Conventional isolation methods: detection of *Salmonella* spp. according to ISO 6579-1:2017

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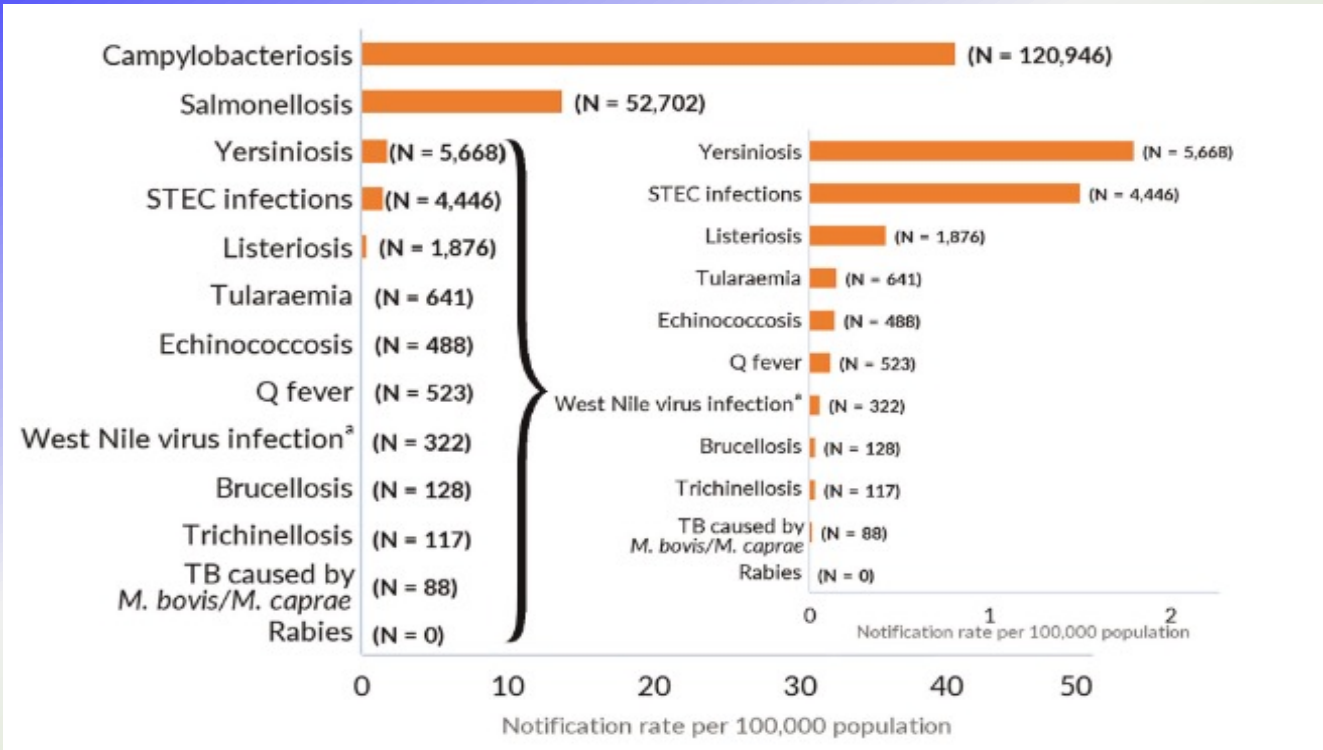
FOOD QUALITY AND CONTROL LABORATORY

7 Veterinarian /Biologist Managers
7 Researchers
24 Trained personnel

CeRTIS
CENTRO DI RIFERIMENTO TIPIZZAZIONE SALMONELLE

5.500 analysis/years

Introduction



Salmonellosis is the second most commonly reported gastrointestinal infection in humans after campylobacteriosis, with 52,073 confirmed cases in humans - **EFSA Journal 2021;19(12):6971**

Introduction

Human cases

Notification rate (per 100,000 population) 13.71

Trend (2016–2020) — Increasing / Decreasing / Stable

52,702 Cases of illness

33,309 Infections acquired in the EU

967 Infections acquired outside the EU

18,426 Unknown travel status or unknown country of infection

6,149 Hospitalisations

57 Deaths

Human cases in foodborne outbreaks

694 Foodborne outbreaks

84 Strong-evidence outbreaks

610 Weak-evidence outbreaks

3,686 Cases of illness

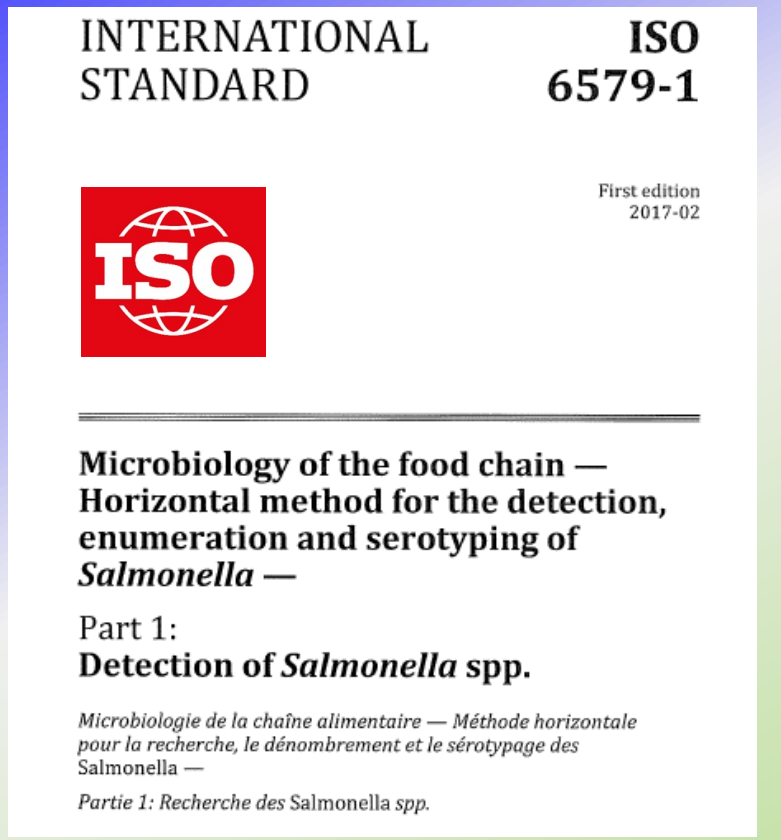
812 Hospitalisations

7 Deaths

Foodborne outbreaks in the EU

EFSA Journal 2021;19(12):6971

ISO: International Organization for Standardization



INTERNATIONAL STANDARD **ISO 6579-1**

First edition
2017-02

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1:
Detection of *Salmonella* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella —

Partie 1: Recherche des Salmonella *spp.*

This document is applicable to:

- ✓ products intended for human consumption and for the feeding of animals;
- ✓ environmental samples in the area of food production and food handling.
- ✓ Samples from the primary production stage such as animal faeces, dust, and swabs

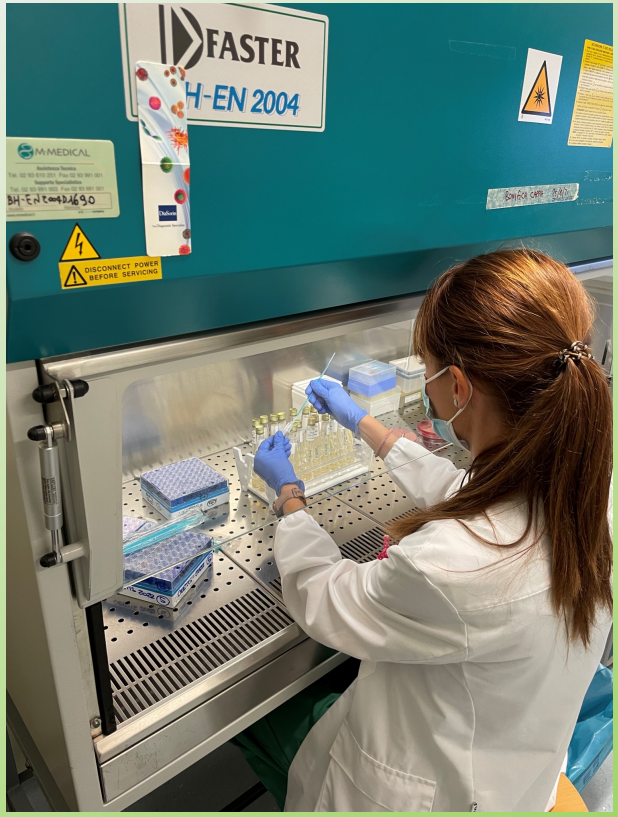
ISO 6579-1:2017

Introductory section.

The prerequisites of this salmonella detection technique are:

1- the laboratory is equipped with the proper instrumentation under the control of a skilled microbiologist;

2- the personnel performing the analysis is familiar with normal laboratory practices.



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ANALYTICAL PHASES:

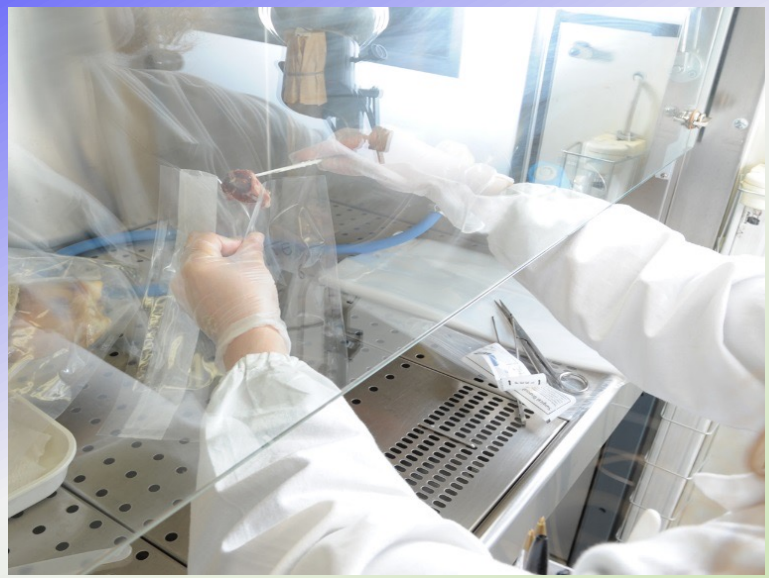
1) PREPARATION OF TEST SAMPLE;
2) PRE-ENRICHMENT;
3) SELECTIVE ENRICHMENT;
4) PLATING OUT;
5) CONFIRMATION.

ISO 6579-1:2017

Sampling

The sampling step, it is not specified in this document, the ISO tells us that the representative sample that we will analyze must not be damaged or replaced with another. Therefore, samples must travel in clean and appropriate containers at refrigeration temperature. In addition, to prevent the sample from being replaced with another, it would be desirable to assign an identification number right away.

ISO 6579-1:2017
PREPARATION & PORTION OF TEST SAMPLE



Sample that should be used is, 25gr in the case of solid matrices and 25 ml in the case of liquid matrices. Samples must be handled using all necessary PPE. All the instruments that meet the sample, e.g., scissors and forceps used during portioning must be properly sterilized and changed between different samples so as to prevent cross contamination episodes. The ISO allows the use of smaller test portion without the need for additional validation, But be sure to provide the 1:10 ratio during the pre enrichment.

ISO 6579-1:2017

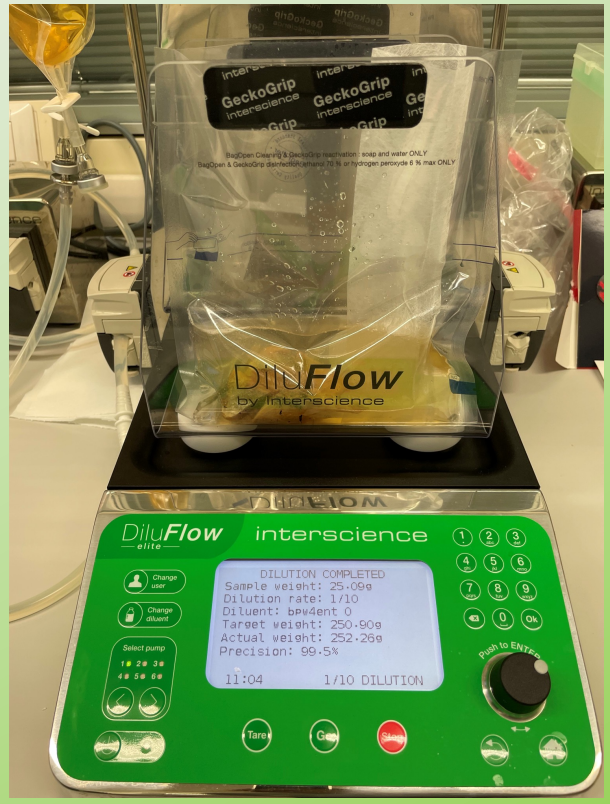
PRE-ENRICHMENT

The pre enrichment phase is divided in a Non selective pre-enrichment and in a Selective pre enrichment. To prepare the initial suspension, add 225 ml of the non-selective pre-enrichment medium, (Buffer Peptone Water-BPW) to 25 g or 25 ml of sample. Homogenize with a stomacher and Incubate between 34°C and 38°C for 18h more or less 2h. It Is possible to store the pre-enriched sample after incubation at 5°C for 72h.

Enrichment:
1:10 Ratio g/mL:
- **25 g/ 225 mL BPW**
- **10 g/ 90 mL BPW**

Incubate:
- 34/38 °C
(93,2/100,4°F)

Store:
- 5°C (41°F) Max 72h



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SELECTIVE ENRICHMENT

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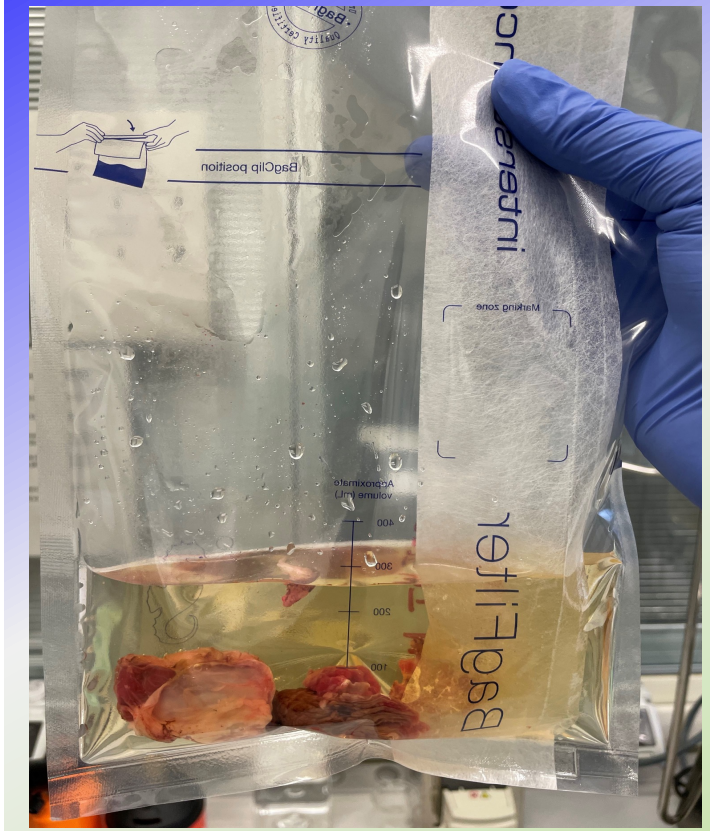

100 µl

Incubate

37 ±1 °C for 24h ± 3h

1000 µl

Incubate

41,5 ±1 °C for 24h ± 3h

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PLATING OUT

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MKTTn

RVS

Incubate

Incubate

37°C ± 3°C for 24/48 ± 3h

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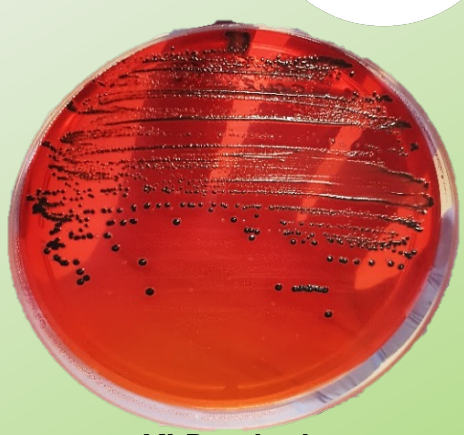
CONFIRMATION

XLD

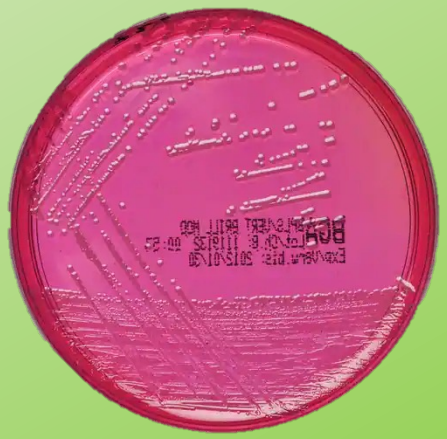
Typical colonies of Salmonella on XLD agar have a black centre and a ligthly zone of reddish color due to the colour chanfe of the indicatore.
Note: Salmonella H2S-negative variants grown on XLD are pink with a darker pink centre. Lactose positive Salmonella grown on XLD are yellow with or without blackening.



XLD colonies

BGA or other from ANNEX E

For example, colonies of *Salmonella* spp. On BGA and Rambach are pink and on Hektoen enteric agar are green/blu with or without halo.



BGA colonies

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CONFIRMATION: BIOCHEMICAL TESTING

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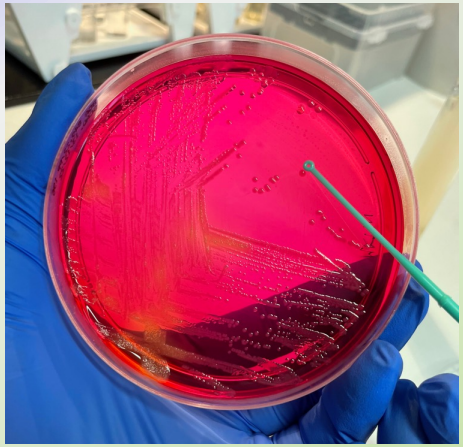
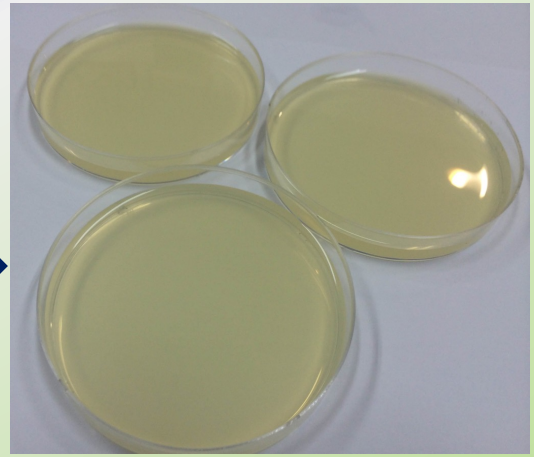
XLD

BGA



Nutrient Agar



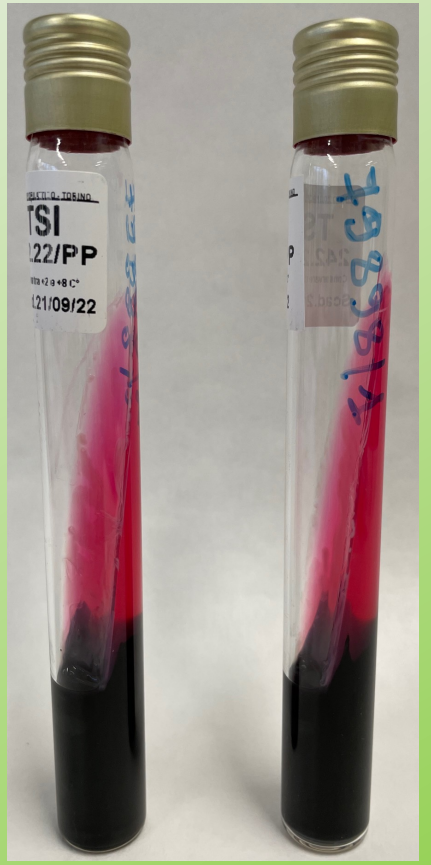
34/38°C ± 3°C for
24 ± 3h

ISO 6579-1:2017
CONFIRMATION TESTS: TRIPLE SUGAR IRON AGAR (TSI AGAR)

TSI Reactions:
Fermentations:
- lactose,
- glucose
- sucrose

Production of:
- hydrogen sulphide)

• alkaline slant-acid butt (**red/yellow**) = fermentation of **glucose** only;
• acid slant-acid butt (**yellow/yellow**) = fermentation of **glucose**, **lactose** and/or **sucrose**;
• alkaline slant-alkaline butt (**red/red**) indicates that the organism being tested is a non-fermenter.
Gas production from glucose is indicated by splitting and cracking of the medium.
A **black** precipitate in butt indicates hydrogen sulphide production



✓ The majority of the typical *Salmonella* spp cultures show alkaline (**red**) slants and acid (**yellow**) butts with gas formation (bubbles) and, in 90% of the cases, formation of hydrogen sulfide (**blackening** of the agar).

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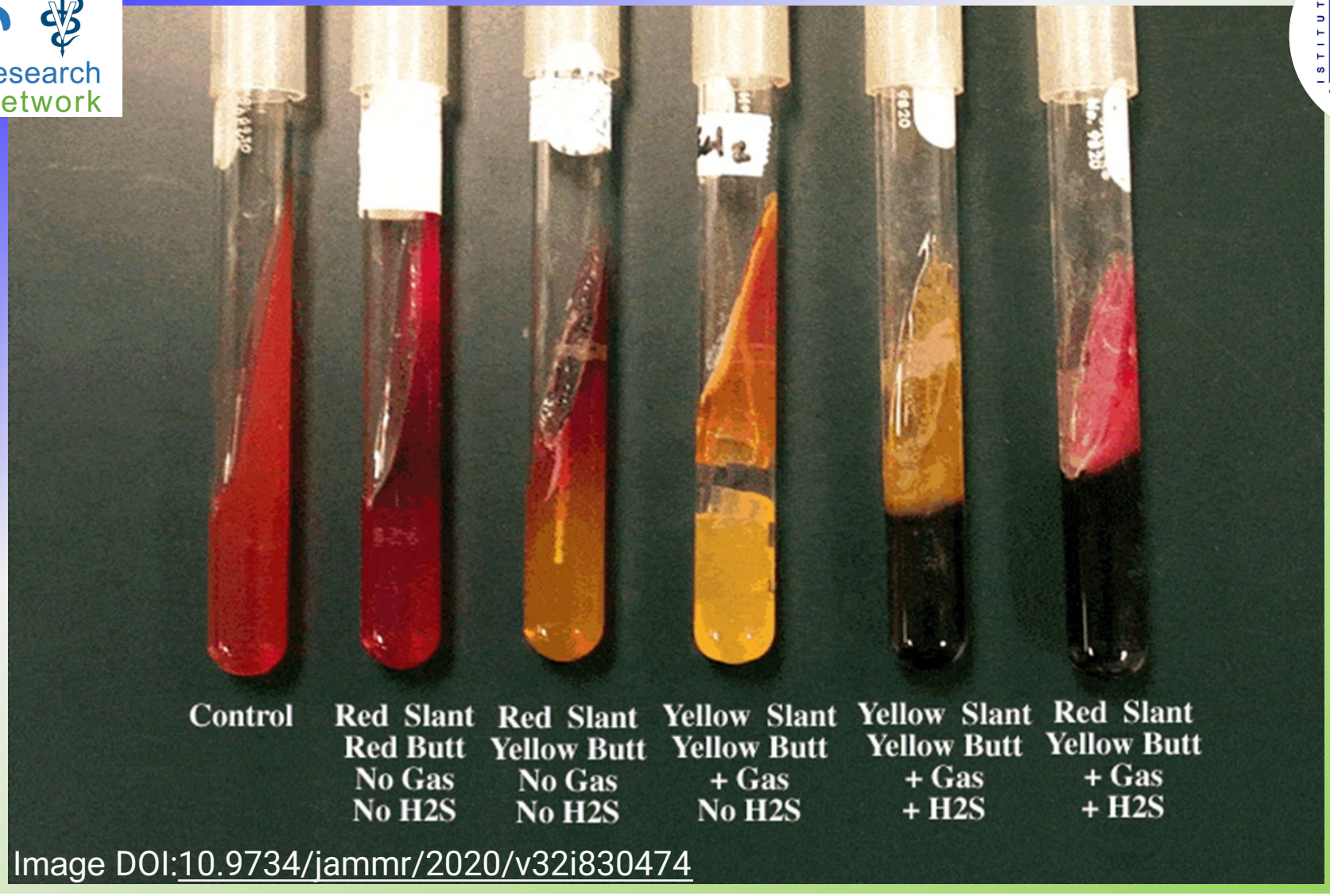

Image DOI:10.9734/jammr/2020/v32i830474

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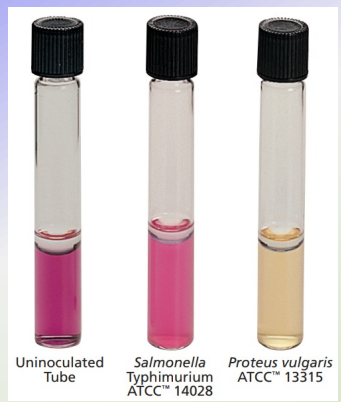
CONFIRMATION TESTS

Urea agar:

Streak the agar slant surface and incubate at 37°C for up to 24h. Typical Salmonella cultures do not hydrolize urea so that the colour of the urea agar will remain unchanged.



L-Lysine decarboxylation medium:

Incoulate below the surface of the liquid medium. Incubate at 37°C for 24h. Purple colour after incubation indicate a positive reaction. Yellow colour a negative reaction.

Optional confirmation tests: Detection of β galactosidase, Medium for indole reaction

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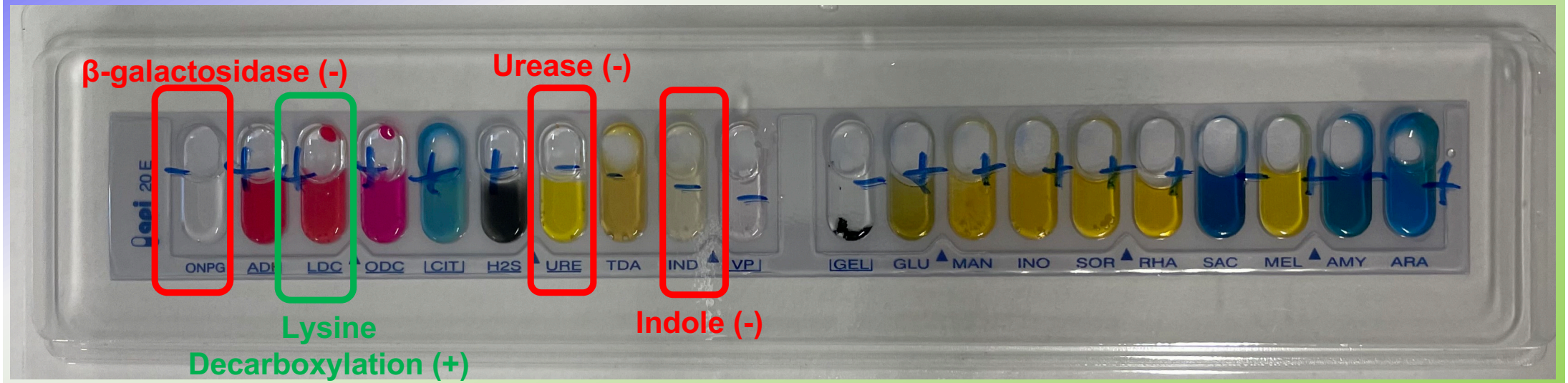
CONFIRMATION TESTS

Optional confirmation tests: Detection of β galactosidase, Medium for indole reaction

If shown to be reliable, miniaturized galleries for the biochemical identification of *Salmonella* may be used



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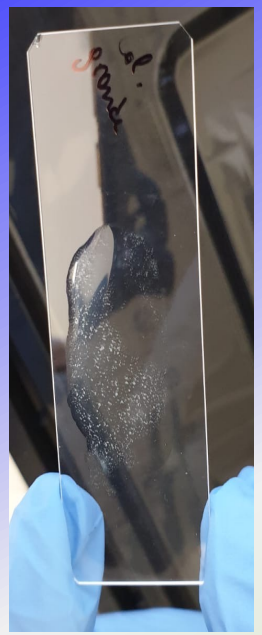
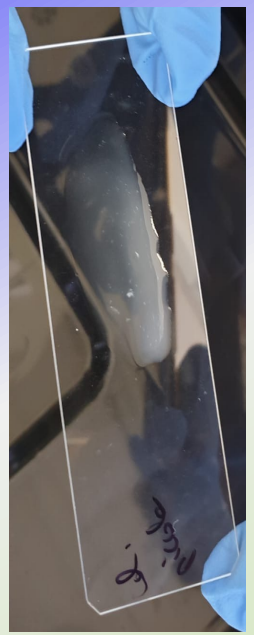
CONFIRMATION: SEROLOGICAL TESTING



Agglutination +



Agglutination -

The pure colonies showing typical biochemical reactions for *Salmonella* are also tested for the presence of *Salmonella* O- and H- antigens (Vi-antigens, if necesary), by using a polyvalent antisiera.

- **The pure colonies are tested for auto-agglutination** → strains that are auto-agglutinable cannot be tested for the presence of *Salmonella* antigens

Elimination of auto-agglutinable strains:
Place one drop of saline solution on a clean glass slide. Using a loop, disperse part of the colony to be tested in the saline to obtain a homogeneous turbid suspension. Rock the slide gently for 5/60s. If the bacteria have formed granules in the suspension that indicate auto-agglutination.

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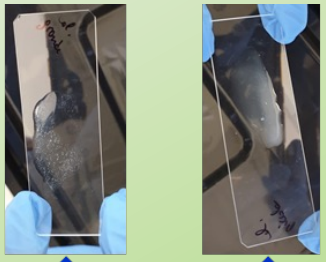
CONFIRMATION: SEROLOGICAL TESTING

The pure colonies showing typical biochemical reactions for *Salmonella* are also tested for the presence of *Salmonella* O- and H- antigens (Vi-antigens, if necesary), by using a polyvalent antisiera.

- **Examination for O-antigens** → using one drop of polyvalent anti-O sera in place of saline solution
- **Examination for H-antigens** → using one drop of polyvalent anti-H sera in place of saline solution

If agglutination occurs, it will be considered a positive reaction



Agglutination + Agglutination -

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CONFIRMATION: SEROLOGICAL TESTING

Biochemical reactions	Auto-agglutination	Serological reactions	Interpretation
Typical	No	O – and H – antigens positive	Strains considered to be *Salmonella*
Typical	No	O – and H – antigens negative	Presumptive Salmonella
Typical	Yes	Not tested because of auto-agglutination	
No typical reactions	-------	-------	Not considered to be *Salmonella*

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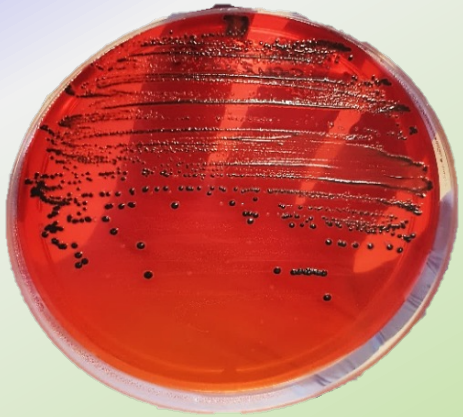
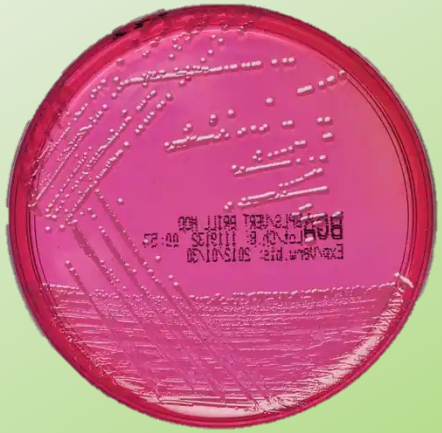
EXPRESSION OF RESULTS

In accordance with the interpretation of the results, report if *Salmonella spp* is detected or not detected, in the test portion, by specifying the mass in grams or the volume in milliliters of the sample tested.





CONCLUSION

Additional characterization of isolated strains:

Recognized National or regional *Salmonella* Reference

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- If the strain is sent to a reference centre, it should be accompanied by all relevant information such as confirmation results, source of which the strain was isolated, and whether it concerns an isolate from an outbreak

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THANKS FOR YOUR ATTENTION!

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