How to choose and prepare media.

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Background

Two synthetic media are now in general use as standards for culturing and crossing Neurospora -- Medium N (Vogel 1956, 1964) for growth, and Synthetic Cross Medium (SC) (Westergaard and Mitchell 1947) for crosses and mating type tests. Variations of these basic media have been derived to meet special needs.

Until 1941, traditional mycological media such as potato dextrose agar and cornmeal agar were used. (See Stevens 1974 for recipes.) Shear and Dodge (1927) employed cornmeal agar both for growing cultures and for making crosses. Cornmeal agar can still be useful as a simple and effective alternative to SC (e.g., Bennett and Howe 1980).

When Beadle and Tatum (1941) required a defined synthetic growth medium ('minimal medium') in their hunt for nutritional mutants, they adopted Fries medium No. 3, which had been devised by the Swedish mycologist Nils Fries (1938) in his studies of fungal nutrition. (Tatum and Fries had become acquainted while both were postdocs working on microbial growth requirements in Kőgl's lab in Utrecht.) Fries medium could be made up only at 2× concentration without precipitating. In the Tatum lab, the 2× stock was made up in 50-liter Pyrex carboys fitted with tubing devised to siphon and dispense the sterile medium. After autoclaving, the heavy carboys were lifted onto a high shelf in the laboratory (not without risk of strained backs). Fries medium continued to be used until 1956, when it was replaced by Medium N, which could be made up as a 50× stock, thanks to chelation by citrate.

Vogel's Medium N has become the medium of choice when a defined synthetic medium is needed for such applications as routine auxanography, stock-keeping, searching for auxotrophic mutants, or growing Neurospora to prepare DNA, mitochondria, etc. However, another medium ("Bird Medium", Metzenberg 2004) has been designed to supplant medium N for critical applications such as preparing samples for microarrays or analyzing subtle phenotypes of new mutants. Bird medium assures constancy of pH during growth of the culture and circumvents certain other problems that arise in the use of Medium N. It is not meant to supplant Vogel Medium for routine use.

In experiments where quantitative precision and reproducibility are important, caution should be exercised regarding batch differences and the purity of chemical constituents. For most purposes, Reagent Grade chemicals are not required, but quality grade should be used. Agar is a natural product, with batches that differ in ability to gel and in their content of minor constituents. It is wise to record Control Numbers.

"Complete' media that would satisfy a wide variety of growth requirements were prepared by supplementing minimal medium with hydrolysates and extracts from various unrefined natural sources. Numerous variations have been devised for use in mutant hunts and for culturing auxotrophic strains. No one formula is optimal for all auxotrophs, largely because cross-inhibitions between the constituents mean that strains with certain requirements cannot grow. (Some of the cross-inhibitions are listed below.) Nevertheless, organic media of undefined content have been found that support growth of a wide variety of auxotrophs. Complete media such as those listed below are convenient for growing up multiply mutant stocks and the progeny of crosses segregating for auxotrophic markers.

Recipes for commonly used Neurospora media have been compiled in publications such as Davis and de Serres (1970), Bennett and Lasure (1991), and Davis (2000). These are repeated below, together with comments and additional recipes from the literature. In addition to formulas for synthetic media, recipes for three organic 'complete' media are given as examples.

Recipes

GROWTH MEDIA

1. ***Medium N*** "Vogel's Medium" (Vogel 1956, 1964)

Recipe for 1 liter 50× salts:

water	755 ml
Na_3 citrate.$2H_2O$	125 g
KH_2 PO_4	250 g
NH_4NO_3	100 g
$MgSO_4$.7 H_2O	10 g
$CaCl_2$. $2H_2O$ (dissolved)	5 g
trace element solution	5 ml
biotin stock solution	2.5 ml

Conveniently prepared in a large Erlenmeyer flask with magnetic stirrer.
Dissolve constituents successively. Make certain that everything is dissolved before adding the next component.
Moderate heating is useful in speeding solution of the citrate and phosphate.
Dissolve the calcium chloride separately in 20 ml water and add the solution slowly. (Alternatively, powdered calcium chloride can be added slowly, but this takes longer.)
Add about 5 ml Chloroform as preservative and store the 50× stock solution at room temperature.

Single strength Medium N is autoclaved after adding sucrose (1 or 1.5%) and, if desired, agar (1.5%).
pH of the single strength medium is about 5.8. No adjustment is necessary.
Grocery-store sucrose is sufficiently pure for most purposes.

***Trace element solution*:**

In 95 ml. distilled water, dissolve successively with stirring, at room temperature:

Citric acid.1 H_2O	5 g
$ZnSO_4$,7 H_2O	5 g
$Fe(NH_4)_2(SO_4)_2$.6 H_2O	1 g
$CuSO_4$.5 H_2O	0.25 g
$MnSO_4$.1 H_2O	0.05 g
H_3BO_3 (anhydrous)	0.05 g
Na_2MoO_4.2 H_2O	0.05 g

Store in a stoppered bottle at room temperature, with 1 ml chloroform added as preservative. The same formula may be used also for other synthetic media. Trivial quantitative differences in published trace element recipes may reflect differences in hydration of the constituents and can be ignored.

***Biotin stock solution*:**

Dissolve 5 mg biotin in 50 ml water or 50% ethanol. Tube 2.5 ml aliquots and store at –20°C.
Alternatively, a 50% ethanol solution can be stored at 5°C.

MODIFICATIONS OF MEDIUM N:

2. *Medium N without ammonium nitrate* (Metzenberg 2003)

Recipe for 1 liter of 50× salts:

water	770 ml
Na_3 citrate.2 H_2O	130 g
KNO_3	126 g
$(NH_4)H_2PO_4$	144 g
KH_2 PO_4	80 g
$MgSO_4$.7 H_2O	10 g
$CaCl_2$. 2 H_2O	5 g
trace element solution	5 ml
biotin solution, 0.1 mg/ml	2.5 ml
chloroform to preserve	a few ml

Because ammonium nitrate in bulk is explosive, obtaining and storing it may be difficult because of safety regulations. This reformulation of Medium N avoids the problem while resulting in a salt solution that is identical with the original.

3. *Sorbose/glucose/fructose medium for plating* (Brockman and de Serres 1963)

Sorbose, which converts spreading wild type mycelial growth into restricted colonies, is toxic in combination with sucrose. Toxicity is overcome, however, if small amounts of glucose and fructose are substituted for sucrose as the carbon source.

To medium N, add:

sorbose	2%
glucose	0.05%
fructose	0.05%
agar	1.5%

Decreasing sorbose to 1% gives less restricted colonies. Colonies are more restricted at 34°C than at 25°C. Glucose at 0.1% may be used, omitting fructose. Other variations have been used.

Medium with fructose becomes somewhat brown when autoclaved, without any obvious detectable effect. If desired, browning can be avoided by autoclaving the sugars separately from the mineral salts and agar, and combining them after autoclaving. Browning is said to be reduced if Synthetic Cross Medium is used rather than Medium N.

Depending on the purpose, conidia or ascospores may be distributed on the surface of sorbose agar using a spreader, or they may be suspended in the medium when plates are poured. Alternatively, to obtain colonies at the same level, prelayer with about 10 ml sterile medium, then add as a second layer 5 ml agar containing conidia or ascospores, kept molten at 45°C in a water bath. Addition of a sterile overlayer delays the aerial growth and conidiation that occur when colonies develop on the surface. 0.75% agar is used for overlayering, 3 to 5 ml per plate. Colonies break through the surface and conidiate much later with 5 ml rather than 3.

For details regarding large-scale quantitative platings and for cautions regarding the effect of crowding, see Davis and de Serres (1970). For a protocol using sorbose in combination with the conditional *cot-1* mutation, see Catcbeside (1966).

4. *Bird medium* (Metzenberg 2004)

Bird medium is a modification of Vogel medium. Just as *amber* mutations were named for Harris *Bernstein* (German for 'amber'), *Bird* medium was named for Henry *Vogel*. The following description is taken from Metzenberg's original Fungal Genetics Newsletter account, with a few minor changes in the text (R. L. Metzenberg, personal communication).

"This medium was designed to circumvent some problems that arise in the use of Medium N (Vogel 1964). These are, among others, the presence of high levels of citrate, a chelator which leaves the concentration of calcium and trace elements uncertain; the use of ammonium nitrate, which leaves the actual source of nitrogen ambiguous; the use of $MgSO_4$, which does not allow the experimenter to vary the concentration of magnesium and sulfur independently; the high activity coefficient for the pK_a values of citrate, which makes the pH unnecessarily sensitive to ionic strength; the use of sucrose, which leaves uncertain the nature and relative amounts of the hexose(s) being used at any particular moment; the need to use chloroform as a preservative, which results in the gradual depletion of the aqueous phase of complexes of trace elements. Molybdate ion is excluded from the trace elements used for Solution 1 because, in concentrated stock solutions, it forms water-insoluble complexes with phosphate plus ammonium ion; instead, it is included in Solution 2. There may still be a light formation of precipitate. If so, it should merely be swirled into suspension before an aliquot is removed for dilution. Finally, concentrations are expressed in moles rather than in grams, which eases the experimenter's task of thinking in terms of stoichiometry and biomass yield.

Bird Medium is not meant to supplant Vogel Medium for routine auxanography, stock-keeping, searches for mutants, or growth of Neurospora for preparing DNA, mitochondria, etc. However, it should be seriously considered for critical applications such as preparation of samples for microarrays and analysis of subtle phenotypes of new mutants.

Bird Medium supports rapid germination of conidia and rapid growth of mycelium in good yield and with apparently normal morphology. It appears at least equal to, or better than, Vogel Medium in this regard. It should be noted, however, that mycelial pads harvested from Bird Medium have a subtly different texture from those grown on Vogel Medium, being somehow more slippery to the touch. It is not evident that more slippery is less "normal" for Neurospora, or more so, than less slippery.

In the stock solution recipes that follow, ingredient quantities for 50 ml of 20× stock solution are, of course, identical to those desired for 1 liter of 1× working medium.

SOLUTION 1 **Ingredient**	**Quantity for 50 ml 20× Stock**	**Concentration at 1× (mM)**
water	45 ml	
MES (Sigma M-8250)	4.85 g	22.75
K_2HPO_4	1.74 g	10
NH_4Cl	1.34 g	25
K_2SO_4	0.174 g	1.
NaCl	0.058 g	1.
10,000× trace element solution without molybdate *	0.1 ml	

SOLUTION 2 **Ingredient**	**Quantity for 50 ml 20× Stock**	**Concentration at 1× (mM)**
water	38 ml	
$MgCl_2.6\ H_2O$	0.203 g	1.
$CaCl_2.2\ H_2O$	0.074 g	0.5
glucose	18 g	100
biotin, 10,000× solution *	0.1 ml	
sodium molybdate, 10,000× *	0.1 ml	

Make up MES, K_2HPO_4, NH_4Cl, K_2SO_4, NaCl, and trace elements solution without molybdate in 45 ml. of warm water, which will produce a volume of 50 ml. (**"Solution 1; 20× final strength"**). There is no utilizable carbon source in this solution. It should be stored at room temperature, without chloroform. Note that 22.75 mM is the correct concentration for 4.85 g/liter MES, taking into account the one molecule of water of crystallization that is present in the commercial product.

Make up $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, biotin, molybdate solution, and glucose in 38 ml. of warm water, which will produce a volume of 50 ml. (**"Solution 2; 20× final strength"**). Store at room temperature over a few ml. of chloroform.

Obviously, it will usually be convenient to make up these two solutions on at least ten times the above scale.

The quantity of constituents in 50 ml 20× stock is that desired for 1 liter 1× medium. Solutions 1 and 2 can be autoclaved separately in their concentrated form, if desired, and diluted into sterile water, or each can be diluted tenfold to 2×, autoclaved, and then combined to give the 1× working medium. The pH of the diluted medium, about 5.8, equals that of Vogel medium and should not be adjusted."

***BIOTIN: 10,000× STOCK SOLUTION**

Ingredient	**Quantity for 100 ml**	**Concentration at 10,000× (mM)**
water	100 ml	
biotin	6.1 mg	0.25

***TRACE ELEMENTS WITHOUT MOLYBDATE: 10,000× STOCK SOLUTION**

Ingredient	**Quantity for 100 ml**	**Concentration at 10,000× (mM)**
water	95 ml	
citric acid.H_2O	4200 mg	20
$ZnSO_4.7H_2O$	5750 mg	20
$Fe(NH_4)_2(SO_4)_2.6H_2O$	980 mg	2.5
$CuSO_4.5H_2O$	250 mg	1.0
H_3BO_3	62 mg	1.0
$MnSO_4.H_2O$	33.3 mg	0.2

***SODIUM MOLYBDATE: 10,000× STOCK SOLUTION**

Ingredient	**Quantity for 100 ml**	**Concentration at 10,000× (mM)**
water	100 ml	
$Na_2MoO_4.2H_2O$	48.3 mg	0.2

5. ***Glycerol minimal medium*** (Charlang 1979)

The amount of growth of wild type in liquid Medium N is reduced to <10% when 2% glycerol is substituted for 2% sucrose. Growth on glycerol is improved significantly by adding an organic nitrogen source and/or ascorbic acid. The yield increases from 30 mg/50 ml on unsupplemented glycerol to 143 mg when both are added.

To Medium N without NH_4NO_3 add the following:

glycerol	2%
L-asparagine	0.5%
Tween 80	(1 or 2 drops per 100 ml)
ascorbic acid	100 μg/ml

The ascorbic acid solution is freshly prepared in sterile distilled water and is filter-sterilized before adding it to the autoclaved medium.

Medium N modified* as *crossing medium

See below under *Crossing media*.

OTHER MINIMAL MEDIA:

6. ***Fries Medium*** (Fries No. 3) (Fries 1938, Beadle and Tatum 1941).

For 1 liter 2× stock solution:

NH_4 tartrate	10 g
NH_4NO_3	2 g
KH_2PO_4	2 g
$MgSO_4.7\ H_2O$	1 g
NaCl	0.2 g
$CaCl_2.2H_2O$	0.2 g
Trace elements	200 μl (0.2 ml)
Biotin stock	100 μl (0.1 ml)
Distilled water	1 liter

Trace elements as for Medium N or Synthetic Cross Medium.
Use 0.1 mg/ml biotin stock solution, conveniently stored frozen in 1.1 ml aliquots.
Store stock solution at 4°C over 2 ml chloroform.

COMPLEX GROWTH MEDIA

7. *Glycerol complete medium (GCP)*

Adapted from the formula devised by Tatum *et al.* (1950) (Medium 2) to satisfy a wide range of requirements while maintaining good conidiation.

For 500 ml:

yeast extract	1.25 g
casein hydrolysate (tryptic digest)	0.5 g
agar	7.5 g
Medium N 50× stock solution	10 ml
glycerol	4 ml
vitamin stock solution	5 ml
water	480 ml
L-arginine (optional)	3.8 mg

GCP inhibits histidine, homoserine, threonine, and some serine mutants. It is suboptimal for growth of some amino acid, purine, and pyrimidine mutants. This can be alleviated if desired, by adding supplement, as with arginine, which is shown in the formula above as an example.

For 100 ml vitamin stock solution:

thiamin.HCl	10 mg
riboflavin	5 mg
pyridoxine.HCl	5 mg
calcium pantothenate	50 mg
p-aminobenzoic acid	5 mg
nicotinamide	50 mg
choline.HCl	100 mg
folic acid	1 mg
inositol	100 mg

Make up in 50% ethanol. Store at 5°C in an amber bottle, to protect riboflavin from destruction by exposure to light.

8. *Proteose-peptone complete medium (PPC)* (Perkins lab, unpublished)

Identical to GCP, but with 0.5 g Difco Proteose-Peptone substituted for casein hydrolysate. Provides better supplementation than NZ-Case (enzymatic digest of casein) for some amino acid requirements.

9. *Horowitz complete* (Horowitz 1947)

To 1 liter minimal medium, add:

glycerol	16 ml
casein hydrolysate	0.25 g
yeast extract	5 g
malt extract	5.g
agar	15 g

This is stated to give good conidiation. Added inositol (200 g/ml) is needed to support inositol mutants. The medium is suboptimal for some other auxotrophs.

10. ***"Neurospora culture agar"*** (Difco)

Dissolve in 1 liter water:

yeast extract	5 g
Proteose-Peptone No. 3 (Difco)	5 g
maltose	40 g
agar	15 g

This medium supports growth of a wide range of auxotrophs (tyrosine excepted). Conidiation is generally poor.

CROSSING MEDIA

11. ***Synthetic Cross medium (SC)*** (Westergaard and Mitchell 1947)

For 2× stock, dissolve the following successively in 3 liters water:

KNO_3	6.0 g
K_2HPO_4 (anhydrous)	4.2 g
(*or* $K_2HPO_4.7\ H_2O$)	(5.49 g)
KH_2PO_4 (anhydrous)	3.0 g
$MgSO_4.7\ H_2O$	3.0 g
NaCl	0.6 g
$CaCl_2.2\ H_2O$ (dissolved separately)	0.6 g
biotin stock solution	0.3 ml
trace element stock solution	0.6 ml

pH of the single-strength medium is about 6.5. No adjustment is needed. Add 2 ml chloroform as preservative and store at 5°C. Before sampling, swirl to resuspend the fine precipitate that forms in the 2× stock.

Different carbon sources were used in the original paper without any one of them being specified as a standard. 1% sucrose is commonly used, but some labs use 0.5% or 0.1% sucrose, or substitute filter paper for the sugar. See *How to make a cross*.

When supplementation is necessary to support growth of auxotrophs, the concentration of amino acids should be kept at a minimum because excess amino nitrogen inhibits crossing.. When one parent is an amino acid auxotroph, it is best used as fertilizing parent to avoid supplementing SC with the amino acid. Alternatively, a prototrophic heterokaryon can be made by combining the amino acid auxotroph with *helper-1* or another helper strain, and the heterokaryon can be used as protoperithecial parent on unsupplemented SC. Crosses homozygous for a nitrate mutant can be made by replacing KNO_3 with 6.25 mM NH_2NO_3 (Catcheside 1981). See *How to make a cross*. See *How to use helper strains*.

SC can be used as a minimal medium for growth, and it has various other applications. For example, it is useful for scoring nitrate nonutilizing mutants.

12. ***Medium N modified*** **for use as a** ***crossing medium*** (Russo *et al.* 1985)

Synthetic Cross Medium as formulated by Westergaard and Mitchell can be made up only at a concentration of 2×, compared with the 50× concentration that is possible for stocks of Vogel's medium N, where citrate acts as a chelator. Russo *et al.* (1985) found that by reducing NH_4NO_3 tenfold in the Medium N formula (from 100 g to 10 g per liter) in the 50× stock, they could modify medium N so as to use it for crossing and mating-type testing.

13. *Cornmeal agar*

- *ATCC formula*: "Add 50 g yellow cornmeal to 1 liter distilled water. Bring to a boil and simmer for 10 minutes. Filter through cheesecloth. Return volume to 1 liter." (Used at half strength for cornmeal agar.) (American Type Culture Collection. http://www.atcc.org/SearchCatalogs/MediaFormulations.cfm)

- *Howe corn infusion medium*: "Suspend 200 to 400 grams white corn meal, unenriched, in 1 liter water. Leach overnight at 5°C. Filter through cheesecloth, and restore to volume. Add agar, but no sugar. The white cornmeal agar medium minimizes conidiation when amounts up to 200 grams per liter are used. At 200 g/liter or above, as many conidia are produced as with Synthetic Cross Medium. The number of perithecia increases proportionately with concentration. On this basis, Difco Cornmeal Agar behaves as though Difco extracted 50g/liter." (Howe and Prakash 1969, H. B. Howe 1970 personal communication to D. D. Perkins.)

- *Difco*: Commercially available Difco dehydrated Corn Meal Agar medium (No. B386) is apparently based on infusion of 50 g of corn meal per liter.

MEDIA FOR SPECIAL PURPOSES

14. *Banding media* *(for rhythm studies)* (Sargent and Kaltenborn 1972 [*bd*], Park and Lee 2004 [bd^+])

For 1 liter minimal medium:

	For *bd* strains	**For bd^+ strains** (Park and Lee)
glucose	3 g (0.3%)	1 g (0.1%)
L-arginine.HCl	5 g (0.5%)	1.7 g (0.17%)
agar	15 or 20 g (1.5% or 2%)	15 g (1.5%)

Maltose (0.5%) has been substituted for glucose (e.g., Lakin-Thomas and Brody 1985). Glucose is omitted by Dragovic *et al.* 2002. Early race-tube experiments obtained clear banding on Medium N salts + 1.2% sodium acetate and 0.5% Difco Casamino Acids (Feldman and Hoyle 1973).

For rhythmic RNA analysis using mycelial mats of *bd* strains grown in liquid culture, concentrations may be reduced tenfold to 0.03% glucose and 0.05% arginine (e.g., Correa *et al.* 2003), or glucose may be increased to 2% (Dragovic *et al.* 2002).

15. ***Storage media for ripening ascospores*** (Metzenberg 1988).

Germination of freshly shot ascospores is initially low but it increases rapidly as they age and ripen.
This can be accomplished by storing ascospores on water agar. However, unsupplemented agar is able to support scanty growth and the agar surface may then be overgrown with hyphae from a spontaneously germinated ascospore or from a casual contaminant. The EDTA storage medium does not support growth.
The following description is based on Metzenberg 1988:

Shot octads (or ordered asci) are collected onto the storage medium. The EDTA at pH ~8.0 completely prevents growth of spontaneous germinants, Neurospora vegetative cells, or casual contaminants. The collection plate or slab is placed in a plastic box (or wrapped) to prevent drying and stored at room temperature for at least a week, or better, for several weeks. Ascospores remain viable for at least several months on these plates.

Stock	Final Concentration	For 100 ml	For 250 ml	For 500 ml
1 M Tris-HCl	0.1 M	10 ml	25 ml	50 ml
50 mM EDTA	2 mM	4 ml	10 ml	20 ml
water		86 ml	215 ml	430 ml
agar	3%	3 g	7.5 g	15 g

For 500 ml, it is convenient to use two 500 ml flasks. Weigh 7.5 g agar into each. Measure the Tris and EDTA solutions into graduated cylinders and add distilled water to 500 ml. Cover with parafilm and invert several times to mix. Pour 250 ml into each flask. After autoclaving, dispense into plates or store at 5°C until needed. Pour plates to generous depth. Wrapping in Saran Wrap keeps the agar from drying and makes isolating ascospores on the plate much easier. After ageing, isolated ascospores are transferred to growth medium before heat shock. A small fragment of storage medium carried over with the isolated ascospore does not impair germination or inhibit growth.

16. ***Media for recovering Neurospora from soil samples*** (R. L. Metzenberg in Glass *et al.* 1990)

For 1 liter of medium, autoclave:

D-xylose	10 g
agar	10 g
KNO_3	1 g
KH_2PO_4	875 mg
K_2HPO_4	160 mg
$MgSO_4.7H_2O$	500 mg
NaCl	100 mg
$CaCl_2$	100 mg
biotin	5 µg (as for Medium N)
trace elements	0.1 ml (as for Medium N)

When cooled to 60°C, add:

chloramphenicol	300 mg
2-furyl alcohol	1 ml of 10% aqueous solution

Soil samples (~2 g) in 50 ml screwcap tubes are charged with 25 ml of the above medium at 60°C. The tubes are shaken vigorously a few times and then held at 60°C for 20 min. After shaking again to resuspend the soil, the contents are poured into petri dishes. See *How to sample natural populations*.

17. Iodoacetate SC for stimulating microconidiation (Ebbole and Sachs 1990)

Synthetic Cross Medium	0.1×
sucrose	0.5%
agar	2%
sodium iodoacetate	1.0 mM

Dispense and sterilize agar medium to 16 × 150 mm tubes. To each tube of molten agar add 60 µl of 0,1M filter-sterilized solution of sodium iodoacetate in water. Mix and slant.

Microconidia from macroconidiating strains grown on this medium are obtained by filtering suspensions through Millipore Durapore Millex 5 µm filters to remove macroconidia and mycelial fragments. Used by Ebbole and Sachs 1990) to purify heterokaryotic transformants. See *How to obtain microconidia*.

18. Chloramphenicol medium to rid strains of bacterial contaminants (Perkins *et al.* 1976)

Prepare chloramphenicol stock solution, 5 mg/ml in 95% ethanol. Use 4 ml/100 ml minimal medium. (Final concentration 20 mg/100 ml.). Autoclavable. Used routinely for newly acquired strains from nature. See *How to sample natural populations***.**

SUPPLEMENTATION OF SYNTHETIC MEDIA

The following are approximations of the growth-factor concentrations required for optimal germination and growth. All values are for supplements added to synthetic medium before autoclaving. The following notes are from the Perkins lab.

Rule of thumb concentrations:

- *Vitamins*: 10 μg per ml except inositol, 200 μg. Riboflavin is destroyed when exposed to light.
- *Amino acids*: 0.2–0.5 mg per ml. Cross-inhibitions are common (e.g., arginine and lysine). See Perkins *et al*. (2000).
- *Purines and pyrimidines*: 0.2–0.5 mg per ml. Guanine mutants are inhibited by adenine.

Stock solutions:

Solutions are made up in water and kept in screw-cap bottles at 5°C, with a few drops of chloroform added to inhibit growth of microbial contaminants. Solubilities permit the concentrations shown below. Numbers in parentheses following each substance.indicate the amount (milliliters) of stock solution to be added (before autoclaving) for 100 ml of final medium.

Single growth factor stock solutions:

Concentration Stock Solution (mg/ml)	**Nutrient** (ml stock/100ml working medium)
0.4	*p*-aminobenzoic acid (1.0)
1	indole (1.0), D-pantothenate (1.0), pyridoxine.HCl, (1.0) riboflavin (5.0), thiamine (1.0).
2	choline chloride (1.5).
3	DL-isoleucine (3.0)
4	L-leucine (4.0), nicotinamide (0.2), L-threonine (2.0).
7	L-valine (3.0).
8	L-tryptophan (2.5)
10	DL-homoserine (2.0), L-methionine (5.0), L-phenylalanine (2.0), L-proline (5.0)
20	L-asparagine (2.0), L-glutamine (2.0), L-lysine (2.5).
25	L-histidine.HCl (2.0)
40	L-arginine (1.25).
50	aspartic acid (1.0), serine (1.0), sodium succinate (1.0).

Multiple combinations:

- Stock to support growth of *ilv* mutants: 3.0 mg DL-isoleucine + 7.0 mg L-valine (3.0).
- Stock for use when *arg* and *lys* mutants are both present: 20.0 mg/ml L-arginine + 40.0 mg/ml L-lysine (2.5).
- Stock to support growth of *aro* mutants: 4 mg/ml L-phenylalanine, 4 mg/ml L-tryptophan, 25 μg/ml PABA (1.0). Then add 0.04 mg/ml tyrosine to the 1× medium.

Stock solutions impractical:

- L-cysteine.HCl oxidizes readily to cysteine in neutral or slightly alkaline solutions.
- Solubility of adenine sulfate, adenosine, tyrosine, uracil, and uridine is too low to make refrigerated stock solutions practical.

AUXOTROPHS SUBJECT TO INHIBITION

In designing media and supplementation, it is important to consider possible inhibitions. For example, if ascospores that require histidine, homoserine, or guanine are germinated on organic complete medium, growth may not go beyond the germination tube. The cross inhibitions are largely due to competition for transport. For amino acid transport systems, see Figure 47 in Perkins *et al.* 2001). Many of the inhibitions are noted by Perkins *et al.* (2001). For example, see entries for *arg*, *gua*, *his*, *hom*, *leu*, *lys*, *phe-1*, *ser-3*, and *thr-2*.

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