1. SCOPE AND PURPOSE:

This method provides procedures for the complete identification of the specific antigenic types of biochemically confirmed pure cultures of *Salmonella* species as delineated in the Kauffman-White scheme (1994) or the White-Kauffman-Le Minor scheme (2007).

2. PRINCIPLE:

2.1. Serological identification of *Salmonella* is based on the detection of specific antigenic components present. First the organism is placed into an "O" (O-Ohne Hauch) group according to its somatic composition and then confirmed by the identification of its flagellar "H" (H-Hauch) antigen.

2.2. The somatic O antigen is a heat-stable polysaccharide associated with the body of the cell. This antigen is determined using the slide agglutination test. Agglutination is typically considered to be positive when 75% or more of the cells have agglutinated. However, there may be instances when the slide agglutination result is weak and the weak result will be considered positive when all other possible antisera have given negative reactions (no visible agglutination).

2.3. The flagellar H antigen is a heat-labile protein located in the flagellum of the motile organism. In the test tube method, cell clumping appears as a loose floccular reaction when cells have agglutinated due to antisera binding to these H antigens. H antigens typically, but not always, consist of two phases: phase 1 and phase 2. Any given culture of *Salmonella* may contain cells in only one phase or cells in both flagellar phases. A phase reversal (GARD) plate contains the antisera of one H phase which immobilizes the sub-population with that particular H phase antigen, allowing the sub-population with the other H phase antigen to remain motile and migrate out from the spot of inoculation on the GARD plate.

2.3.1. Slide agglutination antisera is available for H antigen detection and may be necessary depending on availabilty of antisera.

2.4. Occasionally a third type antigen, called the Vi antigen, may be present in certain *Salmonella* strains. It is a heat-labile envelope antigen surrounding the cell wall, which may block the activity of the "O" antigens. The Vi antigen must be made inactive before the serological grouping of the "O" antigens can be completed.

2.5. Known Interference: The ability to serotype to a specific serovar may be limited due to availability of antisera.

3. PRECISION:

Assay precision for qualitative and quantitative 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. Difco Manual, 11th Edition, 1998, "Bacto *Salmonella* Antisera", Difco Laboratories, Detroit, MI.

4.2. Bacteriological Analytical Manual, 8th Ed., Rev.A, 1998, U.S. Food and Drug Administration

4.3. Bacteriological Analytical Manual, Chapter 5 Salmonella, online, U.S. Food and Drug Administration

4.4. Antigenic Formulae of the *Salmonella* Serovars, 9th edition, 2007, (White-Kauffman-Le Minor Scheme), WHO Collaborating Centre for Reference and Research on *Salmonella*