**SOP Gifu Anaerobic Broth Media preparation**

**Document Number: XX**

**Page 1 of 2**

**Purpose**

This SOP describes the procedure used to make Gifu Anaerobic Broth Media.

**Scope**

For culturing from stool samples, it is a general rich media that allows for growing large majority of anaerobes. Recommended as a general culture medium for cultivation and isolation of anaerobic bacteria.

**Regulatory References:** NA

**Responsibility of experimentalist:** understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results.

**Responsibility of area manager or supervisor:** ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed, and updating the procedure as necessary.

**Definitions/Abbreviations:**

Gifu Anaerobic Broth Media: GAM

Milliliter: ml

**Related Documents:** NA

**Required Equipment and Materials / Reagents**

* GAM (HIMEDIA: Cat# M1801)
* L-cysteine (Sigma Aldrich; Cat# C7532)
* Bottle-top vacuum filter system, pore size 0.2 μm- 1L (Sigma, Cat# CLS430515)
* MilliQ Water

**Precautions**

Personal protection equipment including gloves and lab coat must be worn when executing this procedure

The media expires 3 weeks after preparation

**Procedure**

* Suspend 59.0 grams of GAM powder in 990 ml MilliQ water (to obtain 2X media)
* Mix thoroughly with stir bar and magnetic stirrer (no need to heat the solution)
* Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes
* Let cool at room temperature
* Add 0.5g L-cysteine (to ensure it will easily reduce)
* Prepare Supplements:
  + Hemin Solution:
    - 50mg Hemin (Sigma Aldrich, 51280)
    - 1mL 1N NaOH
    - 100mL MilliQ water
  + Vitamin K1 Solution:
    - 150uL Vitamin K1 solution (Sigma Aldrich, V3501)
    - 30mL 95% Ethanol
* Add 10mL of Hemin solution
* Add 200uL Vitamin K1 solution
* Filter-sterilize with Bottle-top vacuum filter system
* Media can be stored at 4°C for one month (bottle have to be labeled with date of manufacture)

Note 1: the media can be used to pour petri plates: then mix 2X media and 2X agar solutions (1:1)

Note 2: the media can be used for culturing in liquid media: then mix 2X media and sterile water (1:1)

**Version History: NA**

**Worksheets: NA**

**Appendix: NA**