Important practical hints for pouring agar plates:

- Avoid pouring plates in a drafty place. Make sure the bench is clean (wash with soapy water if necessary) and free of dust. Wipe the bench down with 95% ethanol to surface sterilize.
- We routinely use 100 mm Petri plates that have a non-skid lip, so it is possible to pour stacks of plates up to 20 high. This saves a lot of bench space, but means that the agar takes slightly longer to solidify in the middle of the stack. Solid agar is cloudy, whereas molten is clear.
- Most types of media look very similar, exceptions including Rose Bengal and V8. We label our plates by running a Sharpie marker down the side of the stack after pouring. Codes include: one blue line = Minimal Medium; one red line = Complete Medium.
- After a stack of plates is completely cool (not before – it is disastrously messy) the stack should be inverted and left on a clean bench for a day. This allows the agar to dry out slightly, and will reduce future contamination. Except in Type I clean rooms, which cost millions of dollars, you can count on there being spores in all room air, even in apparently clean labs. These will cause contamination if given a chance. Allowing the agar to dry slightly (not enough to cause the agar surface to wrinkle or contract) reduces contamination during storage.
- We store our agar plates inverted, in the bags in which the plates originally were shipped, at 4*C. This way any condensation that does form will collect on the lid, which again helps to reduce contamination. The condensation can be tipped off before turning the plate right side up.

Recipes

"Complete" Medium (a complicated recipe, but good for *Aspergillus nidulans*)
10 g D-glucose (a.k.a. dextrose)
2 g peptone
1 g yeast extract
1 g casamino acids
50 ml 20X nitrate salts (see below)
1 ml trace elements (see below)
1 ml vitamin solution (see below)
Adjust pH to 6.5 with 1 M NaOH.
Add distilled water to 1 litre.
For solid medium add 15 g agar.
Autoclave.

Minimal Medium (a fully defined medium)
50 ml 20X nitrate salts (see below)
10 g D-glucose
1 ml trace elements (see below)
1 ml 1% thiamine
50 ul 0.05% biotin
Adjust pH to 6.5 with 1M NaOH. Add distilled water to 1 litre. For solid medium add 18 g of agar per litre. Autoclave.

20X Nitrate Salts
120 g $NaNO_3$
10.4 g KCl
10.4 g $MgSO_4 \cdot 7H_2O$ (5.2 g if anhydrous)
30.4 g KH_2PO_4
Add distilled water to 1 litre. Autoclave. Store at 5ºC.

1000X Trace Elements
1. Dissolve these salts in 80 ml of distilled water in the order indicated:
$FeSO_4 \cdot 7H_2O$ (ferrous sulphate) 1.0g
Disodium EDTA (ethylene diamine tetra-acetate) 10.0g
Adjust pH upwards with KOH pellets. A golden yellow solution results above ~ pH 5.5, and this is sufficient to proceed.
2. Dissolve these salts in 80 ml of distilled water in the order indicated:
$ZnSO_4 \cdot 7H_2O$ (zinc sulphate) 4.4g
H_3BO_3 (boric acid) 2.2g
$MnCl_2 \cdot 4H_2O$ (manganous chloride) 1.0g
$CoCl_2 \cdot 6H_2O$ (cobaltous chloride) 0.32g
$CuSO_4 \cdot 5H_2O$ (cupric sulphate) 0.32g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (ammonium molybdate) 0.22g
Combine Solutions (1) and (2), and readjust the pH to 6.5 using first KOH pellets, then KOH solutions of decreasing concentration. Bring the final volume to 200 ml with distilled water, and store at 4-8°C. As with traditionally prepared Hutner's TE, this solution is initially bright green, turning purple upon storage. Precipitates are never formed.

Vitamin Solution
0.01 g each of biotin, pyridoxin, thiamine, riboflavin, PABA (p-aminobenzoic acid), nicotinic acid in 100 ml water. Store in a dark glass bottle (riboflavin is light-sensitive) at 4ºC with a drop of chloroform to reduce bacterial spoilage.

5YEG (general purpose media)
5 g yeast extract
10 g D-glucose
Add distilled water to 1 litre. For solid media add 18 g of agar per litre.

5GP (general purpose medium)
10 g D-glucose;
5 g peptone
Add distilled water to 1 litre. For solid medium add 18 g agar

V8 agar (a good natural medium, particularly for plant pathogens – improves sporulation of some strains)
200 ml V8 vegetable juice
3 g $CaCO_3$
20 g agar
1000 ml water

Rose Bengal Agar (good for slowing growth of zygomycete colonies in a mixed culture when making isolations, and for reducing bacterial contaminants)
1000 ml water
10 g D-glucose
5 g peptone
1 g KH_2PO_4
30 mg Rose Bengal (10 ml of a 3 g/l stock solution)
15 g agar
Autoclave and cool
Add 30 mg streptomycin, swirl to mix, and pour

"Organic" medium for oomycetes
10 g D-glucose
1 g peptone
0.1 g yeast extract
1 g KH_2PO_4
0.3 g $MgSO_4.7H_2O$ (or 0.15 g anhydrous $MgSO_4$)
15 g agar (optional)

GY medium for Oomycetes (ATCC medium 1994)
10g glucose (1%0
2.5 g yeast extract (0.25%)
15 g agar (1.5%
distilled water to 1 litre

Dilute Salts (for inducing oomycete zoosporogenesis) makes 1 litre each stock. Store at 4°C.
DSA
13.6 g KH_2PO_4
17.4 g K_2HPO_4
13.2 g $(NH_4)_2SO_4$
DSB
7.4 g $CaCl_2.2H_2O$
10.2 g $MgCl_2.6H_2O$

To use the Dilute Salts stocks, put 500 ml of distilled water in a 1000 ml flask, add 0.5 ml DSA and 1.0 ml DSB, swirl to mix well, and add distilled water to one litre. Swirl again. Mixing stocks directly causes precipitation.

To induce zoosporogenesis: grow oomycete cultures of in liquid OM, overnight, starting with very small inocula, ~1mm^3. Remove OM by aspiration, and replace with dilute salts. Remove replace two or three times in the first hour, and then once an hour. Should have good zoospore release by 4-5 h.

Cotton blue in lactophenol
20 g phenol crystals
20 g lactic acid
40 g glycerine
20 g water
0.1 g cotton blue

NB: lactophenol polymerizes/esterifies with time. This changes the osmolality of the solution so that it causes plasmolysis. Other blue dyes are probably similar to cotton blue -- trypan blue or toluidine blue; Dave Malloch suggests ink blue

Lactofuschin
100 ml 85% lactic acid
0.1 g acid fuschin
Good general cytoplasmic stain, deep pink

IKI solution (for carbohydrates)
100 ml water
5 g potassium iodide (KI)
1.5 g iodine metal
Stains starch deep blue (Amyloid reaction). Stains some basidiomycete spores brown (Dextrinoid reaction).

Czapek's Solution Agar

Sucrose	30 g
$NaNO_3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
Agar	15 g
Distilled water	1000 ml

Czapek's Solution Agar is a synthetic medium widely used in mycological laboratories. Many moulds produce very characteristic colonies on it and may also exude pigmented substances. Aerial growth is often suppressed and sporulation may be enhanced. Some moulds, however, grow poorly on this medium and may even fail to sporulate altogether, often because of their inability to synthesize vitamins.