**Preparation and sterilization of culture media**

There are many types of media which have been developed as general purpose media suitable for growing most fungi, but some are selective and are used for the isolation of particular fungi from plants or soil. Growth media can also be classified as synthetic or natural based on their compositions. Synthetic media (those made entirely from defined chemical compounds) are by nature uniform as their chemical composition is standard whereas natural media (those made from natural material, usually plant extracts) are variable depending on the extract from the plant. If using natural media for distinguishing morphological characters or growth rate studies it is important that the same batch of media is used across all isolates.

**1. Water agar (WA)**

Water agar is the most useful general purpose isolation media often used to isolate and germinate spores, obtain monosporic culture, and verify inoculum viability.

**Preparation:**

**WA (2%)** consists of 20 g agar in 1 L of water and is recommended as the substrate for the

germination of conidia used to initiate single spore cultures. Hyphal growth is sparse on this

medium so it is suitable for cultures from which single hyphal tips are to be taken for the

initiation of new colonies. Sparse growth on WA also facilitates the isolation of fungi from plant

material, particularly roots.

For single sporing and hyphal tipping it is suggested that plates be poured when the medium is

still quite hot so that thin plates can be produced—this restricts fungal growth and makes it easier

to cut out the spores or hyphal tips.

**WA (0.05%)**, 0.5 g agar in 1 L of water, is used in the preparation of soil dilution series. The

small quantity of agar slightly retards sedimentation rates of fungal propagules. The agar is

dissolved in water before being dispensed into McCartney bottles.

**2. Potato-dextrose-agar (PDA)**

Most widely used for isolating, multiplying, and storing fungi because it is suitable for a large

number of species.

**Components:**

Sliced potatoes 250 g

Dextrose 10 g

Agar 20g

Distilled water 1000 ml

Preparation: Boil the potatoes in 500-700 ml of distilled water for 15-20 minutes. Filter through

cheesecloth; pour into a flask and add dextrose, agar, and enough water to reach 1000 ml. Seal

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flask with cotton or aluminum foil and sterilize. Let it cool and pour into Petri dishes (halfway).

Stack dishes one on top of another to avoid condensation.

If commercial powdered PDA is available, weigh 41g and add distilled water to make the

volume of the mixture 1L. Then, follow the above steps.

**3. V-8 agar**

Useful for inducing sporulation in many fungi. V-8 juice is made by the Campbell Soup Co. and

Herdez, a Mexican firm, among others; it contains tomato, celery, beet, parsley, lettuce, spinach,

and watercress extracts. Since there are differences among brands, comparative testing on each

species is recommended. If canned V-8 juice is not available, it can be replaced with a medium

containing leaf extract (such as the following), especially for testing *Helminthosporium triticirepentis*and

H. *teres.*

**Components:**

V-8 juice 200ml

Calcium carbonate 3g

Agar 15-20 g

Distilled water 800ml

Preparation: Place agar and calcium carbonate in a flask; add the juice and mix with water to

complete 1000 ml. Seal the flask and sterilize. Stir the medium before pouring to keep the juice

from precipitating. This medium may be prepared at different concentrations by adjusting the

amount of juice used.

**4. Leaf extract medium**

Used primarily for stimulating growth and formation of asexual structures in some fungi.

Leaves of different crops may be used, depending on the pathogen.

**Components:**

Agar 15-20 g

Distilled water 1000 ml

Fresh leaves (wheat, oats, barley, etc.) 100 g

Sucrose 10 g

Preparation: Boil leaves for 20-30 min and filter the water. Add filtrate to agar and mix with

water to 1000 ml. Sterilize and pour into Petri dishes or test tubes.

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**5. Antibiotics**

Antibiotics may be added to fungal isolation media to prevent the growth of bacteria or

unwanted fungi. Most antibiotics (except chloramphenicol) are unstable if heated and need to be

added to the medium after autoclaving. These antibiotics are dissolved in a small quantity of

sterile distilled water, according to the recipe. For most purposes this may be added directly to

the medium but, for critical work the antibiotic solution should be filter-sterilised before use.

**Commonly used antibiotics**

**Antibiotic Active against Solubility**

Penicillins Gram-positive bacteria Water soluble

Streptomycin Gram-negative bacteria Water soluble

Neomycin Gram-positive bacteria Water soluble

Chloramphenicol Gram-positive and negative bacteria Ethanol soluble