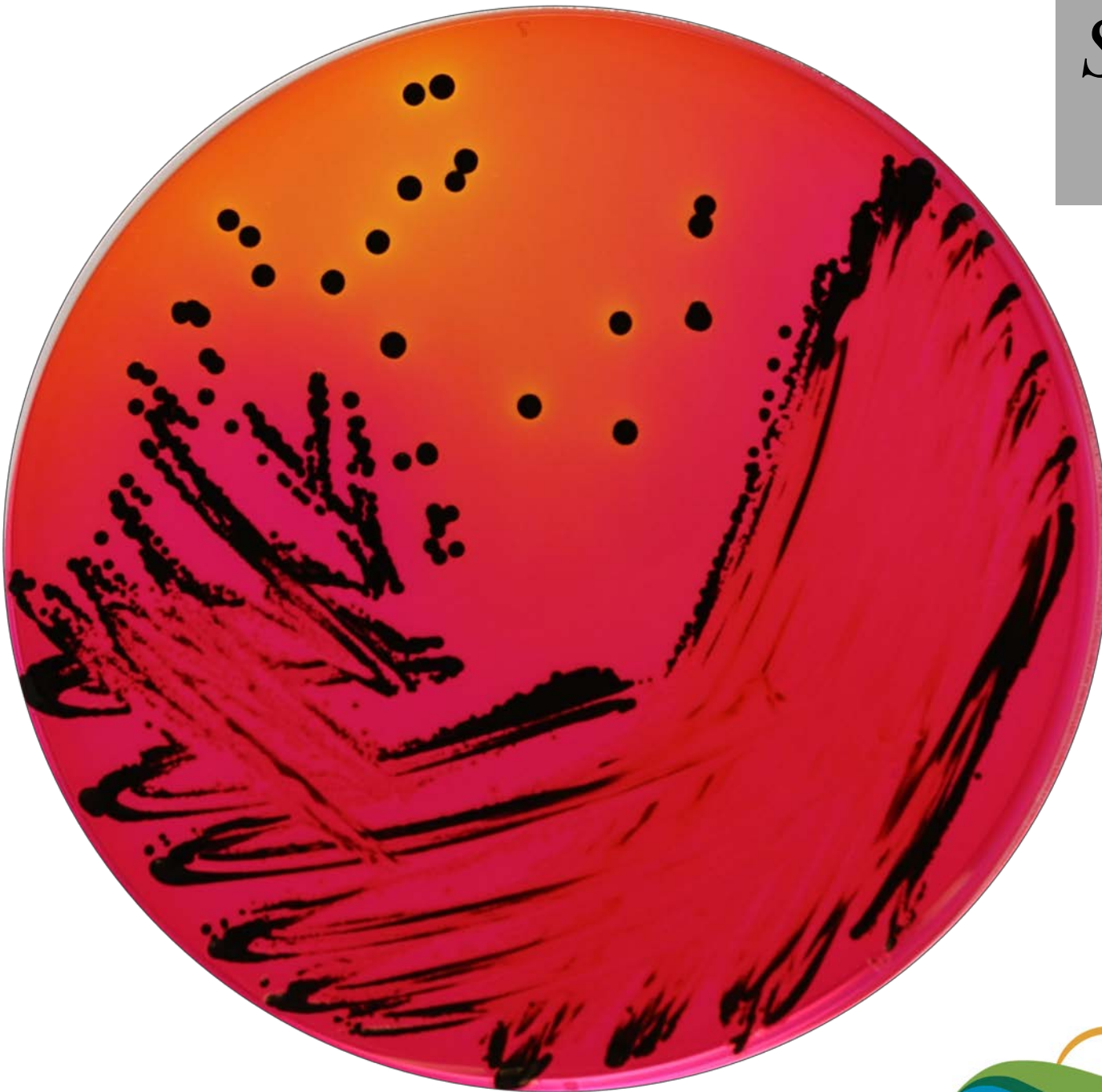


Salmonella Flipbook

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MN Dept. of Agriculture



This flipbook is intended as a general guide to aid analysts in the detection and identification of *Salmonella* growing on the plating media and screening tubes used in the BAM Chapter 5 *Salmonella* method.

For the purpose of this flipbook, “Typical” *Salmonella* refers to *Salmonella enterica* subsp. *enterica* isolates that produce H₂S, are lysine decarboxylase positive, and do not ferment lactose or sucrose.

When using this flipbook, please consider the following:

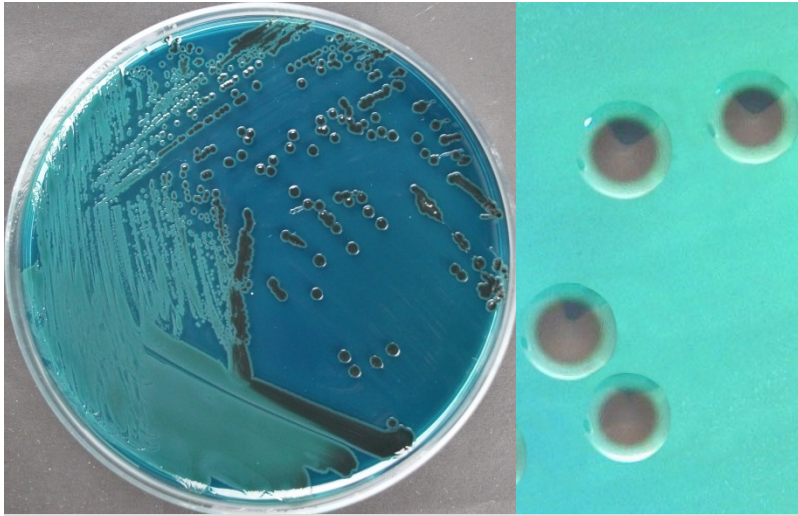
- The appearance of isolates encountered during sample analysis, whether typical or atypical, may vary from the images depicted.
- Atypical *Salmonella* can be rare. Some of the atypical morphologies depicted in this flipbook are so rare that they may not be encountered.
- Some serovars of *Salmonella* may be inhibited on bismuth sulfite agar.
- This flipbook does not address the phenotypic appearance of unique biovars of *Salmonella*, such as *Salmonella* ser. Typhi or *Salmonella* ser. Paratyphi A.
- Use caution if/when printing this flipbook, as the colors of the printed images may be distorted from those pictured on screen.

Salmonella

BAM Plate Morphology
&
Screening Tube Reactions

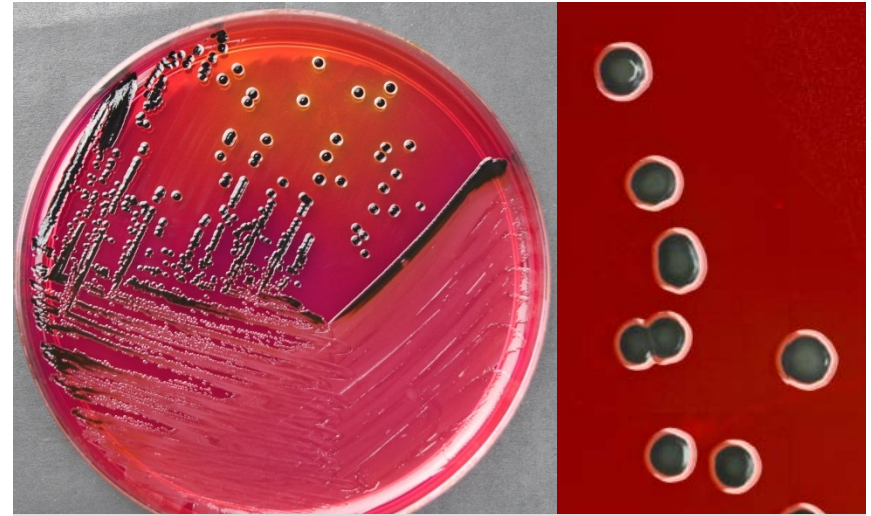
Typical *Salmonella*

BAM Media



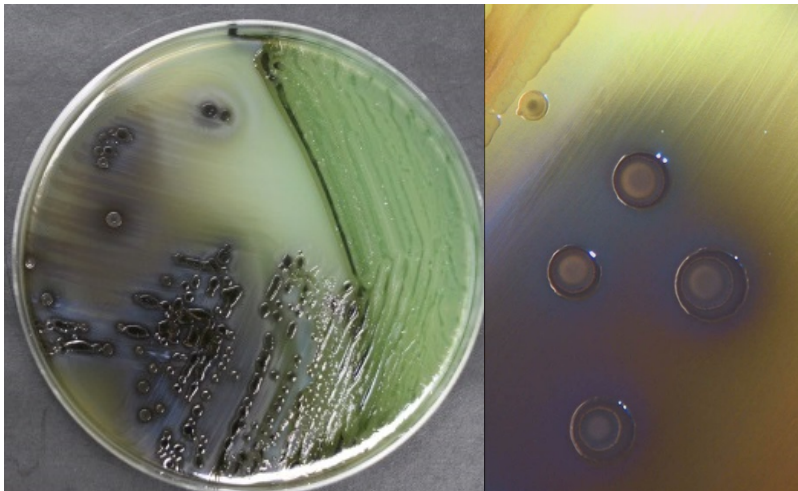
HE

Green to blue—green H₂S+ colonies.



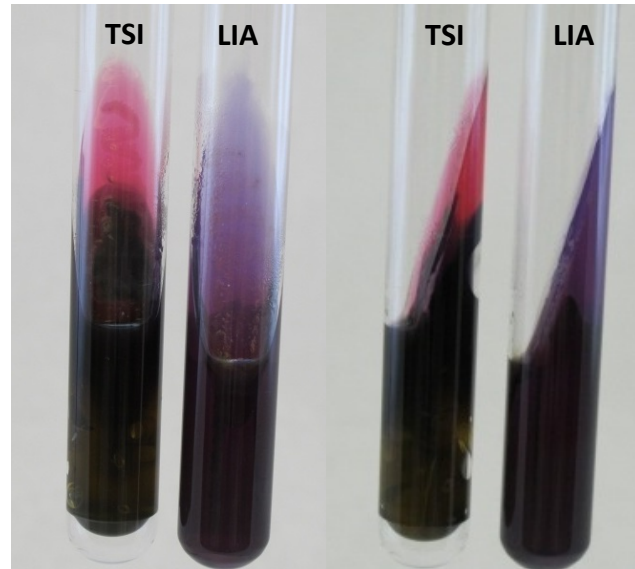
XLD

Opaque /yellow to red H₂S+ colonies.



BS

Brown, grey, or black colonies with darkening around colony.

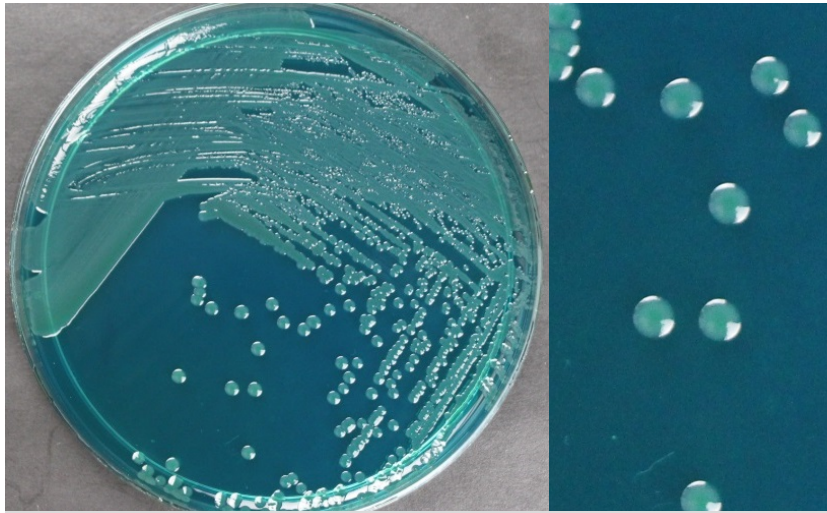


Screening tubes

- TSI
 - ❖ K/A H₂S+
- LIA
 - ❖ K/K H₂S+

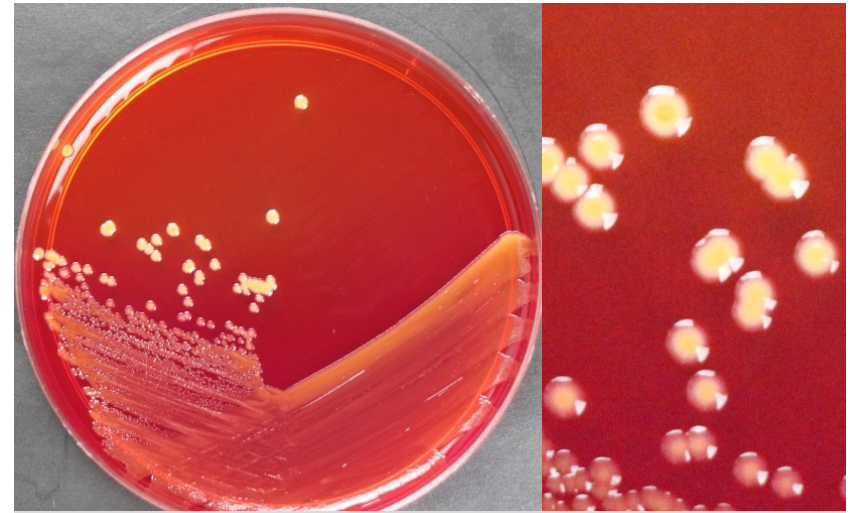
H₂S Negative *Salmonella*

BAM Media



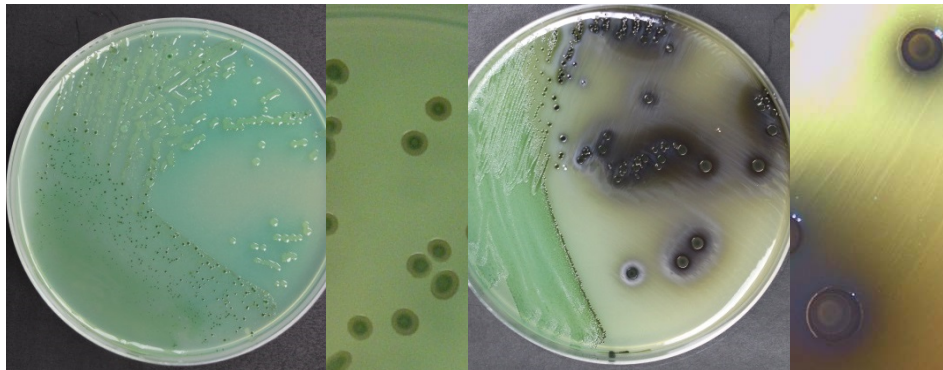
HE

Green to blue - green H₂S(-) colonies.



XLD

Opaque/yellow to red H₂S(-) colonies.

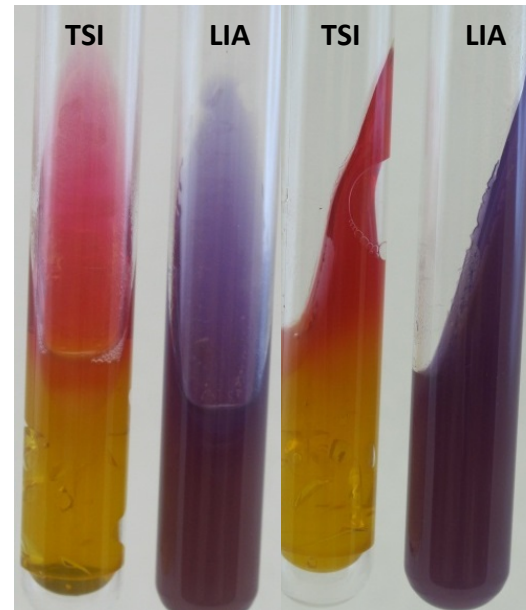


BS

Atypical morphology:
Green colonies, with little or no darkening of the surrounding agar.

BS

Typical morphology:
Brown, grey, or black colonies with darkening of the surrounding agar.



Screening tubes

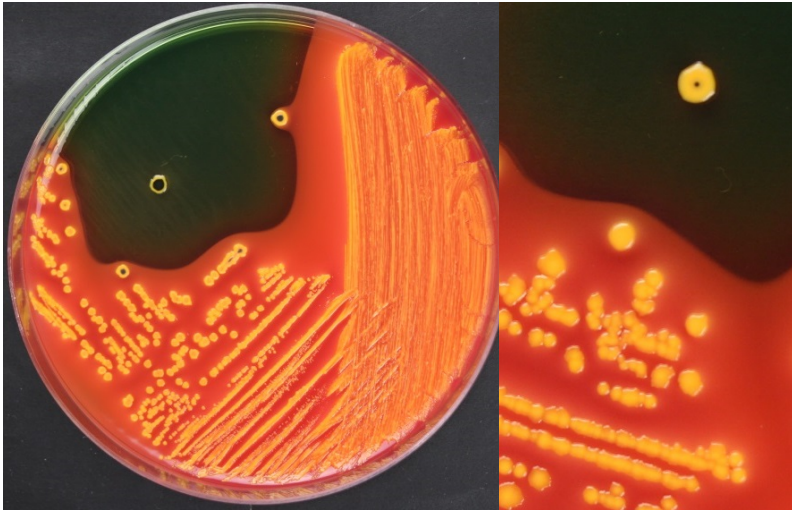
- TSI
 - ❖ K/A H₂S(-)
- LIA
 - ❖ K/K H₂S(-)

Key features:

- H₂S(-) colonies on HE and XLD
- BS may be typical or atypical
- H₂S(-) TSI and LIA reactions

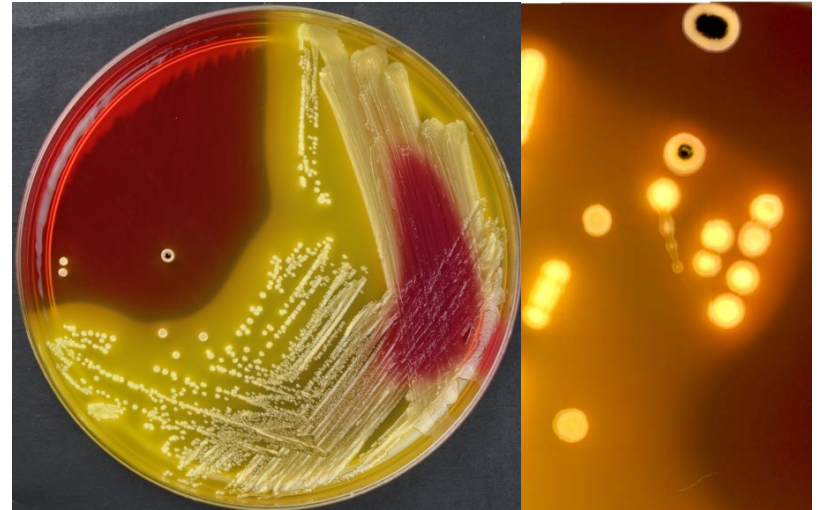
Lactose/Sucrose Positive *Salmonella*

BAM Media



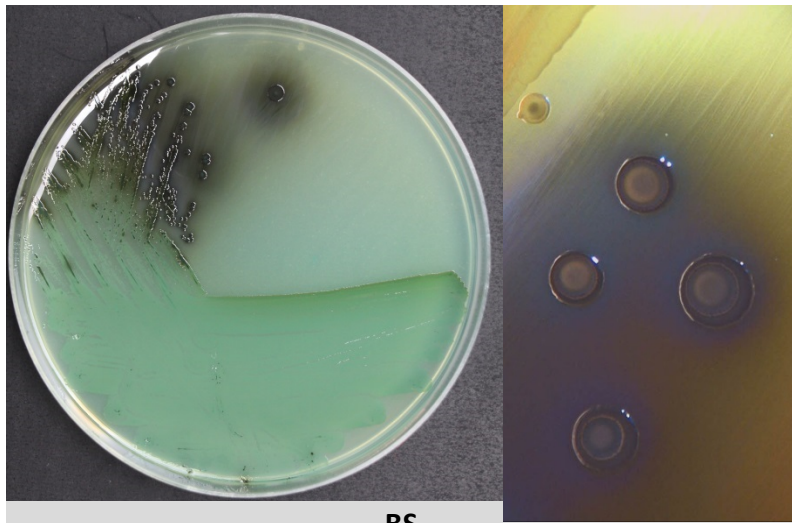
HE

Orange or yellow colonies with or without H₂S.



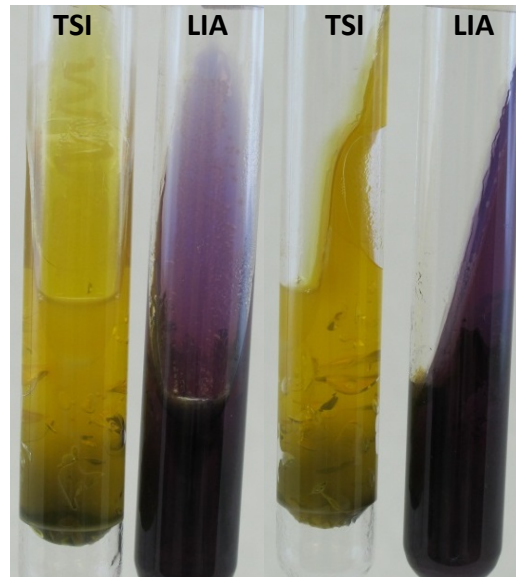
XLD

Yellow colonies with or without H₂S.



BS

Brown, grey, or black colonies with darkening around colony.



Screening tubes

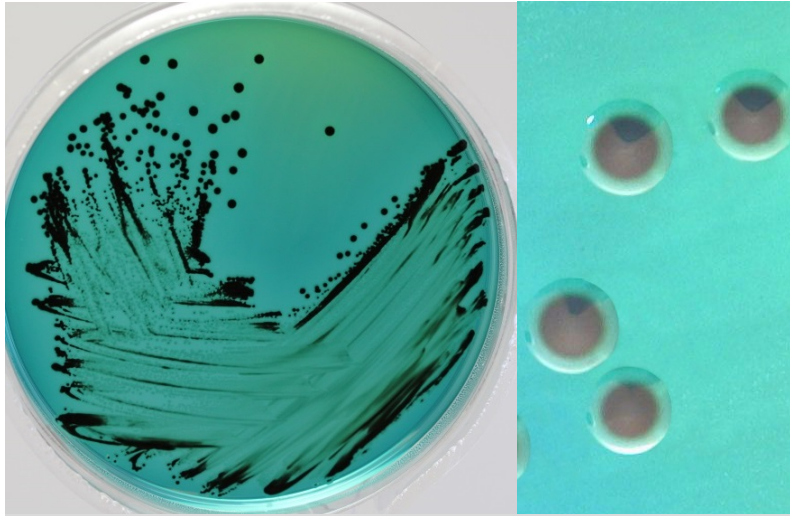
- TSI
 - ❖ A/A H₂S±
 - ❖ May or may not have slight red slant
- LIA
 - ❖ K/K H₂S±

Key features:

- Orange colonies on HE H₂S±
- Yellow colonies on XLD H₂S±
- A/A reaction on TSI H₂S±

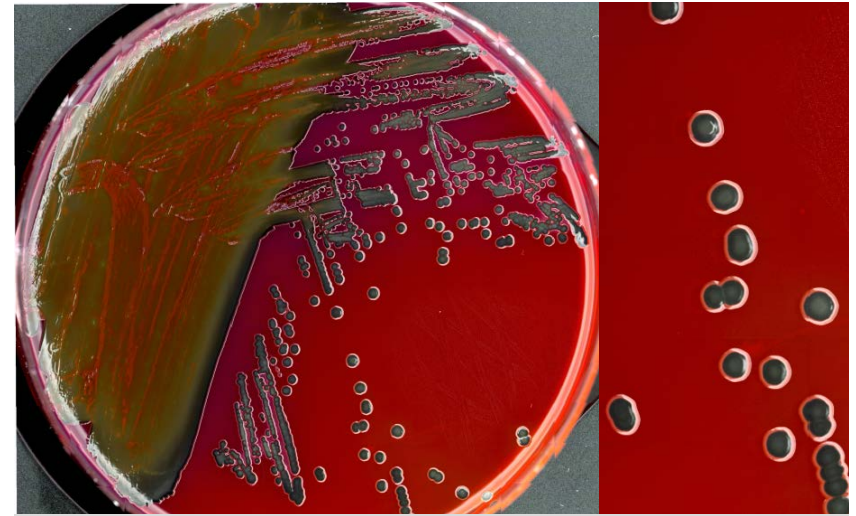
Atypical BS Morphology *Salmonella*

BAM Media



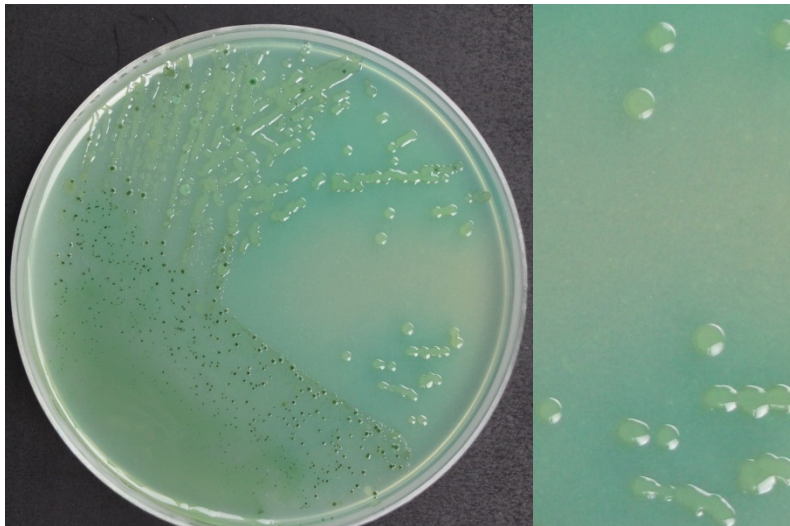
HE

Green to blue—green H₂S+ colonies.



XLD

Opaque/yellow to red H₂S+ colonies.



BS

Green colonies with little or no darkening of surrounding agar.

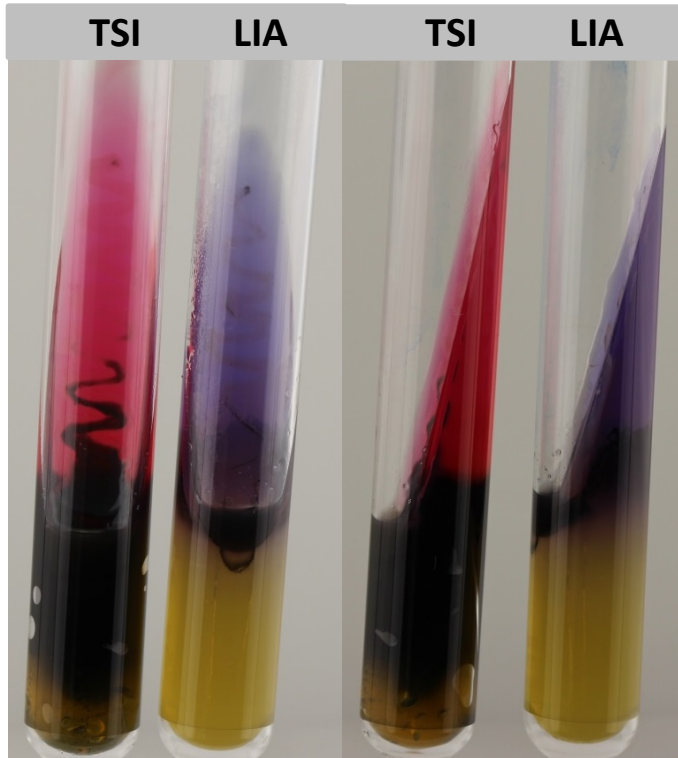
Key Features:

- BS atypical
- HE and XLD both typical

Screening tubes:

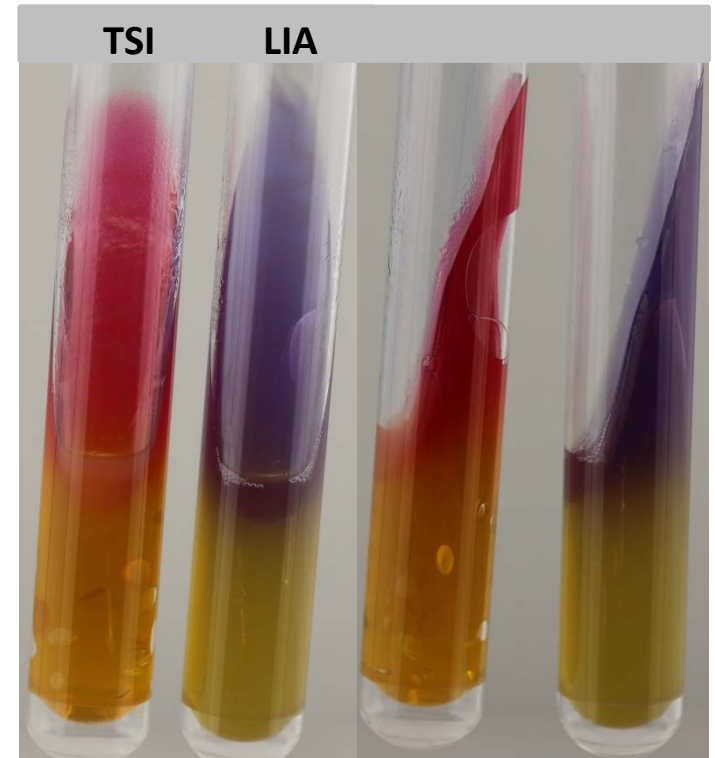
- TSI
 - ❖ Isolates will be typical (K/A H₂S+)
- LIA
 - ❖ Isolates will be positive for H₂S, but could be either K/K or K/A

Lysine Decarboxylase Negative *Salmonella*



Lysine Decarboxylase Negative H_2S Positive

These rare isolates will appear typical on HE.
On XLD, these isolates may appear typical, but
may also cause yellowing of surrounding agar.
On BS, these isolates may be typical or atypical.

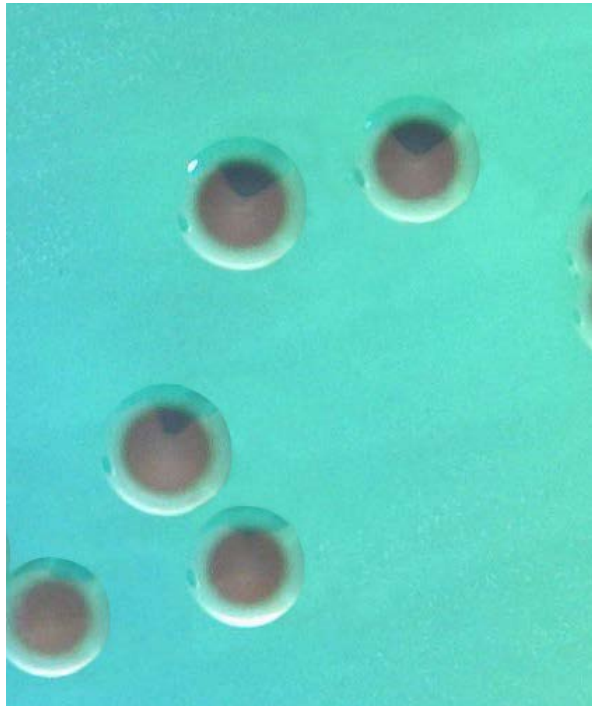


Lysine Decarboxylase Negative H_2S Negative

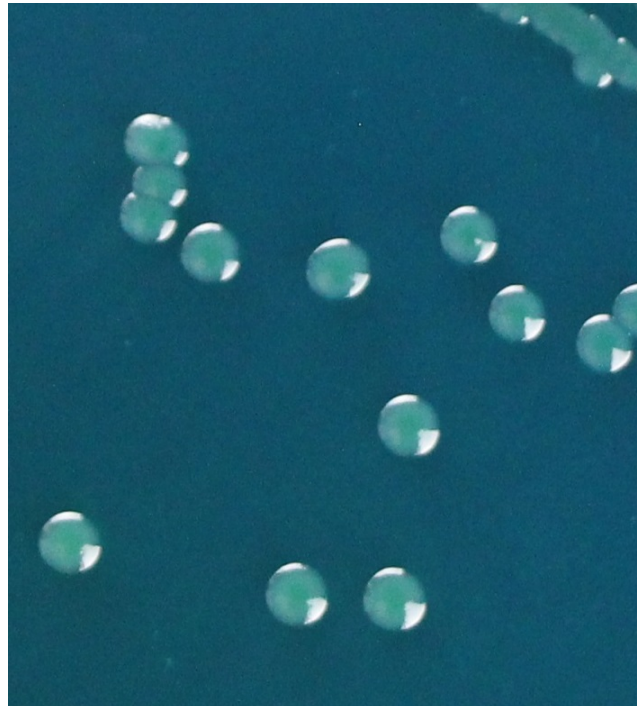
These even more rare isolates will appear H_2S
negative on HE and XLD. On XLD, these isolates
may cause yellowing of surrounding agar. On
BS, these isolates may be typical or atypical.

Hektoen Enteric Agar

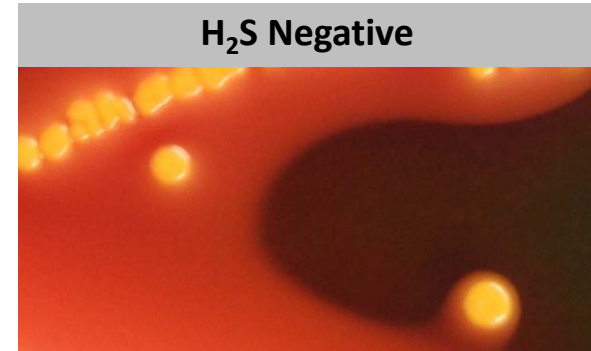
Salmonella Morphologies



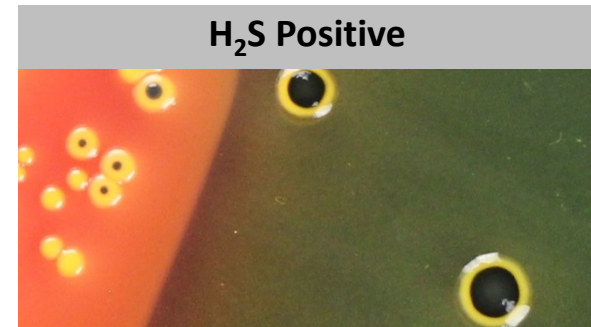
Typical



H₂S Negative



H₂S Negative



H₂S Positive

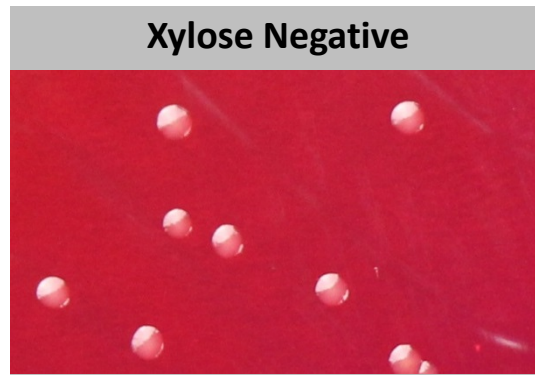
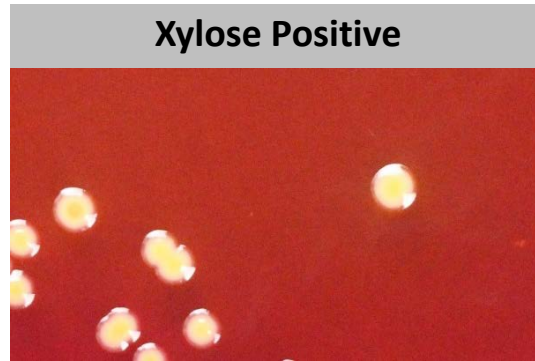
Lactose/Sucrose Positive

Xylose Lysine Desoxycholate Agar

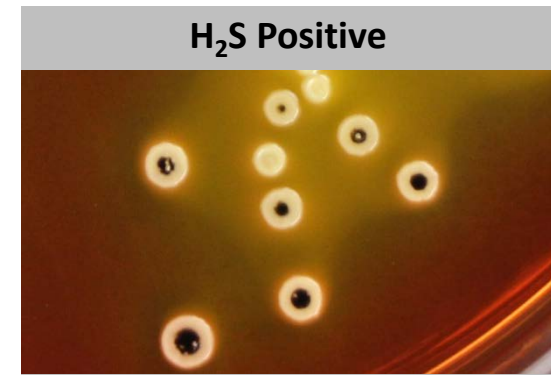
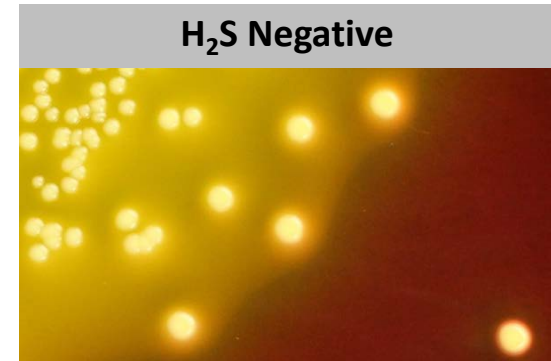
Salmonella Morphologies



Typical



H₂S Negative



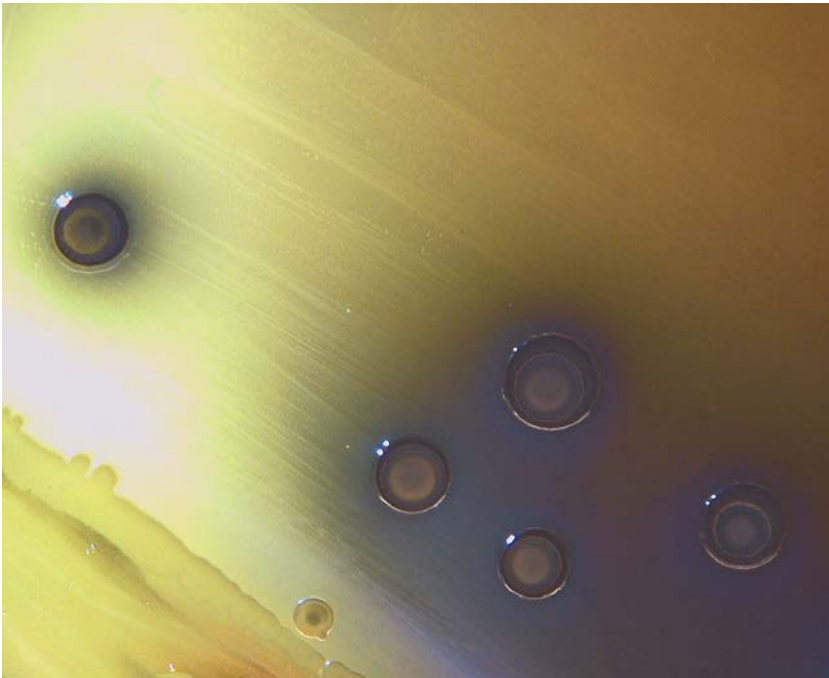
Lactose/Sucrose Positive

Note: *Salmonella* are typically xylose positive, which results in colonies with a dull yellow color after 22-26 hours of incubation, instead of the “pink colonies” described by the BAM. However, atypical xylose negative *Salmonella* will produce pink – red colonies on XLD after 22-26 hours of incubation. The yellow color caused by xylose fermentation is less intense than the yellow color expressed from atypical lactose and/or sucrose fermenting isolates.

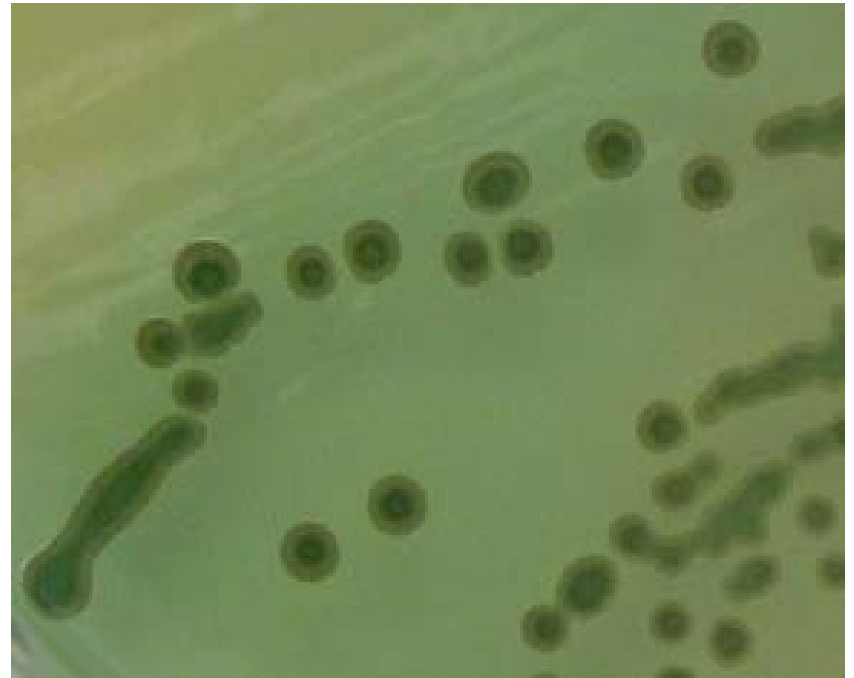
Note: With prolonged incubation, or even letting the plates sit out on the bench, the yellow color caused by xylose fermentation, changes to a pink to red color.

Bismuth Sulfite

Salmonella Morphologies



Typical Morphology

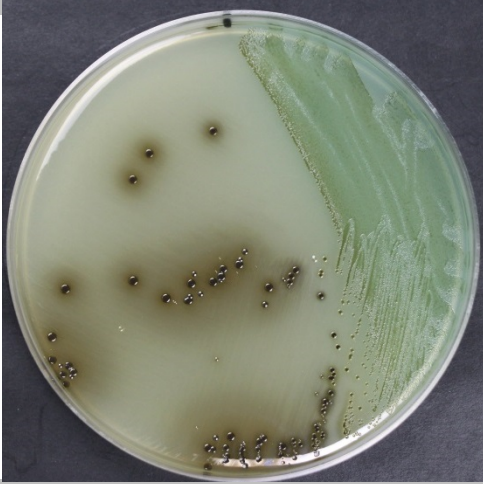


Atypical Morphology

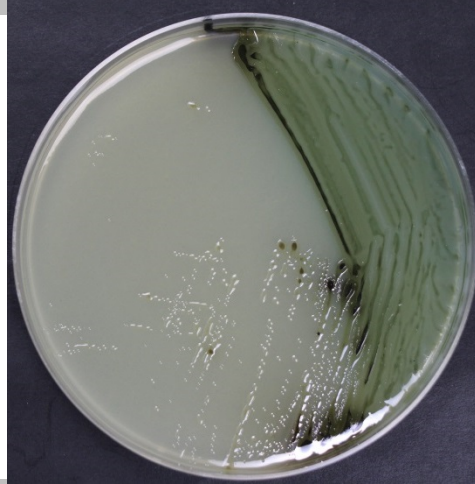
Bismuth Sulfite

Salmonella Morphologies at 24 & 48 Hours

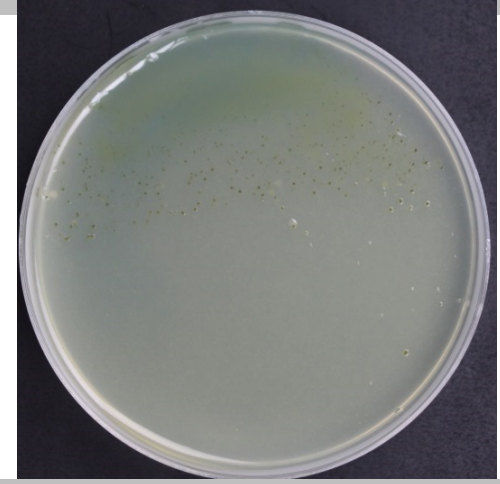
Example 1 at 24 Hours



Example 2 at 24 Hours



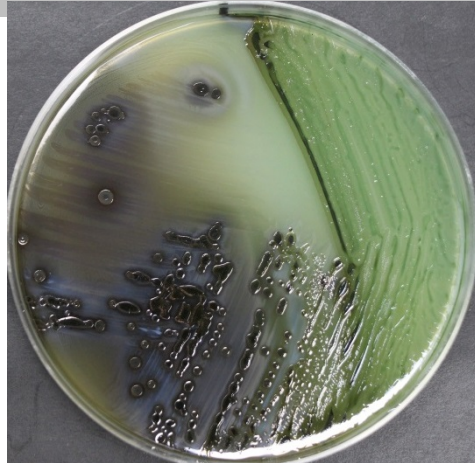
Example 3 at 24 Hours



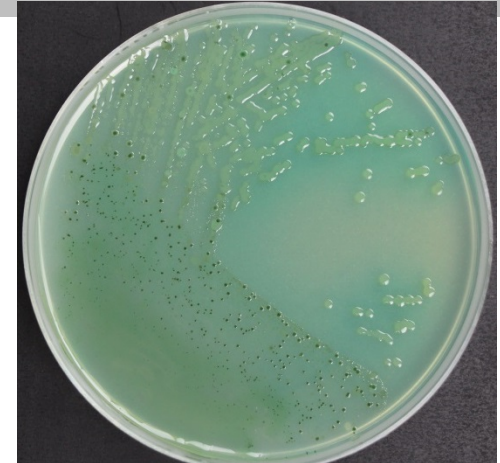
Example 1 at 48 Hours



Example 2 at 48 Hours



Example 3 at 48 Hours



Note: Bismuth sulfite agar must be analyzed after 24 and 48 hours of incubation. In some cases, *Salmonella* may form typical colonies after 24 hours of incubation, as shown in Example 1. However, it is not uncommon for *Salmonella* to only form typical colonies after 48 hours of incubation, as shown in Example 2. In some cases, *Salmonella* may not form typical colonies at all, as shown in Example 3.

Evaluation of Suspicious *Salmonella* Colonies

Examine HE, XLD, and BS plates for presence of colonies that may be *Salmonella*. BS plates should be examined after 24 ± 2 hours and returned to incubator to be examined again after 46 to 50 hours total incubation.

XLD

Typical *Salmonella* appear as opaque/yellow, pink, or red colonies with black centers. Look for colonies with a slightly rough or dimpled H₂S center.

Atypical *Salmonella*

- **H₂S negative *Salmonella*** appear as opaque/yellow, pink, or red colonies without black centers.
- **Lactose or sucrose fermenting *Salmonella*** appear as yellow colonies with or without black centers.

HE

Typical *Salmonella* appear as blue-green to blue colonies with black centers. Many *Salmonella* cultures have large glossy black centers or may appear as almost completely black colonies.

Atypical *Salmonella*

- **H₂S negative *Salmonella*** appear as blue-green to blue colonies without black centers.
- **Lactose or sucrose fermenting *Salmonella*** appear as yellow to orange colonies with or without black centers.

BS

Typical Morphology

- *Salmonella* appear as brown, gray, or black colonies that may have a metallic sheen. Surrounding media is usually brown at first, turning black with increased incubation time.

Atypical Morphology

- *Salmonella* strains produce green colonies with little or no darkening of surrounding media.

Evaluation of Suspicious *Salmonella* Isolates

Salmonella reactions on TSI and LIA tubes.

TSI

Typical *Salmonella* will produce alkaline (red) slants and acid (yellow) butts with hydrogen sulfide (black). K/A +

Atypical *Salmonella*

- **H₂S negative *Salmonella*** will produce alkaline (red) slants and acid (yellow) butts without hydrogen sulfide (black). K/A (-)
- **Lactose or sucrose fermenting *Salmonella*** will produce both acid (yellow) slants and butts or have a slight red slant (SRS) with the balance of the tube yellow, with or without hydrogen sulfide (black). A/A ± with or without SRS

LIA

Typical *Salmonella* will produce both alkaline (purple) slants and butts with hydrogen sulfide (black). K/K +

Atypical *Salmonella*

- **H₂S negative *Salmonella*** will produce both alkaline (purple) slants and butts without hydrogen sulfide (black). K/K (-)
- **Lysine decarboxylase negative *Salmonella*** will produce alkaline (purple) slants and acid (yellow) butts, with or without hydrogen sulfide (black). K/A ±

TSI & LIA Rule-Out Reactions

Isolates giving any of the following reactions may be considered negative for *Salmonella*:

1. Isolates producing a “no change”, or alkaline slant and butt (K/K) in both TSI (red) and LIA (purple).
2. Isolates with the “three A’s you’re out” reaction.
 - TSI = A/A
 - LIA= acid (A) butt
3. Isolates producing a burgundy or brick red slant in LIA.
 - R/A