2% Agar in 1% Formalin

For preprocessing and processing support of small and/or fragile tissues.

Preparation:

1. Combine: 900 ml DDH2O
   20 gm agar (Difco #0140-01. 1 lb. bottle.)

2. Boil gently (in microwave) until thoroughly dissolved.

3. Add 100 ml of 10% formalin.

4. Mix. Pour about 75 ml into 100 ml sterile bottles. Allow to solidify. Store at room temp. (Expiration 2 mo.)

5. Put the quantity to be used immediately into a small bottle with a dropper top. Store this in either a 60 degrees Celsius oven (keeping the bottle tightly stoppered), or while in use in a 60 degrees Celsius water bath.

Method for use:

1. Working on a clean piece of glass, place a small amount of the agar down. Observe the change in it's consistency. When it begins to solidify, place the tissue in it. Orient the tissue as you would if you were embedding into a paraffin block. Drop more agar over the tissue. Continue to do this until the tissue is surrounded by the agar, and a small mound of agar is formed. If the agar is allowed to solidify too much between applications the layers will peel away from each other. Large bubbles should also be avoided during this process.
2. When the mounded agar is solid trim the excess away with a scalpel to form a roughly square shape. Slide the scalpel under the agar/tissue mound, and gently lift it into a processing cassette. Seal the cassette, and process as usual.
3. When embedding, place the flattest side of the infiltrated agar/tissue block down in the mold. Cool blocks, cut sections, and stain as usual.

1/3/91 L Burchell

Preparation of Tissues for the Histology Lab

Cutting Tissues:

Cut tissue slices about 3 or 4 mm for proper penetration of fixatives in the times indicated below. Cover tissue with 20 times its own volume of fixative. For skin samples, make sure the fur is **shaved** off.

Make sure that each bottle has a number and a tissue diagnosis card. Each diagnosis card should also include your name and group, room and phone number.

10% Buffered Formalin:

Fix tissue(s) in cold (4 degree) buffered formalin for 8-12 hrs. After fixation, the tissues should be placed in cold (4 degree) PBS and taken to the Histology lab for processing.

Acetone Fixation:

Fix tissue(s) in cold (4 degree) acetone for 8 hrs. After fixation, the tissues should be placed in fresh cold (4 degree) acetone and taken to the Histology lab for processing.

Carnoy's Fixation:

Glacial acetic acid	10 ml
Absolute ethyl alcohol	60 ml
Chloroform	30 ml

This is a very rapid fixative and should not be allowed to act for longer than 2-3 hrs. on tissue that is 3-5 mm – 12-19 hrs. for tissue that is 10-15 mm. Transfer to absolute ethyl alcohol – change alcohol 3x – 1 hour for each. Take to the Histology lab for processing.

Bouin's Fixative:

Picric acid, saturated aqueous solution (21 gm/ 1 liter DH2O	750.0 ml
37-40% formalin	250.0 ml
Glacial acetic acid	50.0 ml

Fix tissues for 4-12 hours depending on the size. It is important to wash in several changes of 50 % alcohol for 4-6 hrs. – changing the alcohol every hour, agitating constantly, to insure proper removal of the picric acid. Store in 70% alcohol.
NOTE: The removal of picric acid from tissues is essential in order to insure proper staining of the tissue sections.

Frozen Tissues:

Cut tissue slices to size (15 x 20 mm and a thickness of 4 – 7 mm). Cut a piece of filter paper 30 x 45 mm. Write number on bottom right corner. Place filter paper on slab of dry ice. Place fresh cut tissue on top of filter paper (centered) and immediately flip over so tissue is on top of dry ice. Wait until tissue is frozen hard, then store in Whirl-Pak bags and put in –70 degrees freezer.

Make sure that each tissue has a number and a tissue diagnosis card.