1. SCOPE AND PURPOSE:

The *Enterobacteriaceae* Count (EB) Petrifilm is a sample-ready-culture medium system which contains modified Violet Red Bile Glucose (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. Petrifilm EB Plates are used for the enumeration of *Enterobacteriaceae* in the food, beverage, and bottled water industries.

2. PRINCIPLE:

2.1. This procedure is for plating using Petrifilm technology for the overnight enumeration of members of the *Enterobacteriaceae* family of bacteria.

2.2. *Enterobacteriaceae* are oxidase-negative, Gram-negative rods that ferment glucose to produce acid and/or gas. These bacteria are considered good indicator organisms of the overall hygiene and cleanliness of samples. They are also a good indication for the potential presence of pathogenic bacteria like *Salmonella* and *E. coli* as these bacteria are part of the *Enterobacteriaceae* family which also includes other opportunistic pathogen organisms like *Citrobacter* and *Proteus* spp.

2.3. The lowest confidence level of this procedure is 10 Colony Forming Units (CFU) per gram (g) or milliliter (mL) when 1 mL of a 1:10 dilution is plated on EB Petrifilm.

2.4. Known Interferences:

2.4.1. Petrifilm EB plates cannot be used with buffers containing citrate, bisulfate, or thiosulfate.

2.4.2. Chemical preservatives or other inhibitors in a sample can cause inhibition of growth on the lower dilutions of any enumeration technique.

2.4.3. Some species of bacteria may liquefy the gel, which can cause them to spread out and obscure the presence of other CFU's.

2.4.4. The type of matrix being tested may make it difficult to plate on EB petrifilm based on matrix viscosity or other factors. Complete yellowing of the petrifilm may also occur even without bacterial growth present dependent upon matrix.

2.5. Known Limitations:

2.5.1. Petrifilm EB plates do not differentiate any one strain of *Enterobacteriaceae* from another.

2.5.2. 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.

2.5.3. 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, or failure of an injured cell to repair itself.

2.5.4. Some probiotic cultures can produce growth that is suspicious on Petrifilm, are oxidase negative, and ferments glucose. Gram stains & Vitek identifications may be performed upon customer request.

3. PRECISION:

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

4. REFERENCES:

4.1. LI-00.758-01 *Enterobacteriaceae* Enumeration – Petrifilm

4.2. ISO 21528-2: 2017 – Enumeration of Enterobacteriaceae Part 2: Colony-Count Technique