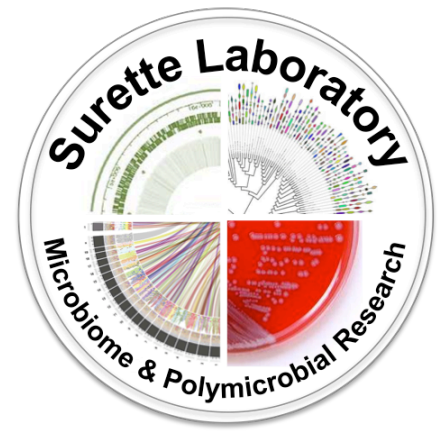

MEDIA RECIPES FOR EXTENSIVE CULTURING

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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
- add 10mL glycerol/ 1L water
- autoclave; cool

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 for 500mL
- autoclave; cool
- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H₂O, 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).

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

- follow Cooked Meat Broth instructions for 500mL and add 6g agar
- autoclave; cool

- add 500uL of 500mg/mL stock of **L-cysteine** dissolved in H₂O, 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)
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CBA – COLUMBIA BLOOD AGAR, ENRICHES FOR FASTIDIOUS ORGANISMS

- follow Columbia blood agar instructions
- 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 400mL:
 - 200mL of GC (BD) Medium powder as directed on bottle
 - autoclave; cool; while cooling prep hemoglobin by warming in 65C water bath for ≤ 5 minutes and prepare and warm IsoVitaleX
 - 200mL of hemoglobin (if too cold it will solidify your agar)
 - IsoVitaleX 2mL per 200mL of GC (BD) Medium powder

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)
- 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

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 filter sterilized stock **Vancomycin**, for 0.0075g/L final concentration
- 25mL Laked sheep blood (12.5ml blood, 12.5ml sterile H₂O, freeze O/N)

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

MSA – MANITOL SALT AGAR, SELECTIVE FOR *STAPHYLOCOCCI*

- follow Manitol Salt Agar instructions

PEA – PHENYLETHYL ALCOHOL AGAR, SELECTIVE FOR GRAM POSITIVE OBLIGATE ANAEROBES

- follow Phenylethyl Alcohol Agar instructions
- 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 YE
- autoclave; cool
- add 500µL of 500mg/mL stock of **L-cysteine** dissolved in H₂O, 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 1µL 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).

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)