One Broth One Plate for *Salmonella* (OBOP-S)

INTENDED USE

One Broth One Plate for *Salmonella* (OBOP-S) offers a rapid method for the enrichment and detection of *Salmonella* spp. using traditional culture methodology.

PRODUCT SUMMARY AND EXPLANATION

The single overnight enrichment step is designed to recover damaged *Salmonella*. The formulation of the base media is compliant to ISO 6579-1:2017.

Salmonella Selective Supplement is provided separately to enable the common use of the medium as a diluent for other analyses from that sample prior to overnight incubation.

Buffered Peptone Water HQ (ISO) follows the ISO formulation and is a nutrient medium, buffered to maintain pH 7.0 for the incubation period. It also provides all nutritional requirements for growth of *Salmonella* spp. When used in conjunction with the OBOP-S Selective Supplement, Buffered Peptone Water constitutes a selective medium for an alternative workflow to ISO 6579-1:2017.

Detection is via Chromogenic agar for *Salmonella* Esterase (CASE), a selective chromogenic agar for the detection of *Salmonella*. It utilises a dual chromogenic system to differentiate between *Salmonella* and non-target organisms that grow on the agar. The first chromogen is a target for esterase activity present in *Salmonella* species. Utilisation of this chromogen results in blue/green colonies. The second chromogen is a target for ß-glucosidase present in non-target organisms that are able to grow on the agar and also possess esterase activity. Utilisation of the second chromogen masks the utilisation of the first resulting in black colonies in non-*Salmonella*. All other organisms are either inhibited or grow colourless on the agar.

The medium is able to detect non-motile *Salmonella* (*S*. Pullorum and *S*. Gallinarum) as well as monophasic variants (1, 4, [5], 12: i :-). The medium is also able to detect serovars that present weak esterase activity (*S*. Dublin), and lactose positive strains (*S*. Arizonae). Although not covered in this protocol, CASE can also be used as the second agar alongside XLD as part of ISO 6579-1:2017*.

* For more information on this please contact your local NEOGEN Account Manager

INTENDED USER

The method is designed for use by personnel with appropriate training.

PRODUCT CODES

Product Name	Format	Pack Size	Product Code
Buffered Peptone Water HQ (ISO)	DCM	500g	NCM0270A
		5kg	NCM0270B
		10kg	NCM0270C
	Prepared Media Bags	3 x 3L	NCM3402
	Ready-to-Reconstitute Media Bags	10 x 20L	NCM3207
Salmonella Selective Supplement	DCM Supplement	10 Test Capsule (Each capsule makes 10mL)	NCM4000-10C
		10g Vial (Each vial makes 100mL)	NCM4000-100
Chromogenic Agar for *Salmonella* Esterase (CASE)	DCM	500g	NCM1006A
		5kg	NCM1006B
		10kg	NCM1006C
	Pre-Poured Plates*	20 x 90mm Plates	NCM3008-20
Salmonella Latex confirmation kit	RTU	50 tests	F42

*Pre-poured plates may not be available in every country. Please ask your Account Manager for further details.

Equipment and Materials Required

- Stomacher or equivalent
- Stomacher-type bags for sample enrichment. Filter bags are recommended (NEOGEN item 6827 or equivalent)
- Graduated cylinder, 250mL (NEOGEN item 9368 or equivalent)
- 1l purified water

TYPICAL FORMULATIONS

Please refer to the specific product information sheets for formulation, reconstitution, QC organisms, interpretation and storage.

Salmonella Selective Supplement	g/10mL
(Reconstituted) Selective mix	1.0

Chromogenic Agar for *Salmonella* Esterase (CASE)	g/L
Growth Mix	10.5
Selective Mix	11.0
Buffer	7.0
Opacity Agents	7.5
Chromogenic Mix	1.4
Agar	12.5

Final pH 7.2 ± 0.2 at 25°C

Method for reconstitution of *Salmonella* Selective Supplement

1. Reconstitute one vial of NCM4000-100 by adding 100mL of Buffered Peptone Water HQ (ISO) or purified water and swirl to dissolve.
2. Alternatively, aseptically take 1 capsule of NCM4000-10C from the container and add or empty the capsule into a sterile universal or sterile container containing 10mL of sterile BPW HQ (ISO) or sterile deionized/RO water and swirl to dissolve.

   Note: The rehydrated supplement may appear to settle upon standing. It is best practice to mix well before use but it is not necessary to mix before every aliquot.

Method for CASE reconstitution

1. Dissolve 49.9g of NCM1006 in one litre of purified water.
2. Mix thoroughly.
3. Bring rapidly to the boil with frequent agitation and temper in a water bath to 50°C.
4. Pour into sterile Petri dishes.

   Note: Media must sufficiently boil or white precipitate will be seen in the agar. This is purely aesthetic, does not affect performance and can be avoided by sufficiently boiling the media.

MINIMUM QC ORGANISMS

Growth Characteristics			
Organism	WDCM	Buffered Peptone Water	CASE Agar
Salmonella Typhimurium	00031	Turbidity (1-2)	Good Growth, Blue/Green Colonies
Salmonella Enteritidis	00030	Turbidity (1-2)	Good Growth, Blue/Green Colonies
Escherichia coli	00013	Turbidity (1-2)	
Enterobacter aerogenes	00175		Growth, Black Colonies
Pseudomonas aeruginosa	00026		Inhibited

INTERPRETATION

Growth Characteristics on CASE		
Organism	Growth	Colour
Salmonella spp.	Good Growth	Blue / Green
Enterobacter spp., *Klebsiella* spp.	Growth	Black
Escherichia coli	Suppressed	Colourless
Shigella spp.	Suppressed	Colourless
Proteus spp.	Suppressed	Colourless to Brown

The organisms listed are the minimum that should be used for quality control testing.

PRECAUTION

Refer to SDS.

https:/www.neogen.com/solutions/microbiology/harlequin-chromogenic-agar-salmonella-esterase/

STORAGE

Product Name	Product Code	Storage Conditions
Salmonella Selective Supplement	NCM4000	2-8°C, away from direct sunlight, until expiry date
Reconstituted Supplement	NCM4000	Up to 7 days at 2-8°C
Reconstituted BPW + Supplement	NCM0270 + NCM 4000	Use within 1 day of preparation
DCM CASE	NCM1006	2-8°C, away from direct sunlight until expiry date
Reconstituted CASE	NCM1006	2-8°C, in the dark, for up to 14 days
Pre-Poured CASE	NCM3008-20	2-8°C, in the dark until expiry date

OBOP-S FLOW DIAGRAM

x g or *x* mL of sample in 9 x *x* mL of Buffered Peptone Water HQ (ISO) + OBOP-S Selective Supplement 18h ± 2h at 41.5°C ± 1°C

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Detection using 10µL on 1 CASE agar plate

Incubation 24h ± 3h at 34-38°C

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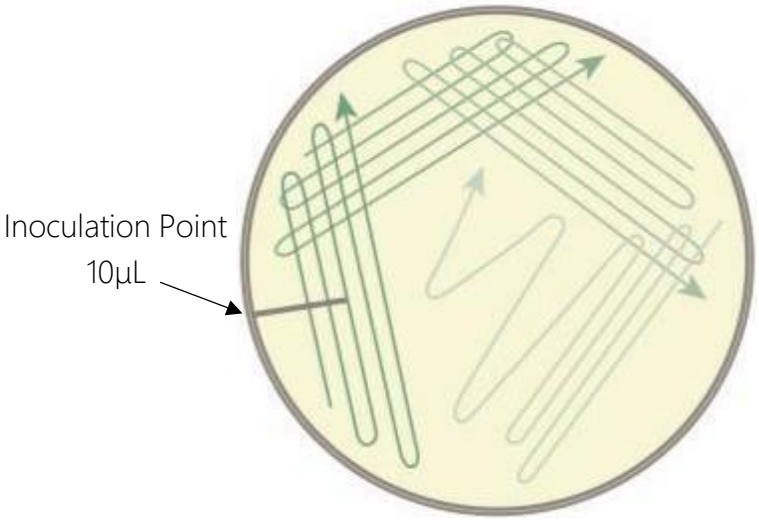

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Confirmation using a *Salmonella* latex (Microgen F42), ISO 16140-6 validated and certified methods or standard tests described in the standardized CEN or ISO methods

TEST PROCEDURE

1. Dilute ***x*** g or ***x*** mL of the sample in 9 x ***x*** mL of Buffered Peptone Water HQ (ISO), i.e. add 25g or mL of sample for 225mL of Buffered Peptone Water HQ (ISO).
2. Homogenise in a Stomacher blender.
   Note: If you use BPW as a common diluent for additional analyses from the same sample, aseptically remove the volumes required using a pipette prior to the addition of the OBOP-S Selective Supplement. The diluted sample in BPW (stock solution) can be stored up to one hour at room temperature to permit other analyses.
3. Up to one hour later, add ***x*** g (or ***x*** mL) x 0.04mL of the reconstituted OBOP-S Selective Supplement to the sample.
   i.e. add 1mL of reconstituted supplement for every 225mL of Buffered Peptone Water or the correct amount of supplement, if the sample differs to 25g. Please note that the supplement contains a green dye which acts as an indicator to demonstrate that it has been added to the enrichment sample.
   Note: If only *Salmonella* analysis is taking place, the supplement can be added before step 2 above.
4. Homogenise by agitating vigorously.
   Note: Samples can be maintained at room temperature for two hours when carrying out the analysis.
5. Incubate aerobically at 41.5°C ± 1 °C for 18h ± 2h.
   Note: It is possible to store the enriched Buffered Peptone Water HQ (ISO) between 2-8°C for 72 hours maximum, following the last incubation at 41.5°C.
6. Using a sterile inoculating loop, from the enrichment broth, isolate onto the surface of a CASE agar plate with a loop on half of the plate, then streak on the other half of the plate coming back onto the first half as described in the scheme above (4 quadrants streak method).
7. Incubate the plate at 34-38°C for 24h ± 3h.
   Note: After incubation, the CASE plates can be stored in a refrigerator (2-8°C) for 72 hours, before reading and confirmation.

INTERPRETATION AND CONFIRMATION

Blue/green colonies are presumptive positive for *Salmonella* and can be confirmed.

All positive culture media screening results need to be confirmed in one of three ways:

1. Using standard tests described in the standardized CEN or ISO methods (including the purification step).
2. Perform a *Salmonella* Latex test (Microgen F42 or OXOID), using an isolated colony.
3. Using ISO 16140-6 validated and certified methods, starting from a single colony.

Note: In the event of results that are not in agreement, between the detection method and one of the confirmation options listed above, the laboratory should use another confirmation test in the approved list above. If the second confirmation test gives a negative result, it means a false positive result on the CASE agar plate and the sample is negative. If the result of the second confirmation test is positive, the sample is positive.

PREPARATION PRECAUTIONS & LIMITATION OF THE METHOD

1. Selective agents are blended into the CASE agar media meaning it does not require any supplementation. As such excessive heating during sterilisation or prolonged holding may result in a loss of selectivity, volumes up to 1 litre should be brought to the boil in no longer than 30 minutes, and should be held no longer than 1 hour after sterilisation. Contact NEOGEN technical support for further guidance on larger volume preparation. Please note the opacity agent requires proper boiling (i.e. visible bubbling) to present a smooth appearance in the plate. Failure to do this can result in a faint whiteprecipitate. This is purely aesthetic and has no impact on performance.
2. Although the most prevalent *Salmonella* strains can be detected by Microgen Latex kit F42, it must be noted that the Microgen kit doesn't allow the detection of 100% of *Salmonella* strains.
3. Use good microbiology laboratory practices, such as ISO 7218.
4. Prepare samples according to standards for the product concerned (ISO 6887 series).
5. For heavily-loaded dishes, a re-isolation of suspicious colonies on a CASE agar plate may be considered.

EXPIRATION

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original colour. Expiry applies to medium in its intact container when stored as directed.

DISPOSAL

Enrichment cultures should be disposed of as biohazard waste. The preferred method of treatment for biohazard waste is autoclaving. Items that cannot be autoclaved may be disinfected with bleach solution. Consult with the safety advisor for your facility for detailed instructions.

CUSTOMER SERVICE

NEOGEN Customer Services and Technical Services can be reached by using the contact information below. Training on this product, and all NEOGEN test kits, is available upon request to your Account Manager.

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