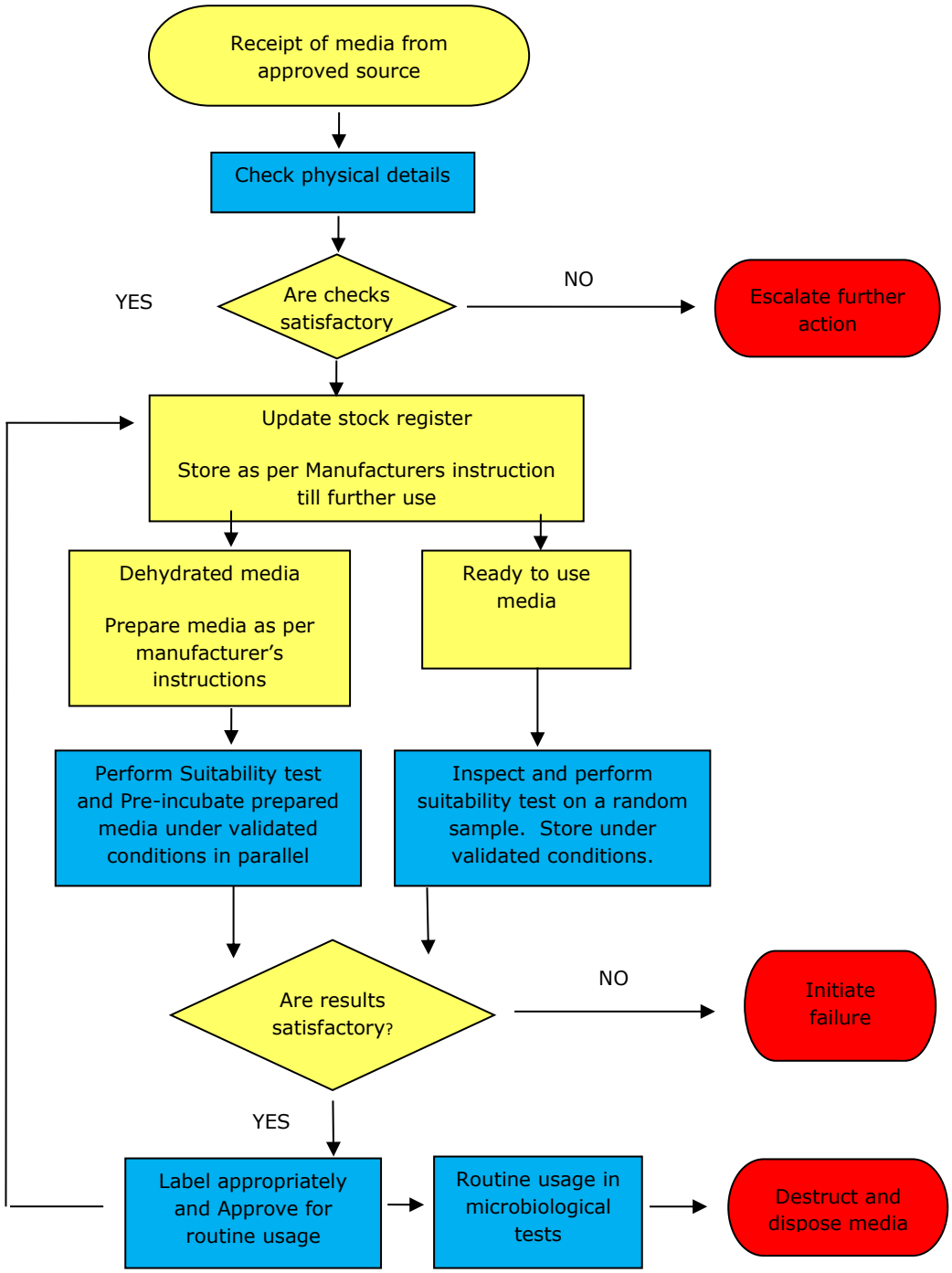
Best Practices in the Growth Potential Testing (GPT) of Microbiological Media

It seems that there is a struggle to perform GPT in a co-ordinated and well documented approach. GPT testing is a regulatory expectation from a cGMP microbiology laboratory and there have been some issues with trepidations and data integrity observations in the microbiology labs around the subject of GPT testing. This blog suggests a best practice of GPT testing with some advice on major concerns that one can reflect upon.

To begin with the below flow chart gives the process flow for GPT from the receipt of media, storage, testing and approval:



From the top, receipt any material with a new lot number and mark as quarantined pending release so it cannot be used before. Before inspecting make sure on receipt of either the raw material or pre-prepared media that there is a certificate of analysis from the supplier which has information about pH and if appropriate their own growth potential results. Inspect the raw materials for appearance, colour, clarity (for liquids), consistency (free flowing powders) and completeness of information on the label. Check the lot number on the label matches that on the C of A. Store the material at the correct temperature and humidity in a controlled area so only authorised personnel have access to the material until approved for use. When storing use the first in first out (FIFO) principle with your batches. If applicable document a rationale for tailgate samples for testing before accepting lot numbers from suppliers.

When checking ready to use media before performing GPT check the following:

- Variable amounts of medium in dishes/tubes/bottles
- Uneven distribution of media in petri dishes (Affecting colony size)
- Discolouration or haemolysis
- Validated storage location – in terms of humidity, light & temperature
- Integrity of packaging
- Broken or cracked petri dishes
- Quality and accuracy of labelling
- Condensation in petri dishes
- Dehydration (split or retracted medium, dry surface)
- Sloped or uneven filling of petri dishes
- Contamination
- Gel strength
- Pitted surface or large bubbles
- Presence of leakage
- Shelf-life required

It is important to check the above as there is no point in performing GPT on media when there are defects that may affect its performance. If any of the above are defective investigate why they are defective, in all these cases contact the manufacturer.

If the pre testing checks meet your criteria then you are ready to perform the GPT. The correct techniques required are too numerous to mention and are the subject of my next blog.

Post testing; clearly document GPT results, ensure documentation is simple to provide all relevant details required. Make all batch acceptance decisions based on the results. Any adverse results need to be investigated via deviation/non-conformance QA systems. Assure the batch is labelled as passed or failed and quarantine any failed batch until the investigation is complete.

Following on from my previous blog, part 2 gets into the detail of performing the growth potential test itself. You want guarantee the test is both consistent and the results reproducible. The following advice will hopefully give you a few tips on how to do this.

Firstly choose the correct test organisms. Use ATCC lines or the equivalent with documented evidence of suitability. Store them in appropriate validated conditions. Ensure the culture you are using is no more than four generations from the original strains of isolates and show appropriate documented evidence of its control. Ensure the isolate or its diluents get to ambient temperatures before reviving or using. The dilution should obtain 10-100cfu when plated out for GPT analysis. Use wild type flora from a persistent local micro-flora with high incident rates within local cleanrooms water plant or other utilities for GPT testing. This is on top of the required ATCC strains.

You will require a media reference standard as a comparator. You can use the previous lot (assuming it passed all tests) as the reference batch. The reference batch should be stored in a validated controlled environment; away from temperature, light and humidity variations.

Onto the actual test itself, allow the test articles and reagents to come to ambient temperature before testing. If not this will lead to volume contraction or expansion of the air cushion inside the pipette tip and pipette which will impact precision and accuracy of the dispense. Use low retaining pipette tips with filter caps to mitigate risks of occurrence contamination events of pipettes and aerosol contamination of test articles. Maintain a consistent pipette angle of not more than 20° as depicted in below figure 1 and pre-wet the pipette tip by aspirating and dispensing about three times. This will equilibrate the temperature difference and humidify the dead air space inside the pipette tip. Optimise the volume range to use 35 and 100% of the nominal volume. Touch off after dispensing as per below figure 1.

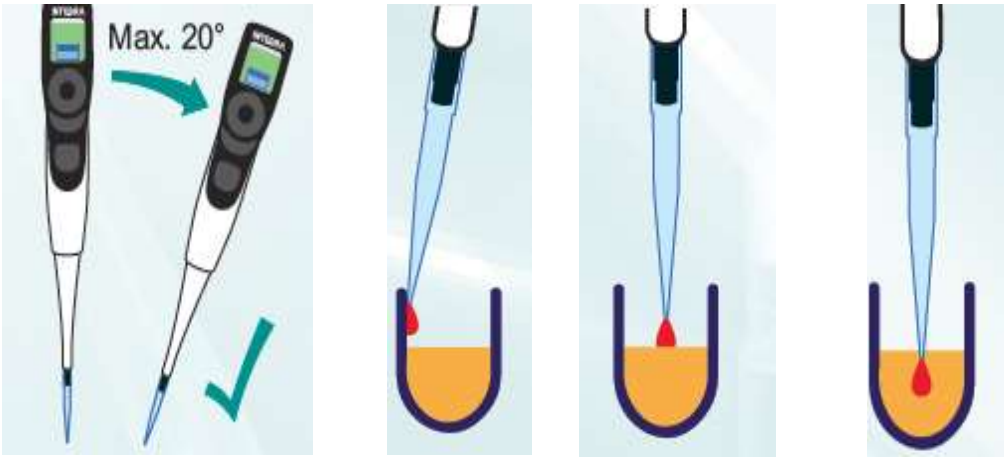


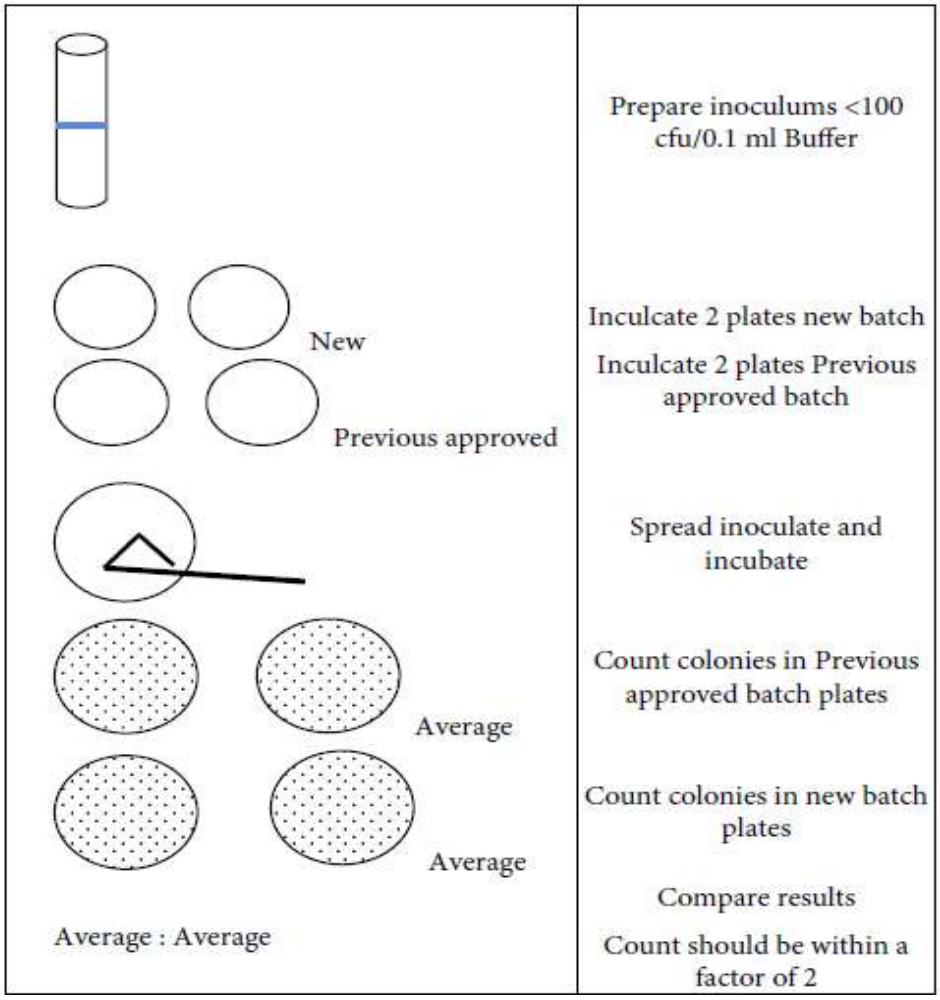
Figure 1 – depicted maintenance of pipette angle whilst using a pipette and how to dispense and touch off inoculum.

Regarding GPT on agar plates, ensure the agar matrix is dry from any condensation and droplets. Use adequate aseptic technique to ensure all the cell culture dispensed spreads over the entire surface of the agar but also ensure it does not spread to the edge of the petri dish. Ensure the agar matrix absorbs all the cell culture aliquot before you turn the plate over for incubation. All this will ensure your colonies are entire and easy to enumerate. Document temperature incubation duration and do not leave the plates for overgrowth or it may be extremely difficult to enumerate your plates.

Use a colony counter with adequate natural light to accurately count the colonies. Utilise automated colony counters which are validated to count accurately. Use photographic evidence to indicate purity of microbial characteristics' of each strain type.

Below is a figure 2 to summarise the testing procedure

Growth promotion test (microbial enumeration test)



I hope you have found this advice informative and have given you food for thought when looking at ways to improve the accuracy and consistency of your growth potential testing.