	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	MQIP MILK QUALITY IMPROVEMENT PROGRAM

Title: Antibiotic Code System and Preparation of Antibiotic Stock Solutions			
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Antibiotic Code System and Preparation of Antibiotic Stock Solutions

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this SOP is to set forth standard signage for labelling agar plates containing antibiotics, and to define the preparation of antibiotic stock solutions commonly used in the lab. Additional details on the use of these stocks to prepare antibiotic-containing media are also included.

1.2 Scope

This SOP applies to the Food Safety and MQIP lab.

1.3 Definitions

AMP – Ampicillin
CHL – Chloramphenicol
ERY – Erythromycin
KAN – Kanamycin
RIF – Rifampicin
SPT – Spectinomycin
STR – Streptomycin
TET – Tetracycline

1.4 Safety

Allergic reactions to some classes of antibiotics (e.g. β-lactams) may occur; if you have an allergy to certain antibiotics please ask a senior lab member to help you prepare any media containing the antibiotic to reduce exposure via inhalation when preparing media.

All BSL-1 antibiotic containing waste needs to be autoclaved or bleached to ensure inactivation of the antibiotic prior to disposal. BSL-2 antibiotic containing waste should be disposed of as BSL-2 waste. All unused antibiotic containing media should be disposed of as BSL-1 waste.

SECTION 2 MATERIALS

- **Molten agar media (cooled to 60°C) or Broth media**
- **Antibiotic stock solutions (thawed on ice)**
  - prepared in either ultra-pure water (Ampicillin, Kanamycin, Streptomycin, or Tetracycline) or 96% ethanol (Chloramphenicol, Erythromycin, Rifampicin, and Spectinomycin)
  - Stock solutions will vary, but most will be 50 – 100 mg/mL
- **Sterile pipette tips**
- **Pipettes**
- **Stir plate**
- **Sterile plates**
- **Bunsen burner**

SECTION 3 PROCEDURES

3.1. Prepare antibiotic stock solutions.

(1) Clean a spatula with ethanol and flame sterilize it. After allowing the spatula to cool, use it to weigh out the desired amount of antibiotic into a clean weigh boat. Below are some examples of amounts of antibiotic and solvent volumes for the desired stock concentration:

Amount of antibiotic	Volume of Solvent	Final Concentration
100 mg	1 mL	100 mg/mL
250 mg	5 mL	50 mg/mL
500 mg	5 mL	100 mg/mL

(2) Add approximately half of the amount of solvent (96% EtOH or Ultra-pure H_2O) to a clean tube. Use a 1.5 mL Eppendorf tube for final volumes $\leq$ 1.2 mL, and 15 mL conical vials for final volumes > 1.2 mL.
(3) Carefully pour your powdered antibiotic into the Eppendorf or conical tube with ½ of the solvent. Mix by pipetting or vortexing.
(4) Add the remaining ½ volume of the solvent to the tube and repeat pipetting and/or vortexing to mix.
(5) For stocks rehydrated in Ultra-pure H_2O, use a 0.22 µM filter to filter sterilize the stocks.
(6) Tubes containing antibiotic stock solutions should be wrapped in aluminum foil to reduce degradation due to light. Stock solutions should be stored at -20°C.

3.2. Addition of antibiotic stock solutions to agar media.

(1) Calculate the amount of antibiotic stock to be added to media to achieve the desired final concentration. Use the following formula:

$$V_{Stock} = \frac{[C_{Final} * V_{Final}]}{C_{Stock}}$$

Where V_{stock} is the volume of the stock solution to be added to the volume of media (V_{Final}) to achieve the final concentration (C_{Final}).
(2) Label plates by marking the side of the plate lid with a single vertical line. Use the following color code to select the color that corresponds to the antibiotic(s) used:

Antibiotic	**Color**
Ampicillin (AMP)	Blue
Chloramphenicol (CHL)	Red
Erythromycin (ERY)	Brown
Kanamycin (KAN)	Black
Rifampicin (RIF)	Green
Spectinomycin (SPT)	Orange
Streptomycin (STR)	Purple
Tetracycline (TET)	Light Blue

(2) Cool molten agar on a stir plate, stirring just fast enough so as not to introduce bubbles but to facilitate even cooling of the agar. Once the agar reaches ~55-60°C, use a sterile pipette tip to transfer the calculated amount of antibiotic into the vessel containing the molten agar and allow for the stir bar to mix the solution for at least 1 minute.
(3) Pour agar plates next to a Bunsen burner, and allow agar to set (will depend on the number and position of plates in a stack).
(4) Include a label for each different agar media type (or for each "sleeve of plates") that includes the following information: media type, concentration of the antibiotic added along with the three letter abbreviation of the antibiotic, name of the person who prepared the media, and the date the media was prepared. Below is an example of the label.

LB + KAN 50 µg/mL
SAL 22APR20

(5) Agar media containing antibiotics should be stored at 4°C if not used immediately.

3.3 Addition of antibiotic stock solutions to liquid media.

(1) Calculate the amount of antibiotic stock as in 3.2 step 1.
(2) Working near a flame, use a pipette to aseptically transfer the desired amount of stock antibiotic solution into the container with your liquid media.
(3) Mix the solution by vortexing or pipetting.
(4) Label as described in section 3.2 step 4 and store unused media at 4°C.

SECTION 4 TROUBLESHOOTING

(1) Many antibiotics are heat sensitive. To reduce degradation of the antibiotic, only add antibiotic to solutions < 60°C (i.e. allow molten agar to cool, do not add antibiotics immediately after removing from autoclave). Stock solutions should be stored at -20°C and should be thawed at room temperature.

(2) Make sure that you are using the correct solvent to prepare stock solutions. Not all antibiotics can be dissolved in water. Check to make sure that you are using the appropriate solvent for preparing the stock solution.

SECTION 5 REFERENCES

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1			Original document
Version 2	22 APR 20	ram524	New SOP