



Recovery of Enumerated Microorganism Challenges on Selective Media versus Non-Selective Media

MicroBioLogics Inc. is a manufacturer of microorganism preparations that serve as quality control challenges in clinical and industrial microbiology laboratories. A frustration that MBL and our customers often share is the apparent discrepancy between established mean assay value of a challenge microorganism preparation and the colony forming unit (CFU) recovery on selective media.

To better serve our customers, a brief discussion regarding the selectivity and recovery of assayed microorganism challenges on selective media will be informative and beneficial.

Statement of Fact: Two simple statements of fact are necessary for a meaningful dialogue.

“Selective media rarely demonstrates 100% CFU recovery in comparison to identical enumerated microorganism challenges on non-selective media.”

“Acceptable minimum recovery limits need to be established for each selective medium employed in each individual laboratory.”

A **“Certificate of Assay”** is provided with each enumerated MicroBioLogics lyophilized microorganism preparation. The statements of fact simply support the conclusion that the actual and stated mean assay value will rarely be recovered on selective media.

Certificate of Assay:

MicroBioLogics' enumeration method employs:

- an automated plating device and colony counting device to reduce the influence of analyst and manual deviations. Both devices have met process validation control criteria.
- only non-selective nutrient or enriched agar media to perform colony counts to reduce the inhibitory influences of selective media. Each non-selective medium is subjected to demanding quality assurance specifications and performance (e.g. Growth Promotion).
- a statistical validated sampling plan for the number of samples to be assayed and the number of replicate assays. Mean value, standard deviation, coefficient of variation, and confidence level calculations support each stated assay value.

Note:

In parallel with the enumeration assay method cited above, MicroBioLogics does challenge common in-house selective media preparations, commercial pre-poured selective media, and commercial detection devices based on selectivity. It is not unusual to demonstrate, within a given log concentration (e.g. 10 CFU to 100 CFU or 100 CFU to 1,000 CFU), 10% to 50% reduction in CFU recovery on selective media.

Selectivity and Recovery:

An important lesson can be learned. Each in-house selective media preparation, commercial pre-poured selective medium, or commercial detection device based on selectivity must be quality controlled. In addition to such specifications and performance as sterility, pH, positive and negative differential reaction and colony morphology, evaluation of selectivity and recovery properties must be established and documented.

The following is a simple procedure that can be used to demonstrate the recovery of challenge microorganisms on selective media.

1. Select reference stock cultures or working stock cultures, traceable to authentic reference cultures, which are appropriate to challenge the medium's selective properties.
2. Prepare dilutions of the challenge microorganism to achieve a challenge concentration of 100 CFU.
If a selective agar medium or device is used as a "pour plate", 100 CFU per 1.0 mL is recommended.
If a selective agar medium or device is used as a "streak or spread plate", 100 CFU per 0.1 mL is recommended.
3. Inoculate three selective agar media with 100 CFU challenge microorganism per 1.0 mL or 0.1 mL.
Inoculate three non-selective standard methods agar media with 100 CFU challenge microorganism per 1.0 mL or 0.1 mL.
4. Incubate both sets of selective and non-selective media in accordance with the requirements for the selective medium.
5. Following incubation, count and average the CFU for the selective and non-selective media. Calculate the average and divide the average value of the non-selective CFU recovery into the selective CFU recovery. Multiply the answer by 100 to determine the Percent Recovery.

Precaution and Advisory:

The fact that a selective medium may not demonstrate exact correlation with CFU recovery in comparison with a non-selective medium should not be interpreted as a quality assurance performance failure. The value of detecting a target microorganism in a mixed population may justify less than complete CFU recovery due to selective or inhibitory properties in a medium. Two important considerations must be addressed:

1. **Selectivity and Recovery**
Everyone must be aware of the fact that, by its composition or formula, a selective medium may demonstrate some degree of selectivity or inhibitory influence against the microorganism(s) for which it is designed.
2. **Acceptable Minimum Recovery Limits**
Each laboratory, based on the specific use of a selective medium, should establish the recovery limit. The performance from batch to batch of a selective medium can vary due to influences such as incorrect formula components, excessive heating or sterilization, and microbiology suitability of the water. This consideration is important so reliable results are obtained in a reproducible manner.