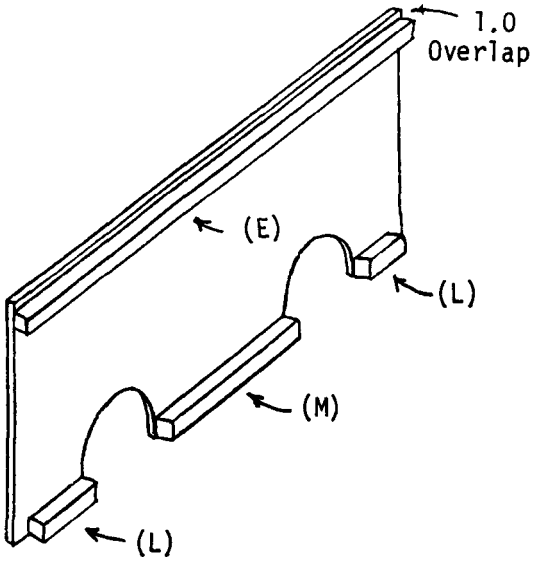
Endpiece (C)

Cut the two pieces (C) as shown. Use these pieces as endpieces for the chamber.

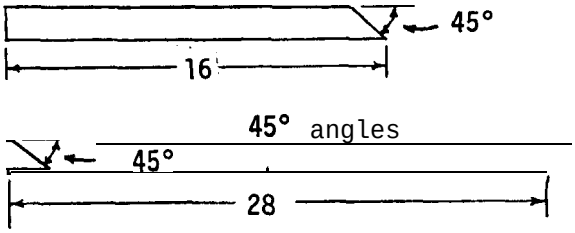


Frame

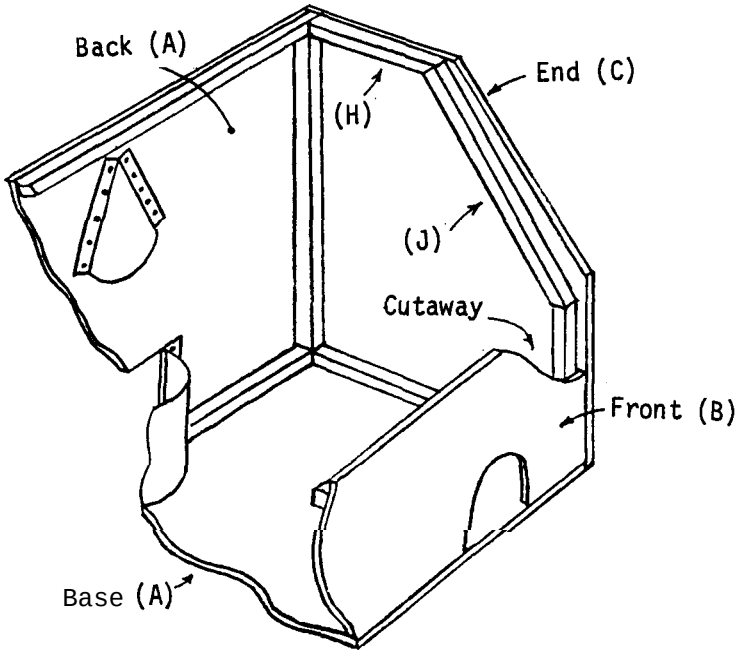
Nail two strips (G), two strips (I), and one strip (E) to the back and base. Then nail the two endpieces (C) into position. Nail the two strips (K) to the front edge of the end, being careful to leave a 0.75 cm overlap for the frontpiece to fit into.



Next, nail the two strips (L), and the other strip (E) and strips (M) to the back of the frontpiece (B) as shown. Properly done, this piece can now be nailed into the front of the chamber. Be sure there is about a 1.0 cm overlap of the plywood over the 60 cm strip.

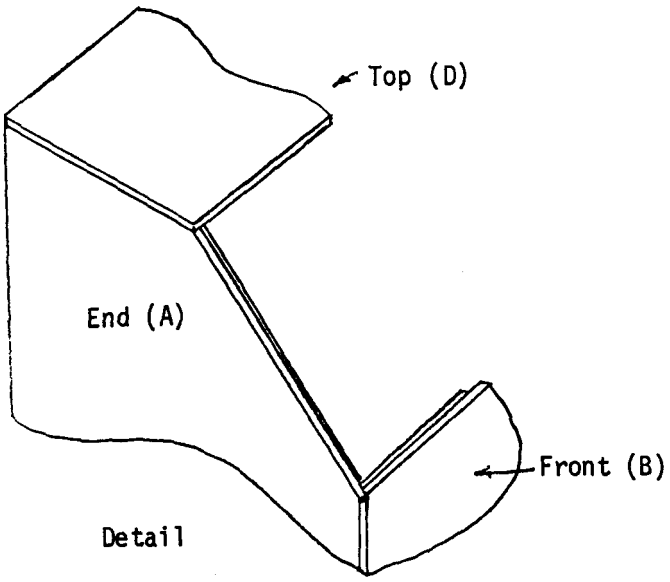


Cut one end of the wood strips (H) and wood strips (J) off at



Cutaway View of One End

Nail one each of strips (J) and (H) to the endpieces (C) insuring they fit as shown in the drawing.



To complete the body, nail the last piece of plywood (D) to be the top, even with the edges of the back and sides.

(2) Glass

Simply rest the glass (P) on the frame made of the three wood strips, one on the front (E) and one each (J) on each endpiece. There should be no gaps between the glass and frame.

c. Notes

(i) Use the transfer chamber when transferring microbiological cultures from one container to another. With it, such techniques can be performed in a draft-free environment, thus reducing the possibility of airborne contamination. The students' or instructors' arms fit through the armholes in front while the glass permits all operations to be viewed easily.

(ii) The holes in the back serve for ventilation when the chamber is used with a bunsen burner.