1. SCOPE AND PURPOSE:

This method employs procedures for the isolation and identification of *Salmonella* species in presumptive detected results from rapid method assays, enrichment or selective broths, or plate samples.

2. PRINCIPLE:

2.1. There are over 2,400 serovars within the genus *Salmonella*. The genus *Salmonella* consists of facultatively anaerobic, gram-negative, non-sporulating rods. Most are motile, but nonmotile serovars occur.

2.2. The use of rapid screening methods (e.g. SBAX and SVEZ) have reduced the test time to ≤48 hours for a negative *Salmonella* result. If the rapid screening test is presumptive detected, confirmation of *Salmonella* must be carried out through the procedure outlined in Section 8.

2.3. In principle, methods for examining products for the presence of *Salmonella* employ similar procedural steps of pre-enrichment, selective enrichment, differential and selective plating, isolation, and biochemical and serological confirmation or identification of the selected isolates.

2.4. Selective and Differential Plating:

2.4.1. After enrichment, the sample is struck onto several selective and differential agar media to isolate any *Salmonella*. Most plating media contain lactose plus an indicator system that distinguishes between the non-*Salmonella* lactose fermenting colonies and the non-lactose fermenting colonies that may be *Salmonella*.

2.5. Biochemical and Serological Confirmation:

2.5.1. After incubation of selective/differential media, suspect colonies are transferred to tubes of nonselective media (Tryptic Soy Agar [TSA], Triple Sugar Iron Agar [TSI], Lysine Iron Agar [LIA], and Trypticase Soy Tryptone Broth [TST]) for biochemical and serological confirmation.

2.5.2. Suspect growth from TSA is evaluated for presumptive *Salmonella* identification on a commercial biochemical test system (Vitek 2 GN card).

2.5.3. Suspect growth from TSI and TST is screened serologically by the antigenic composition. Agglutination reactions with polyvalent somatic (Poly O) and flagellar (Poly H) antisera are used as part of *Salmonella* culture identification. If determination of individual O and H antigens is performed for specific serovar identification with antisera (See NPAL Method: SSID), then Poly O and Poly H testing is not needed.

2.6. Serotyping: Upon customer request (SSID), the genus *Salmonella* is differentiated into serovars, serologically characterizing the antigenic composition (somatic -O Group and flagellar -H Group) through the use of antisera and determining the serovar using the White-Kauffman-Le Minor Scheme.

2.7. *Salmonella* Confirmation: Upon customer request *Salmonella* will be isolated and identified from plate or broth cultures using pertinent portions of section 8 in this method.

2.8. Known Interferences: Variability and discrepancies may occur in one or more of the steps for isolation and identification of *Salmonella*. The following are precautions and limitations that should be considered:

2.8.1. Suspicious colonies on selective agar must be taken through confirmation procedures.

2.8.2. A pure culture is required in order to establish valid biochemical test reactions. If positive serological test reactions and negative biochemical test reactions are obtained, check the

culture for purity.

2.8.3. Occasionally, *Salmonella* cultures showing atypical biochemical reactions may be isolated (e.g. *Salmonella* that are H_2S negative, lactose positive, or dulcitol negative). The classification of an isolate rests ultimately upon the antigenic structure, not unqualifiedly on its biochemical characteristics. Most *Salmonella* show a relatively consistent pattern of biochemical characteristics, but variants are frequently encountered.

3. PRECISION:

Assay precision for qualitative test 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:

4.1. ISO 6579-1:2017 Microbiology of the Food Chain – Horizontal Method for the Detection, Enumeration, and Serotyping of *Salmonella*

4.2. ISO/TR 6579-3:2014 Microbiology of the food chain – Horizontal Method of the Detection, enumeration, and serotyping of *Salmonella* – Guidelines for serotyping of *Salmonella* spp.