MEDIA RECIPES FOR EXTENSIVE CULTURING

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BACKGROUND

- The following recipes allow for the growth of a wide variety of organisms. Some media are enriching, others are selective to promote the widest variety of growth.

EQUIPMENT

- Scale, stir plate
- Autoclave

AIA – ACTINOMYCETE ISOLATION AGAR, ENRICHES FOR ACTINOMYCETES

- follow Actinomycete Isolation Agar instructions (11g powder)
- add 5mL glycerol/ 500mL water
- autoclave; cool

BBE

- 20g Trypticase Soy agar
- 10g Oxgall
- 0.5g esculin hydrate
- 0.25g ammonium iron (III) citrate
- 500mL dH2O, autoclave
- 10uL 1:1 Vitamin K in EtOH
- 1.25mL gentamicin (0.4g in 10mL for stock, filter sterilized) 100ug/mL final
- 1.25mL hemin (0.025g + 0.5mL 1N NaOH, up to 5mL total, autoclave short cycle)

BEEF + SUPPLEMENTS (0.5G/L L-CYSTEINE, 10MG/L HEMIN, 1MG/L VITAMIN K) –ENRICHES FOR LACHNOSPIRACEAE ORGANISMS

- follow Cooked Meat Broth instructions, 28.5 g for 500mL and add 6g agar
- autoclave; cool
- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H_2O, filter sterilized to 500mL of media (final concentration of 0.5g/L)
- add 1mL of 5mg/mL stock of **hemin** made up in 25mM NaOH, filter sterilized to 500mL of media (final concentration of 10mg/L)
- add 1uL of 1:1 ratio of **Vitamin K**:ethanol to 500mL of media (final concentration of 1mg/L)

- **When culturing sputum from CF patients can add colistin to inhibit the growth of *Pseudomonas* so other organisms have a chance to grow. Add 1mL of 5mg/mL filter sterilized stock of **colistin** to 500mL of media (final concentration of 10mg/L).

BHI1

- 26g BHI agar with 500mL dH2O
- Autoclave, cool

BHI2

- 26g BHI agar in 500mL dH2O, autoclave, cool
- Add 100uL colistin (50mg/mL stock, filter sterilize, use fresh) 10mg/mL final
- 50uL nalidixic acid (0.2g in 3mL dH2O, add NaOH dropwise to dissolve, up to 4mL with dH2O for a 50mg/mL stock, filter sterilize) 5mg/mL final

BHI3 - BHI + SUPPLEMENTS (0.5G/L L-CYSTEINE, 10MG/L HEMIN, 1MG/L VITAMIN K) – BRAIN HEART INFUSION AGAR, TO PROMOTE GROWTH OF ANAEROBES

- follow Brain Heart Infusion Agar instructions – 26g- for 500mL
- autoclave; cool
- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H_2O, filter sterilized to 500mL of media (final concentration of 0.5g/L)
- add 1mL of 5mg/mL stock of **hemin** made up in 25mM NaOH, filter sterilized to 500mL of media (final concentration of 10mg/L)
- add 1uL of 1:1 ratio of **Vitamin K**:ethanol to 500mL of media (final concentration of 1mg/L)
- **When culturing sputum from CF patients can add colistin to inhibit the growth of *Pseudomonas* so other organisms have a chance to grow. Add 1mL of 5mg/mL filter sterilized stock of **colistin** to 500mL of media (final concentration of 10mg/L).

BHI4 – BHI + SUPPLEMENTS + COLISTIN + NALIDIXIC ACID

- follow Brain Heart Infusion Agar instructions – 26g- for 500mL
- autoclave; cool
- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H_2O, filter sterilized to 500mL of media (final concentration of 0.5g/L)
- add 1mL of 5mg/mL stock of **hemin** made up in 25mM NaOH, filter sterilized to 500mL of media (final concentration of 10mg/L)
- add 1uL of 1:1 ratio of **Vitamin K**:ethanol to 500mL of media (final concentration of 1mg/L)
- Add 100uL colistin (50mg/mL stock, filter sterilize, use fresh) 10mg/mL final
- 50uL nalidixic acid (0.2g in 3mL dH2O, add NaOH dropwise to dissolve, up to 4mL with dH2O for a 50mg/mL stock, filter sterilize) 5mg/mL final

BHI5 (aer and ana)

- 26 g BHI agar
- 1 ul vitamin K
  ~ 500 ml dH2O, autoclave ~
- 0.5 ml L-cysteine-HCl (500 mg/ml stock, sterile filter)
- 1 ml hemin (5 mg/ml stock in 10% 1 N NaOH, autoclave short cycle)
- 250 µl gentamicin (0.4 g in 10 ml (40 mg/ml stock), sterile filter à 20 mg/L final)

BHI6 (only in Jen's)

- 26 g BHI agar
- 1 ul vitamin K
  ~ 495 ml dH2O, autoclave ~
- 0.5 ml L-cysteine-HCl (500 mg/ml stock, sterile filter)
- 1 ml hemin (5 mg/ml stock in 10% 1 N NaOH, autoclave short cycle)
- 5 ml propionic acid (1% final)

BHIcell (aer and ana)

- 3.8 g BHI broth
- 7.5 g agar
  ~ 450 ml dH2O, autoclave ~
- 50 ml sterile cellulose (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5g/L final. Autoclave short cycle). Note: cellulose is not soluble in water, ensure stock tube is well mixed before adding. Cellulose will remain suspended in the plates as granules

BHIinu (ana)

- 3.8 g BHI broth
- 7.5 g agar
  ~ 450 ml dH2O, autoclave ~
- 50 ml sterile inulin (5 g in 500 ml dH2O (10 g/L stock) à 1 g/L final. Autoclave short cycle)

BHImuc (aer and ana)

- 3.8 g BHI broth
- 7.5 g agar
  ~ 450 ml dH2O, autoclave ~
- 50 ml sterile mucin (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5g/L final. Autoclave short cycle)

BHIpect (ana)

- 3.8 g BHI broth
- 7.5 g agar
  ~ 450 ml dH2O, autoclave ~
- 50 ml sterile pectin (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5g/L final. Autoclave short cycle)

BHIstar (aer and ana)

- 3.8 g BHI broth
  7.5 g agar
  ~ 450 ml dH2O, autoclave ~
- 50 ml sterile starch (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5g/L final. Autoclave short cycle)

BSM (ana)

- 27.75 g BSM agar
  ~ 490 ml dH2O, autoclave ~
- Add 58 mg BSM supplement (dissolve in 10 ml sterile water first)

BHI+CO (10mg/L Colistin, 5mg/L Oxolinic Acid) – Selective for Streptococci. Inhibits growth of Gram-negatives, Staphylococci, Bacillus, and Corynebacteria.

- follow Brain Heart Infusion Agar instructions for 500mL
- autoclave; cool
- add 10mL of 0.5mg/mL stock of **colistin** dissolved in H_2O, filter sterilized to 500mL of media (final concentration of 10mg/L)
- add 5mL of 0.5mg/mL stock of **oxolinic acid** made up in 0.1M NaOH, filter sterilized to 500mL of media (final concentration of 5mg/L)

CBA – Columbia Blood Agar, enriches for fastidious organisms

- follow Columbia blood agar instructions
- Add 21.25 g CBA powder to 500mL dH2O
- autoclave; cool
- add 5% sheep blood (25mL of sheep's blood per 500mL)
- pour immediately (blood cools the media quite a bit)

CHOC – Chocolate agar, enriches for growing fastidious pathogenic respiratory bacteria, such as *Haemophilus influenzae*, *Neisseria meningitidis* and *Strep pneumo*

- For 500mL:

- 18g of GC (BD) Medium powder as directed on bottle
- 250mL dH2O
- autoclave; cool; while cooling prep hemoglobin by warming in 65°C water bath for ≤ 5 minutes and prepare and warm IsoVitaleX
- 250mL of hemoglobin (if too cold it will solidify your agar)
- IsoVitaleX 2.5mL prepared ahead of time

CNA – Columbia agar with colistin, nalidixic acid and blood, selective for Gram positive cocci

- follow CNA agar instructions (colistin and nalidixic acid are in the powder already)
- 21.25g CNA powder
- 500mL dH2O
- autoclave; cool
- add 5% sheep blood (25 mL of sheep's blood per 500mL)
- pour immediately (blood cools the media quite a bit)

FAA – Fastidious anaerobe agar, enriches for anaerobes

- follow Fastidious Anaerobe agar instructions
- 22.85g FAA powder
- 500mL dH2O
- Autoclave

KVLB – Kanamycin Vancomycin Laked Blood - Selective for Gram-negative, anaerobic bacilli such as *Prevotella* spp. *Fusobacteria* spp. and *Bacteroides* spp.

- For 500mL:
  - 22.5g trypticase soy agar
  - 2.5g yeast extract
  - autoclave; cool
  - 10µL of 1:1 ratio of **Vitamin K**:ethanol, for 10mg/L final concentration
  - 500µL of 5mg/mL stock **Hemin**, for 5mg/L final concentration
  - 400µL of 500mg/mL filter sterilized stock **L-cysteine**, for 0.4g/L final concentration
  - 500µL of 100mg/mL filter sterilized stock **Kanamycin**, for 0.1g/L final concentration
  - 37.5µL of 100mg/mL filer sterilized stock **Vancomycin**, for 0.0075g/L final concentration
  - 25mL Laked sheep blood (12.5ml blood, 12.5ml sterile H_2O, freeze O/N)

M9inu (ana)

- 7.5 g agar
  ~ 340 ml dH2O, autoclave ~
- 100 ml 5X M9 salts (autoclaved ahead of time)
- ~~10 ml 20% glucose, sterile filtered~~
- 1 ml sterile 1M MgSO4
- 0.05 ml sterile 1M CaCl2
- 50 ml sterile inulin (5 g in 500 ml dH2O (10 g/L stock) à 1 g/L final. Autoclave short cycle)

M9muc (ana)

- 7.5 g agar
  ~ 340 ml dH2O, autoclave ~
- 100 ml 5X M9 salts (autoclaved ahead of time)
- ~~10 ml 20% glucose, sterile filtered~~

- 1 ml sterile 1M MgSO4
- 0.05 ml sterile 1M CaCl2
- 50 ml sterile mucin (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5 g/L final. Autoclave short cycle)

M9pect (ana)

- 7.5 g agar
  ~ 340 ml dH2O, autoclave ~
- 100 ml 5X M9 salts (autoclaved ahead of time)
- ~~10 ml 20% glucose, sterile filtered~~
- 1 ml sterile 1M MgSO4
- 0.05 ml sterile 1M CaCl2
- 50 ml sterile pectin (2.5 g in 500 ml dH2O (5 g/L stock) à 0.5 g/L final. Autoclave short cycle)

M9star (aer and ana)

- 7.5 g agar
  ~ 340 ml dH2O, autoclave ~
- 100 ml 5X M9 salts (autoclaved ahead of time)
- ~~10 ml 20% glucose, sterile filtered~~
- 1 ml sterile 1M MgSO4
- 0.05 ml sterile 1M CaCl2
- 50 ml sterile starch (2.5 g in 500 ml dH2O stock à 0.5 g/L final. Autoclave short cycle)

MAC – MacConkey agar, selective for Gram-negative and enteric bacilli such as *Escherichia*, *Pseudomonas* and *Klebsiella*

- follow MacConkey agar instructions

McKAY – selective for Streptococcus Milleri Group bacteria

- complex recipe; see separate document

MRS (aer and ana)

- 35 g MRS powder
  ~ 500 ml dH2O, autoclave ~

MSA – Manitol Salt agar, selective for *Staphylococci*

- follow Manitol Salt Agar instructions
- 55g MSA powder
- 500mL dH2O
- Autoclave

PEA – Phenylethyl Alcohol agar, selective for Gram positive obligate anaerobes

- follow Phenylethyl Alcohol Agar instructions
- 21.25g PEA agar
- 500mL dH2O
- autoclave; cool
- add 5% sheep blood (25mL of sheep's blood per 500mL)
- pour immediately (blood cools the media quite a bit)

TSY + supplements (0.5g/L L-Cysteine, 10mg/L Hemin, 1mg/L Vitamin K) – Tryptic Soy Yeast agar, to promote growth of Anaerobes

- follow tryptic soy agar instructions + 0.3% yeast extract
- For 500mL:
  - 20g TSA
  - 1.5g Yeast Extract
- autoclave; cool
- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H_2O, filter sterilized to 500mL of media (final concentration of 0.5g/L)
- add 1mL of 5mg/mL stock of **hemin** made up in 25mM NaOH, filter sterilized to 500mL of media (final concentration of 10mg/L)
- add 1uL of 1:1 ratio of **Vitamin K**:ethanol to 500mL of media (final concentration of 1mg/L)
- **When culturing sputum from CF patients can add colistin to inhibit the growth of *Pseudomonas* so other organisms have a chance to grow. Add 1mL of 5mg/mL filter sterilized stock of **colistin** to 500mL of media (final concentration of 10mg/L).

YPD (aer) (not in Jenn's paper)

- 12 g agar
- 10 g peptone
- 5 g yeast extract
  ~ 450 ml water ~
- 50 ml 20% glucose (sterile filtered)

YPDpH5 (aer) (not in Jenn's paper)

- 12 g agar
- 10 g peptone
- 5 g yeast extract
- pH to 5.5 before autoclave
  ~ 450 ml water ~
- 50 ml 20% glucose (sterile filtered)

GMM (only in Jen's paper)

- 1 g tryptone
- 0.5 g yeast extract
- 2.5 g meat extract
- 7.5 g agar
- 0.5 mg resazurin
- 1 µl Vitamin K (1:1 Vitamin K : EtOH stock)
  ~ 420 ml dH2O, autoclave ~
- 4.055 µl sterile filtered 1 M MgSO4
- 2.4 ml sterile filtered 1 M NaHCO3 (4.2 g in 50 ml dH2, filter sterilize)
- 137 µl sterile filtered 5 M NaCl
- 5 ml cellobiose (5 g in 50 ml dH2O, filter sterilize)
- 1 ml 20% glucose (0.4 g total, sterile filter the 20% solution)
- 0.5 ml 500 mg/ml L-Cys:HCl
- 5 ml maltose (5 g in 50 ml dH2O, filter sterilize)
- 5 ml fructose (5 g in 50 ml, filter sterilize)
- 50 ml KH2PO4 (1 M stock is 68 g in 500 ml, pH = 7.2, filter sterilize. Note: only add ~200 ml water to start because it takes a LOT of NaOH to get up to pH, but very important to get to at least 7.2 to counteract the acids added later)
- 36 µl 1 M CaCl2 (0.8% final, 0.008 g total, filter sterilize)
- 0.5 ml FeSO4 (0.004 g in 10 ml stock, sterile filter)
- 0.5 ml histidine/hematin solution (1.2 mg hematin/ml in 0.2 M histidine, sterile filter. 0.062 g L-His in 1 ml dH2O, then added 2.4 g hematin. Add NaOH drop by drop to dissolve hematin, before topping up with water to 2 ml)
- 1 ml Tween-80 (25% stock solution in dH2O, sterile filter)
- 5 ml ATCC vitamin mix (already sterile)
- 5 ml ATCC trace mineral mix (already sterile)
- 0.85 ml acetic acid
- 50 µl isovaleric acid
- 1 ml propionic acid
- 1 ml butyric acid (Note: add these 4 SCFAs in the fumehood, preferably after everyone has gone home – VERY SMELLY)
  ~ Ensure pH is not lower than ~6.5, otherwise may need to add sterile concentrated NaOH ~

REFERENCES

Sibley CD, Grinwis ME, Field TR, Eshaghurshan CS, Faria MM, et al. (2011) Culture Enriched Molecular Profiling of the Cystic Fibrosis Airway Microbiome. PLoS ONE 6(7): e22702. doi:10.1371/journal.pone.0022702

BD BBL™CDC Anaerobe Laked Sheep Blood Agar
with Kanamycin and Vancomycin (KV)

Lau JT, Whelan FJ, Herath I, Lee CH, Collins SM, Bercik P, Surette MG. 2016. Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. Genome Medicine 8:72.

Check M9 mucin
Check glucose in M9 media