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Aug 11, 2020

Salmonella Typhimurium isolation from specimens

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1 Works for me dx.doi.org/10.17504/protocols.io.bjjdkki6

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ABSTRACT

Salmonella are motile, flagellated rod-shaped Zoonotic pathogens which may survive with or without oxygen and do not absorb crystal violet stain.They are decolourised by alcohol due to their outer lipopolysaccharide membrane and thin peptidoglycan layer.They belong to the family Enterobacteriaceae and is implicated with typhoid fever and food-borne illnesses.This pathogen is associated with intestinal disease which may become fatal and has negatively impact the health of individuals and various economies globally.The poultry industry is most impacted and vulnerable to the onslaught of this pernicious microbe.The Lipopolysaccharide Somatic O antigen, flagellar H and virulent V antigenic structure determines the serotype designate of *Salmonella* species [1]. A wide range of antisera is used in the location of the O antigen associated with the carbohydrate constituent of Lipopolysaccharide and H protein antigen linked with the flagella of the bacteria.

Salmonellosis is an inflammation of the intestinal mucosal lining by *Salmonella* bacteria infiltration resulting in painful symptoms.Both man and animal are vulnerable.Salmonellosis is caused by ingestion of *Salmonella* with food (Wall et al., 1994).The main route of transmission of *Salmonella* microbe is fecal-oral. Ingestion of small concentration of *Salmonella* such as 6 cells with contaminated food or water will lead to salmonellosis infection [2].

References

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DOI

dx.doi.org/10.17504/protocols.io.bjjdkki6

PROTOCOL CITATION

Angel A Justiz-Vaillant, Suzette E. Curtello 2020. Salmonella Typhimurium isolation from specimens. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjjdkki6

CREATED

Aug 11, 2020

LAST MODIFIED

Aug 11, 2020

PROTOCOL INTEGER ID

40261

MATERIALS

NAME	CATALOG #	VENDOR
Difco™ Dehydrated Culture Media: Brilliant Green Agar	DF0285177	Thermo Fisher
Salmonella Shigella Agar (SS Agar) / XLD Agar Biplate	PO5210E	Thermo Fisher
MacConkey Agar Medium, MacConkey Agar, MacConkey Agar Medium, MacConkey Agar	R01562	Thermo Fisher
Remel™ Rappaport-Vassiliadis Salmonella Enrichment Broth MLT, E - 10mL	R117661	Thermo Fisher
Denka Seiken™ Agglutinating Sera Salmonella O Factor, Salmonella O Factor 16	R679333	Thermo Fisher

SAFETY WARNINGS

For the risk associated with a particular microbe including Salmonella spp laboratory staff or members should consult the website of the CDC: (http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf)

1 The Salmonella Typhimurium isolation was carried out as followed: the exterior of the hen cloaca was first cleaned with a sterilized and moistened cotton balls before application of the cotton tips of each swab applicator.

2 The swabs, caeca, and stomach tissues were immediately placed in a sterile screw-cap test tube containing 9 ml of pre-enrichment broth (buffered peptone water 1%).

3 At least 2.5 g of each type of specimen was dissolved in 250 ml pre-enrichment broth.

4 The inoculated pre-enrichment broth was incubated at 37 ºC for 24 hrs following this incubation it was thoroughly mixed using a vortex mixer.

5 A 1.1 ml aliquot of buffered peptone water 1% was added to 9 ml of enrichment broth (selenite broth, selenite cystine broth, and tetrathionate broth) and further incubated at 37 ºC for 24 hrs.

6 After vortexing 0.16 ml and a 3 mm loopful of inoculum was used to inoculate the differential plating media such as Salmonella Shigella agar selective media, MacConkey agar, bismuth sulphite agar and brilliant green agar that were incubated at 37 ºC for 24-48 hrs. .

7 Following the incubation, as typically, the cultures were examined, and non-lactose fermenting colonies were selected and used to inoculate Kleiger iron agar and urea agar slants.

8 After a further 24 hours incubation period at 37ºC colonies that gave the typical Salmonella Shigella reaction, were inoculated to the routine line of sugars and again incubated.

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9 Confirmation was followed by slide agglutination with somatic “O” and flagella “H” antigens of Salmonella.

10 Serological typing of Salmonella Typhimurium was performed.

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