ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilising agar.

Preparation of agar plates is central to effectively and economically expose our students to the wonderful world of micro-organisms. School science labs have long questioned the best physical method of control to sterilise agar prior to pouring into Petri dishes. Methods used for sterilizing include pressure cookers, sterilisers or autoclaves. Most schools have limited financial resources available, and sourcing processes and equipment that are cost effective is a priority. So which method should you choose and why?

Sterility of processes and equipment forms the very essence of good microbiological laboratory practice and the teaching of these methods to students. This includes the preparation and presentation of agar plates and other equipment used either before, during or after the practical has been run. Teaching aseptic technique to students can be part of the unit of learning; however can be a difficult process to master for first timers. Outcomes of student practicals may be misleading or incorrect if external contaminates are introduced at any stage prior to or during inoculation. Unfortunately, this is often not evident until much time and effort by the student and lab technician has passed and the results are interpreted. Ascertaining the source of any contaminant can be difficult and inconclusive.

While many types of agar plates are available commercially, many laboratory technicians prepare plates in-house thus reducing the cost to schools. Although it can be time consuming, many schools find the cost benefit far outweighs time spent. Many school science preparation areas are used for many disciplines of science and are not usually specific to microbiology or necessarily a 'clean' area. Schools may be permitted by their state jurisdictions to use micro-organisms from Risk Group 1* and/or culture environmental samples. It is impossible to predict what, if any, further contaminants may be introduced to an agar plate during a practical session. Hence the need to 'get it right' by initial effective sterilisation of agar to ensure plates distributed to students do not contain any contaminants.

Universally, micro-organisms are ubiquitous. Effective sterilisation of a liquid such as agar is achieved when all viable organisms are eliminated[1]. The most effective and suitable method of sterilising agar is by using moist heat in the form of steam under pressure i.e. 121^{o}C for 15 minutes at 15 pounds per square inch (psi). This method will denature & coagulate enzymes and other cell constituents in the bacterial cell. Sterilization can be guaranteed only when these parameters are reached.

Sterilisation of agar and plates is usually done in an autoclave or a commercially available pressure cooker with a gauge and the capacity to reach 15 psi, which provides these conditions. Microwave ovens will not sterilise as they do not provide these conditions and therefore are not a suitable alternative to a pressure cooker or autoclave. Water boils at 100^{o}C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases. In an autoclave or pressure cooker the water is boiled in a closed chamber. As the pressure rises, the

boiling point of water also raises. At a pressure of 15 psi inside the autoclave, the temperature is said to be 121°C. Exposure of articles to this temperature for 15 minutes sterilises them.[2]

Due to the action of a microwave oven, micro-organisms will not be killed. Microwaves penetrate unevenly and there are also 'hot spots' caused by wave interference. The whole heating process is different because you are 'exciting atoms' rather than 'conducting heat'.[3] The heat and pressure required to effectively sterilise agar will be insufficient and cannot be maintained for the required period of time. The agar will boil over before any of the required parameters are reached.

As laboratory technicians, we are constantly on the lookout for more efficient ways of finding good quality relevant resources for our students and teachers within budgetary constraints. Sourcing equipment such as a pressure cooker or autoclave is important to ensure the validity of student results and is imperative for microbiological safety.

***WHO Risk Group 1** (no or low individual and community risk). A micro-organism that is unlikely to cause human disease or animal disease (AS 2243.3)

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[1] Todar, Kenneth 2008, 'Control of Microbial Growth', Todar's Online Textbook of Bacteriology http://textbookofbacteriology.net/control_1.html (Accessed 01/04/2014)

[2] Rao, Sridhar 2008 'Sterilization and Disinfection', Department of Microbiology, JJMMC, Davangere www.microrao.com/micronotes/sterilization.pdf (Accessed 01/04/2014)

[3] Brain, Marshall 'How microwave cooking works', howstuffworks.com, http://home.howstuffworks.com/microwave2.htm (Accessed 01/04/2014)