

| EFFECTIVE DATE                | NP Analytical Laboratories                            | METHOD CODE |
|-------------------------------|---|-------------|
| REVISED: 06/27/25             | LABORATORY TEST METHOD SUMMARY                        | EBAC        |
| REPLACES: 12/08/24            | ENTEROBACTERIACEAE ENUMERATION – POUR PLATE PROCEDURE | PAGE 1 OF 1 |
| KEY WORDS: Enterobacteriaceae |   |             |

## 1. SCOPE AND PURPOSE:

- 1.1. This method employs procedures based upon an international standard and is used for the enumeration of *Enterobacteriaceae* via pour plate technique in foods, animal feeds, food and feed ingredients, and environmental samples in the area of primary production, food production and food handling.
- 1.2. *Enterobacteriaceae* are good indicator organisms of the overall hygiene and cleanliness of samples, and this family includes pathogenic bacteria like *Salmonella* and *E. coli*, so this procedure is a good indication of the potential of these pathogens to be present.

## 2. PRINCIPLE:

- 2.1. This plating procedure is for the overnight enumeration of members of the *Enterobacteriaceae* family by using Violet Red Bile Glucose agar.
- 2.2. The lowest confidence level of this procedure is 1 Colony Forming Unit per gram (g) or milliliter (mL) when 10 mL of a 1:10 dilution is plated.
- 2.3. Known Limitations:
  - 2.3.1. Microbial cells often occur as clumps of cells. A colony may have resulted from a clump of cells. The results from plating methods are an estimation of the number of colony forming units, not an actual cell count from the product.
  - 2.3.2. The accuracy of colony count methods may be limited by the failure of some microorganisms to form visible colonies on the agar medium. This failure can result from nutritional deficiencies of the medium, unfavorable oxygen tension, unfavorable incubation temperature or length of incubation, or failure of an injured cell to repair itself.

## 3. PRECISION:

Assay precision for plating procedures may vary with test matrix and the physiological condition of the microorganisms in the test sample. Guidelines used to describe method precision are defined in NPSOP3040, *Verification of Microbiological Tests*.

## 4. REFERENCES:

ISO 21528-2:2017 Enumeration of *Enterobacteriaceae*