Plenary: What is wrong?

**Standard operating procedure**

**cover sheet**

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| **SOP number** | 100023 |
| **SOP title** | **Preparing agar plates** |

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|  | **Name** | **Signature** | **Date** |
| **Author** | S. Johnson |  | 03.06.20 |
| **Reviewer** |  |  |  |
| **Authoriser** |  |  |  |

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| **SOP effective Date:** | 03.06.20 |
| **SOP review Date:** | 03.06.25 |

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**Standard operating procedure for:**

**Preparing agar plates**

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| Purpose: |
| The SOP is designed for use by experienced science teachers and technicians only. |
| Scope: |
| Nutrient agar plates must be produced aseptically to ensure all plates are free of microorganisms and other contaminants.  When used for culturing, agar must be set at room temperature.  Nutrient agar plates must only be used to culture organisms permitted in your facility. Once used, cultured plates must not be opened and must be disposed of correctly. |
| Responsibilities: |
| Laboratory technician is responsible for preparing sterile agar plates. |
| Safety notes: |
| Maintenance – all equipment must be maintained according to manufacturer’s instructions.  Handling – ‘hot equipment’ warning signage to be displayed.  Storage – check the purchased nutrient agar container is tightly sealed before storing.  Cleaning – ensure hands are thoroughly washed before and after the procedure and work area is disinfected. Note: 70% Ethanol used in disinfectant is flammable. |

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| Equipment: | |
| PPE (disposable apron, safety glasses)  Autoclave  Balance  Measuring cylinder  Conical flask with non-absorbent cotton wool plug | Hotplate stirrer and magnetic flea (stirring bar)  90mm diameter sterile plastic petri dishes  Disinfectant: 70% ethanol  Nutrient agar |

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| Procedure: |
| To make 1 litre of nutrient agar, (enough for 50–60 plates):   1. Using a digital balance, prepare the agar medium according to the manufacturer’s instructions. 2. Place water in a 1 litre beaker. Using the hotplate stirrer and magnetic stirring bar, mix ingredients with the water whilst stirring to prevent clumping. Heat whilst stirring until the agar dissolves at …..°C. The agar is then poured into conical flasks and plugged with non-absorbent cottonwool. 3. Sterilise the conical flask by autoclave for 20 minutes at 121°C, 15psi, according to manufacturer’s instructions for use of this equipment. 4. The pressure in the vessel should be at zero prior to opening the equipment. The sterilized agar is allowed to cool to 50°C–55°C, this is the optimal temperature for pouring to minimise condensation. (Note agar solidifies at 42°C.) 5. Thoroughly wash and dry hands. Wear a disposable apron. 6. Create a sterile area by thoroughly wiping the bench with 70% alcohol and a paper towel. Air conditioners, fans and fume cupboards must be turned off, and windows and doors closed, to reduce the chance of contamination while pouring. 7. Label the sterile Petri dishes and organise them base down on the bench. 8. Hold the flask containing sterile agar medium in liquid form with your right hand. Turn your left hand palm side up and clamp the cotton plug between two fingers. 9. Use the hand holding cotton plug to lift the lid of the Petri dish. Pour approximately 15 to 20cm3 of the sterilised nutrient agar medium into the base of the Petri dish, until it is about half full. Hold the Petri dish lid so that it partially covers the bottom of the dish as you pour to prevent microorganisms and air borne dust particles from dropping into the sterile plate and contaminating it. 10. Immediately place the lid on the base at an angle so that steam is able to escape. 11. Pour the remaining plates the same way. 12. Allow the plates to cool to room temperature for approximately 30 minutes before packing upside down and refrigerating. Plates can then be stored for several weeks prior to use. |
| Links to Other Policies: |
| Risk Assessment  Manufacturer’s Safety Data Sheets for use of nutrient agar  Manufacturer’s instructions for autoclave |

Change History

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