Tsai Lab SOP – General Instructions for preparing media/Good Lab Practice

1. Wear gloves, lab coat. Take an appropriate size mixing container and stir bar, rinse both with deionized water.

2. Begin with 1/3rd the water of the final volume. Turn on the stir plate to stir water and help uniform mixing of the components.

3. Refer the media prep check list near media prep station. Take out all the media components that you need to add before autoclaving. Remember, few chemicals and antibiotics cannot be autoclaved and are to be added later right before pouring and do not open autoclaved media, boxes and filter sterilized chemicals outside clean hood.

4. Weigh and add media components in the order as they appear on the media prep checklist. Make sure that you let all the added components dissolve before adjusting pH and never add MES buffer before adjusting pH.

   Adjust pH then add MES then again check/adjust pH to the desired value.

Use clean weighing paper and spatulas and do not cross contaminate media source by using same spatulas, or weighing paper.

Before and after adjusting pH, rinse pH electrode with deionized water kept next to the pH meter and gently pat dry the electrode using Kim wipe, never rub the electrode. Also, make sure that there is enough electrolyte solution in the electrode and in the electrode dipping bottle.

5. Makeup volume of the media to the final volume using a cylinder.

6. Divide the media in autoclavable bottles or conical flask. Select the size of the container so that there is at least 2/3 empty space above media to avoid boiling over during sterilization cycle.

7. Now add gellan gum to each container and maintain the final concentration.

8. Cover containers properly either with screw caps for bottles or aluminum foils for flasks.

Wipe clean thoroughly media prep station and keep all the media components back to their storage spots immediately. Wash weighing accessories with soap and water and rinse thoroughly to wash off any soap residue (soap interferes with research chemicals).

9. Autoclave stir bars with media @ 25 minutes.

10. After autoclaving let media cool while continuously stirring in the clean laminar flow hood. (Wipe clean stirs plates and take them to hood).

   Laminar flow hood should be UV sterilized for at least 45 minutes prior to use. Pay attention to the hood as well, it should be running properly (if ever in doubt ask somebody in lab). Wipe clean the hood surface with ethanol and change gloves or spray ethanol on your gloves and rub till air dry.

11. When media reaches optimum (50-60°C) temperature to add heat sensitive chemicals i.e. when you can hold between your palms without burning yourself, it is time to add them.
12. Open media in clean hood and using autoclaved tips, add filter sterilized (these chemicals and antibiotics are filter sterilized by other senior lab members or lab manager; you do not need to filter sterilize them at the spot) required chemicals.

13. Let media stir properly for uniform distribution of additives.

14. While opening and closing sterile tubes, boxes, petri dishes and glassware etc. be extra careful not to accidently touch inside the caps to avoid contamination.

15. Organize boxes and petri plates in hood so that you do not reach over the open sterile items and as unknowingly you might shed particles leading to contamination.

16. Label boxes/petri plates before pouring media. Also, write the date made and your initial on each container.

17. After letting media gel, close boxes properly and store them at 4°C.

18. Bag all the dried/gelled plates in original plastic sleeve. The plates should sit upside/topside up in the cold room. Write date, type of media and your initial on the bag as well.

19. Wipe clean the hood surface with ethanol once again and turn off the blower if not needed anymore.

20. Rinse all the media prep plastic, glassware etc. with hot water until remaining media is washed off. Check containers against light as the gelled media is hard to visualize. Dump any remaining gelled media in regular trash can and never in sink.

21. Properly rinsed media prep glassware can be kept in wash tubs around sinks in lab for washing later (in case you run out of time to do so. But they have to be rinsed with hot water right after you finish pouring media).