CASE 4: ONE TOO MANY HAMBURGERS
THE MICROBIOLOGY LABORATORY

PATH 417

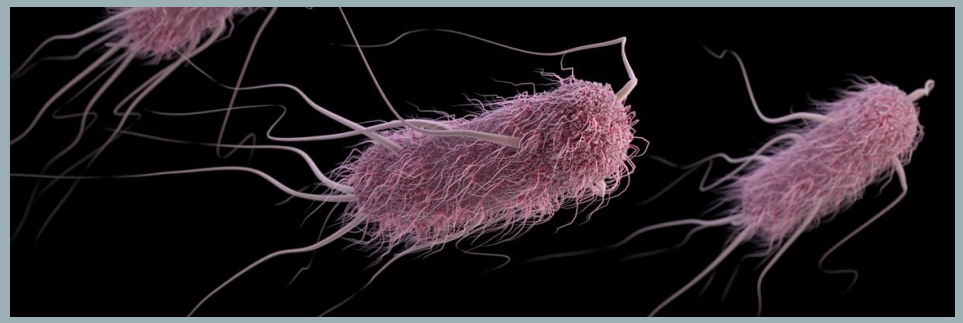
FRANK LAM

Q1: *NARROWING IN ON THE BACTERIAL CAUSES, WHAT ARE THE MOST COMMON BACTERIAL PATHOGENS ASSOCIATED WITH THIS INFECTIOUS SCENARIO?*

THE MOST COMMON PATHOGENS ASSOCIATED WITH THIS SCENARIO

- ESCHERICHIA COLI O157:H7
- CAMPYLOBACTER JEJUNI
- SALMONELLA TYPHIMURIUM
- SHIGELLA SONNEI

ESCHERICHIA COLI O157:H7



From: UK Biobank, 2011

Characteristics

- Gram negative, rod-shaped, facultative anaerobic

Primary reservoirs

- GI tract of animals such as cattle, goats & sheep

Common diseases

- Hemorrhagic colitis, Hemolytic uremic syndrome

Cause of infection

- Ingestion of improperly prepared beef products (contamination and undercooking)

Symptoms

- Bloody stool, bloody diarrhea, diarrhea, abdominal pains, vomiting, mild fevers

Other

- Produces Shigella toxin,

CAMPYLOBACTER JEJUNI

From: CDC

Characteristics

- Gram negative, spiral-shaped, microaerophilic,

Primary reservoirs

- GI tract of animals such as poultry and cattle

Common diseases

- Gastroenteritis, "food poisoning"

Cause of infection

- Ingestion of improperly prepared poultry products

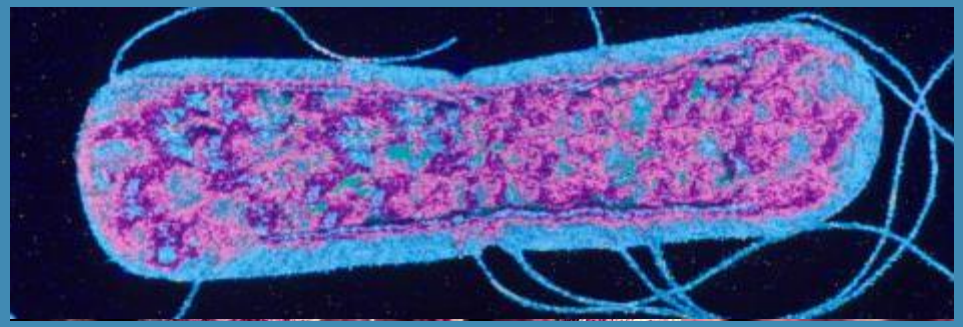
Symptoms

- Bloody stool, bloody diarrhea, diarrhea, abdominal pains, vomiting, nausea mild fevers

Other

- #1 cause of diarrhea in the USA

SALMONELLA TYPHIMURIUM



From: www.barfblog.com

Characteristics

Gram negative, rod-shaped, facultative anaerobic

Primary reservoirs

GI tract of animals such as poultry, pigs and cattle

Common diseases

Salmonellosis, Tyhpoid fever

Cause of infection

Ingestion of contaminated food products, improper food preparation

Symptoms

Bloody stool, bloody diarrhea, diarrhea, abdominal pains, vomiting, nausea mild fevers (Salmonellosis)

High fever, weakness, abdominal pain, headache, loss of appetite (Typhoid fever)

SHIGELLA SONNEI

From: CDC

Characteristics

- Gram negative, rod-shaped, facultative anaerobic

Primary reservoirs

- Humans

Common diseases

- Shigellosis

Cause of infection

- Ingestion of contaminated food or water

Symptoms

- Bloody stool, bloody diarrhea, diarrhea, abdominal pains, abdominal cramps, fever

Other

- 90% of all Shigella infections are caused by the *sonnei* and *flexneri* serotypes. *Sonnei* is the most common serotype in North America and will be the serotype considered for this case

Q2: *WHAT SAMPLES ARE TAKEN FOR LABORATORY TESTING AND HOW IMPORTANT IS THE MICROBIOLOGY LABORATORY IN THE DIAGNOSIS OF THIS DISEASE?*

HOW IMPORTANT IS THE MICROBIOLOGY LAB?

Very important!

As there are several candidates that could be the potential pathogen for Ronnie's illness, the microbiology lab is needed to isolate and identify the bacteria so that his doctor can provide the proper treatment.

Although all four of our suspect pathogens cause similar signs and symptoms, they do not respond similarly when exposed to antibiotics. Some may be resistant towards certain antibiotics, while others may be more susceptible to them. The identification of the pathogen will allow Ronnie's physician to create the right treatment plan to clear his infection quickly and effectively.

In addition, some pathogens cause some serious secondary infections that Ronnie's physician may want to keep an eye out for.

STOOL

Timing

- Early, within first 48hrs if possible during an active period of diarrhea

Collection

Whole stool

- Pick bloody, watery or slimy portions
- Ensure stool is not contaminated with toilet paper, barium or urine

Rectal swab (Alternative)

- Not as good as a stool sample for culturing but can be used if stool cannot be collected
- Moisten swab and insert 1-1.5" into rectum, then rotate

Storage

Whole stool

- Storee in a clean, dry container with a tight resealable lid
- Label with patient's name and DOB
- Keep cool at 4°C
- Can freeze at -15°C if used for antigen testing and/or PCR

Rectal swab

- Place in a transport medium (Stuart's, Aimes, Cary-Blair)
- Keep cool at 4°C
- Can be frozen if needed

Transportation

- Best if stool sample is processed within 2hrs of collection
- Transport medium (Cary-Blair) can be used for whole stool if needed
- Transport to the lab should be done ASAP

BLOOD

Timing

- Early, during onset of symptoms

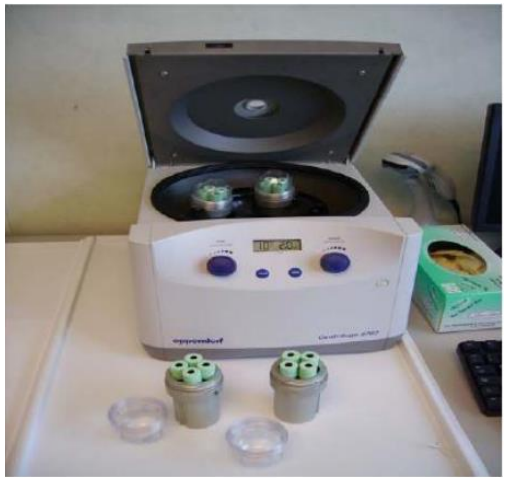
Collection

- Sterilize puncture site
- Apply tourniquet 1" away from site
- Draw blood into vacuum tube, a venous site is preferred
- Collect around 1-5mL of blood
- Label tube with patient's name and DOB

From: WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy, 2010

Storage

- Store at 4-25°C
- Anticoagulants, gels or additives may be present in collection tube to prepare for lab testing
- Separate serum from cells within 2hrs of collection, usually via centrifuge



From: UK Biobank, 2011

Transportation

- Can be transported in tube rack inside a transport box
- Transport box should be cooled by a cold pack

URINE

Timing

- Early, during onset of symptoms

Collection

- Try to collect first passed urine in the morning mid-stream if possible
- Collect a10-50mL sample into a leak-resistant sterile container/tube
- Label with name and DOB

Storage

- Keep cool at 4-6°C
- If urine sample cannot be processed within 2hrs of collection, chemical preservatives should be considered

Transportation

- Should be transported to the lab and processed within 48hrs

Q3: *EXPLAIN THE TESTS THAT WILL BE PERFORMED ON THE SAMPLES IN ORDER TO DETECT (ALL OF) THE BACTERIAL PATHOGENS THAT MAY BE CAUSING THIS DISEASE.*

STOOL CULTURE

MacConkey (MAC) Agar

TARGET PATHOGEN FOR ISOLATION

Gram negative, enteric bacteria

KEY FEATURES

Selective → Crystal violet and bile salts inhibit growth of gram$^+$ bacteria

Differential → Organisms that ferment lactose will appear **PINK** while those that do not will appear colourless

Fermentation decreases the local pH producing acid, which turns the indicator **PINK**

COMPOSITION OF MEDIUM

Peptone lactose ▪ Sodium chloride
Proteose peptone ▪ Bile salts ▪ Neutral red
Crystal violet ▪ Agar

MacConkey (SMAC) Agar with Sorbital

TARGET PATHOGEN FOR ISOLATION

E. coli (O157:H7)

KEY FEATURES

Selective → Crystal violet and bile salts inhibit growth of gram+ bacteria

Differential → Differentiates O157:H7 *E. coli* serotype based on its inability to ferment **sorbital**

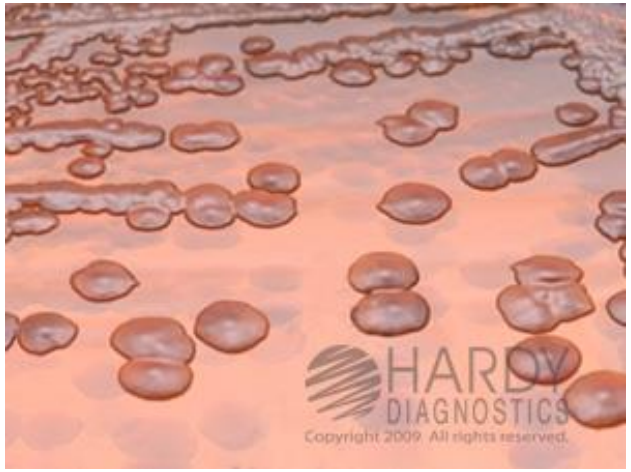
The lactose is switched out of MAC with Sorbital to make SMAC.

O157:H7 is not able to ferment sorbital in the medium and their colonies will be colourless

Other strains of *E. coli* (any other other bacterial species) that can ferment sorbital will show up as **PINK**

COMPOSITION OF MEDIUM

Pancreatic digest of gelatin ▪ **Sorbitol** ▪ Sodium choloride ▪ Pancreatic digest of casein ▪ Peptic digest of animal tissue ▪ Bile salts ▪ Neutral red Crystal violet ▪ Agar



Eosin Methylene Blue agar (EMB), Levine

TARGET PATHOGEN FOR ISOLATION

Gram negative, enteric bacteria

KEY FEATURES

Selective → Eosin Y and methylene blue inhibit most gram+ bacteria

Differential → Differentiates enteric bacteria based on its ability/inability to ferment lactose

Lactose fermenting bacteria will appear as **brown** to **blue-black**

Bacteria that can rapidly ferment lactose and form strong acids like E. coli will show as a **green metallic** sheen.

Non-lactose fermenting bacteria will appear colourless on this media

COMPOSITION OF MEDIUM

Pancreatic digest of gelatin ▪ Lactose ▪ Dipotassium phosphate ▪ Eosin Y ▪ Methylene blue ▪ Agar

Bismuth sulfite agar

TARGET PATHOGEN FOR ISOLATION

Salmonella

KEY FEATURES

Selective → Bismuth sulfite and brilliant green inhibit most commensal gram+ and gram- bacteria

Differential → Differentiates Salmonella based on its ability to metabolize iron sulphate and produce H_2S

Ferrous sulphate reacts with H_2S and acts as an indicator, producing a **brown/black** colour with a metallic sheen

Other strains are inhibited, or produce only dull **green/brown** colonies without any sheen

COMPOSITION OF MEDIUM

Pancreatic digest of casein ▪ Peptic digest of animal tissue ▪ Beef extract ▪ Dextrose ▪ Disodium phosphate ▪ Bismuth sulfite indicator ▪ **Ferrous sulfate** ▪ Brilliant Green ▪ Agar

Campylobacter blood agar

TARGET PATHOGEN FOR ISOLATION

Campylobacter jejuni

KEY FEATURES

Selective → Antimicrobial agents such as vancomycin, polymyxin B, nystatin, trimethoprim inhibit growth of normal microbial flora

Vancomycin inhibits most gram^{+} bacteria

Polymyxin B inhibits most gram^{-} bacteria

Nystatin is antifunal

Campylobacter blood agar is highly selective for *C. jejuni*

C. jejuni colonies will show up as non-hemolytic, **greyish** colonies with irregular edges

COMPOSITION OF MEDIUM

Agar ▪ Trimethoprim ▪ Pancreatic digest of casein Pancreatic digest of animal tissue ▪ Sodium chloride ▪ Yeast extract ▪ Dextrose ▪ Vancomycin Nystatin ▪ Polymyxin B ▪ Sodium bisulfite ▪ Sheep blood

HardyChrom Salmonella Shigella (SS) NOPRO Agar

TARGET PATHOGEN FOR ISOLATION

Salmonella & Shigella

KEY FEATURES

Selective → Bismuth sulfite and brilliant green inhibit most commensal gram$^+$ and gram- bacteria

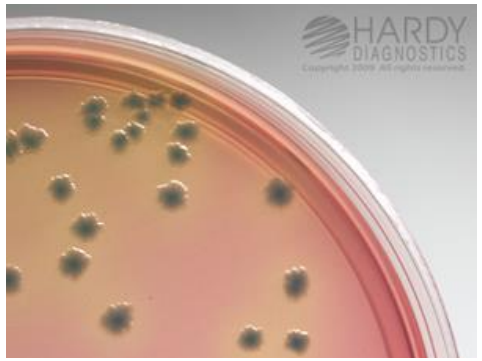
Differential → Differentiates Salmonella based on its ability to metabolize iron sulphate and produce H_2S

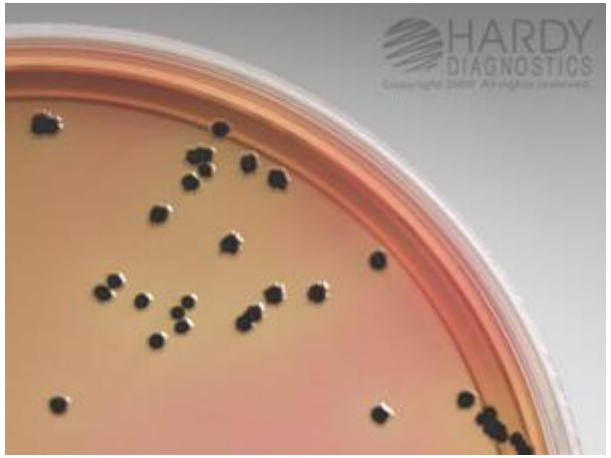
Ferrous sulphate reacts with H_2S and acts as an indicator, producing a **brown/black** colour with a metallic sheen

Other strains are inhibited, or produce only dull **green/brown** colonies without any sheen

COMPOSITION OF MEDIUM

Pancreatic digest of casein ▪ Peptic digest of animal tissue ▪ Beef extract ▪ Dextrose ▪ Disodium phosphate ▪ Bismuth sulfite indicator ▪ **Ferrous sulfate** ▪ Brilliant Green ▪ Agar





SLANTED MEDIA TESTS

Lysine Iron Agar (LIA) Test

TARGET PATHOGEN FOR ISOLATION

Enteric bacteria that can demonstrate decarboxylation or deanimation of lysine. Typically used to identify Salmonella species.

PRINCIPLE

The medium is mixed in a test tube and allowed to solidify at a slant

Decarboxylation or deanimation of lysine is identified by colour of the slant and butt of the medium

+ Decarboxylation (anaerobic, occurs in Butt of media)
Butt - **PURPLE** (alkaline)

- Decarboxylation
Butt - YELLOW

+ Lysine deanimation (aerobic, occurs in SLANT of media)
Slant – **DARK RED**

- Lysine deanimation
Slant – **PURPLE**

+H_2S → Black precipitate

COMPOSITION OF MEDIUM

L-Lysine hydrochloride ▪ Peptone ▪ Yeast extract
Dextrose ▪ Ferric ammonium citrate ▪ Sodium thiosulfate
Bromcresol purple ▪ Agar

Triple Sugar Iron (TSI) Test

TARGET PATHOGEN FOR ISOLATION

Enteric bacteria that is able to ferment lactose, glucose, sucrose and H_2S – Typically Salmonella and Shigella

PRINCIPLE

The medium is mixed in a test tube and allowed to solidify at a slant

Lactose and sucrose are usually present in 10x the amount of quantity as compared to glucose

Fermenting any of these sugars will generate acid byproducts, which changes the phenol red indicator to yellow (acid, A) in the butt and slant of the tube.

If the bacteria cannot ferment any of these sugars, the medium colour will not change and remain **RED** (alkaline, K).

If ONLY glucose is fermented, the butt will turn yellow and the slant will turn **RED**. The glucose at the slant is quickly used up, and the reaction becomes alkaline which causes the slant to turn **RED**.

If the bacteria is able to ferment Sucrose or Lactose in addition to glucose, the butt and slant will turn yellow. There is excess sucrose and lactose available for fermentation which sustains a acidic reaction at the slant.

If gas is produced this will be visible as **cracks or bubbles** in the medium.

If H_2S is produced, this reacts with ferrous ammonium indicator and shows up at as a **black** precipitate.

COMPOSITION OF MEDIUM

Pancreatic digest of casein ▪ Lactose ▪ Sucrose ▪ Sodium chloride ▪ Peptic digest of animal tissue ▪ Yeast extract Beef extract ▪ Dextrose ▪ Ferric ammonium citrate Sodium thiosulfate ▪ Phenol red ▪ Agar

STOOL SAMPLE WORKUP

Gram Stain

ISOLATION PARAMETER

Gram$^-$ or Gram$^+$ bacteria

PRINCIPLE

Gram+ bacteria have a thicker peptidoglycan layer that can retain crystal violet-iodine complex

Gram- have a thinner peptidoglycan that cannot retain the dye complex

When counterstain is applied Gram$^+$ shows up as **PURPLE** while Gram$^-$ shows up as **RED**

Oxidase Test

ISOLATION PARAMETER

Presence of Cytochrome c oxidase

PRINCIPLE

Cytochrome c oxidase is an enzyme involved in the bacterial electron transport chain

Wet filter paper is soaked with 1% reagent solution and the culture is rubbed onto the filter

The redox reaction carried out in the presence of this enzyme turns the reagent **PURPLE**.

If cytochrome c oxidase is not present it remains colourless

STOOL SAMPLE WORKUP

Catalase

ISOLATION PARAMETER

Presence of catalase

PRINCIPLE

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen:
$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

The sample colony is transferred into a solution of H_2O_2

Bubbles indicate the presence of catalase

Polymerase Chain Reaction (PCR)

ISOLATION PARAMETER

Bacteria with specific gene sequences – Stx1 & Stx2 in this case.

PRINCIPLE

The presence of a gene is tested by attempting to amplify it and then having it analyzed.

The sample DNA is denatured by heating it up to 95°C, which allow the strands to separate.

Complementary DNA primers can bind to their target DNA gene if it is present and begin the annealing process.

DNA polymerase extends the complementary strands to complete the strand.

This process is then repeated 20-40 times to exponentially amplify the number of DNA strands.

If the target gene was present it will be detected in analysis.

STOOL SAMPLE WORKUP

Enzyme-linked immunosorbent assay (ELISA)

ISOLATION PARAMETER

A specific antigen

PRINCIPLE

The sample antigen is attached to the surface of a microtiter well

Antibodies that can bind to the antigen is added.

The plate can be washed to remove any unbound antibodies, leaving the bound antibody-antigen complexes.

The antibody is typically linked with an enzyme that can link with a substrate that produces a visible effect (colour change) that will allow us to identify the antibody that is being bound to the antigen, revealing the identity of the antigen.

Antibiotic Sensitivity Test

ISOLATION PARAMETER

Sensitivity to antibiotics

PRINCIPLE

This test is a measure of how sensitive a pathogen is to an antibiotic

Various discs infused with antimicrobial agents are pressed down a plate inoculated with bacteria and incubated for16-24hrs

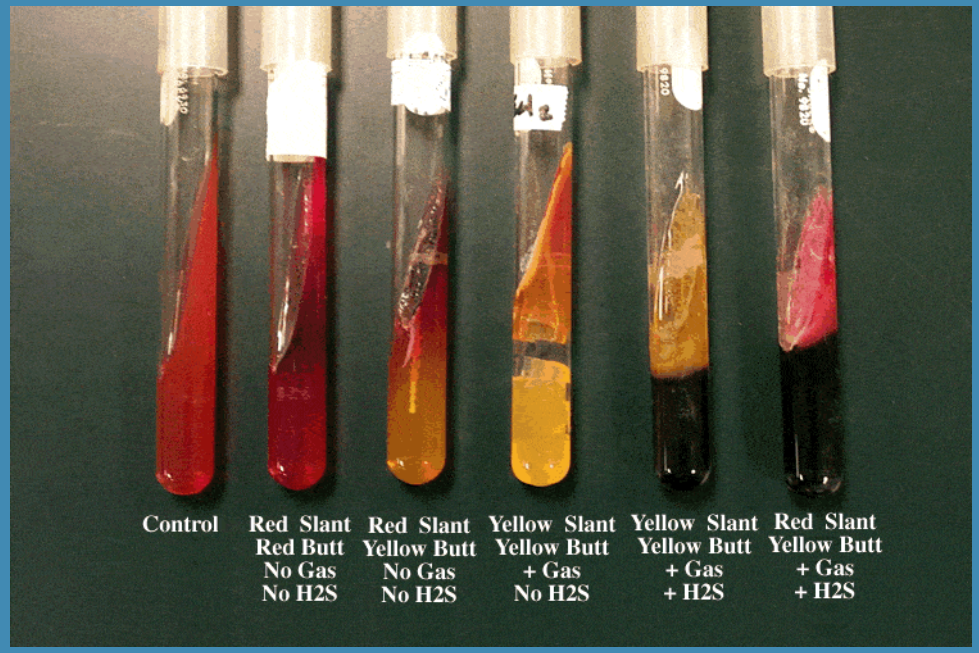
The diameter of the area where bacteria did not grow is measured. This is the **zone of inhibition.**

Larger zones indicate a higher sensitivity to an antibiotic.

Q4: *FOR EACH POTENTIAL PATHOGEN, WHAT ARE THE EXPECTED RESULTS FROM THESE TESTS?*

E. COLI (O157:H7)

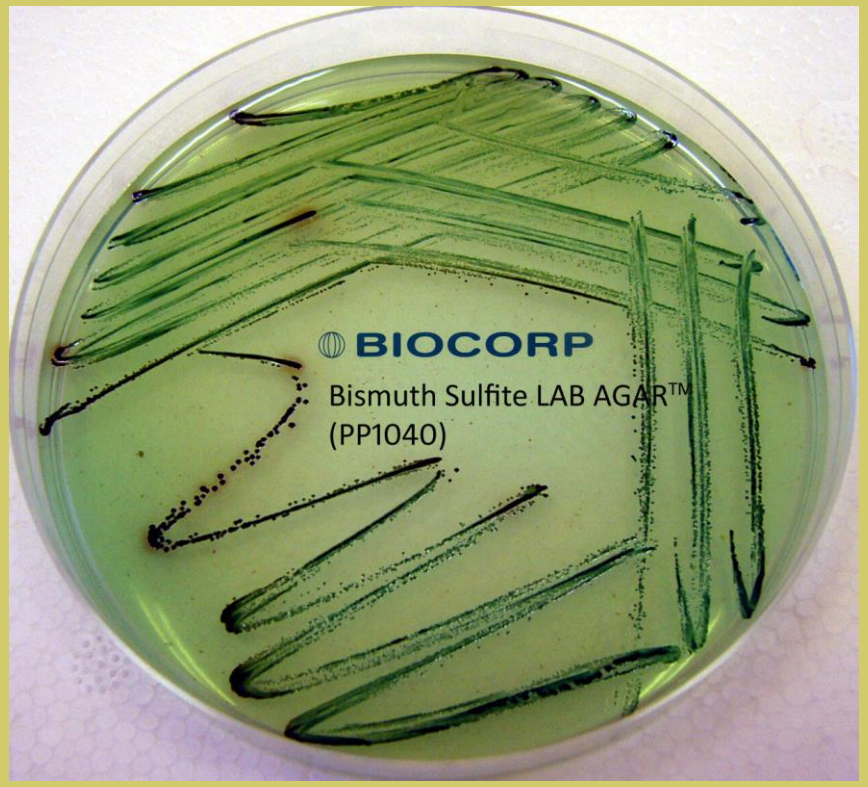
Test	Results
MAC	Pink
SMAC	Colourless
EMB	Blue-black with green metallic sheen
Bismuth sulfite agar	Minimal growth, green/brown
Campylobacter blood agar	No growth
HardyChrom SS NOPRO Agar	Pink
TSI	A/A, Yellow/Yellow (Slant/Butt), -Gas, -H_2S
LIA	Purple/Yellow
Gram stain	Negative, Red
Oxidase	Negative, no colour change
Catalase	Positive, Bubbling seen
PCR (Stx biased)	Positive
ELISA (Stx biased)	Positive
Antibiotic sensitivity	+ (susceptible) nitrofurantoin, ciprofloxacin, and norflaxocin
	- (not susceptible) tetracycline, erythromycin, and amoxicillin



From: Microbeonline
TSI Test

CAMPYLOBACTER JEJUNI

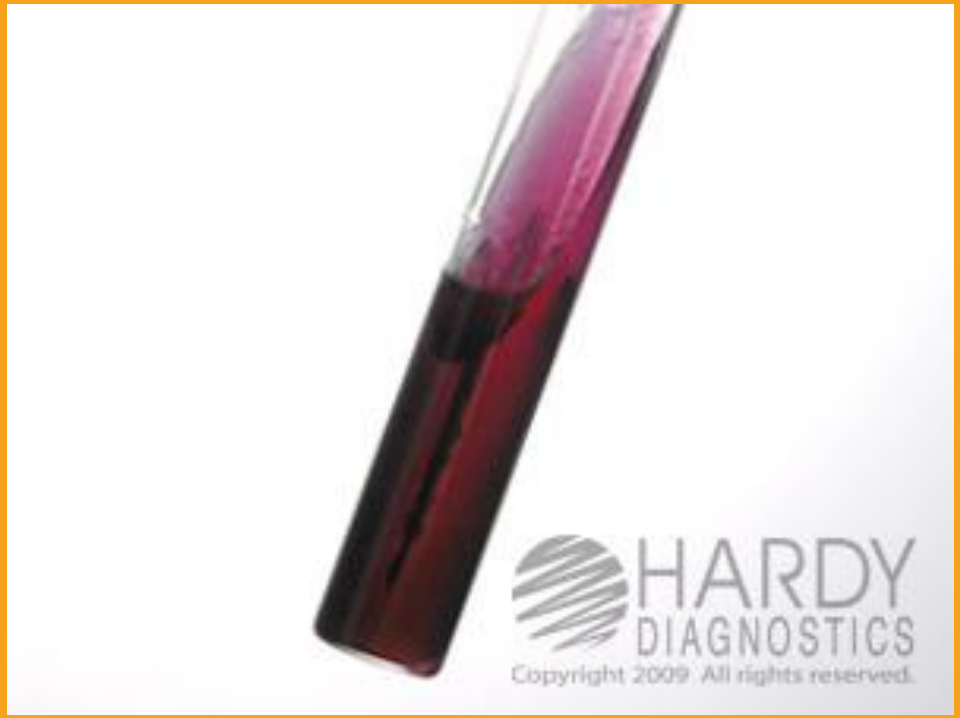
Test	Results
MAC	Colourless
SMAC	N/A
EMB	Colourless
Bismuth sulfite agar	Minimal growth, green/brown
Campylobacter blood agar	Growth seen, non-hemolytic, greyish colonies
HardyChrom SS NOPRO Agar	N/A
TSI	K/K, -Gas, -H_2S
Gram stain	Negative, Red
Oxidase	Positive, reagent turns Purple
Catalase	Positive, Bubbling seen
PCR (Stx biased)	Negative
ELISA (Stx biased)	Negative
Antibiotic sensitivity	+ erythromycin
	- ciprofloxacin



Bismuth Sulfite Agar

SALMONELLA TYPHIMURIUM

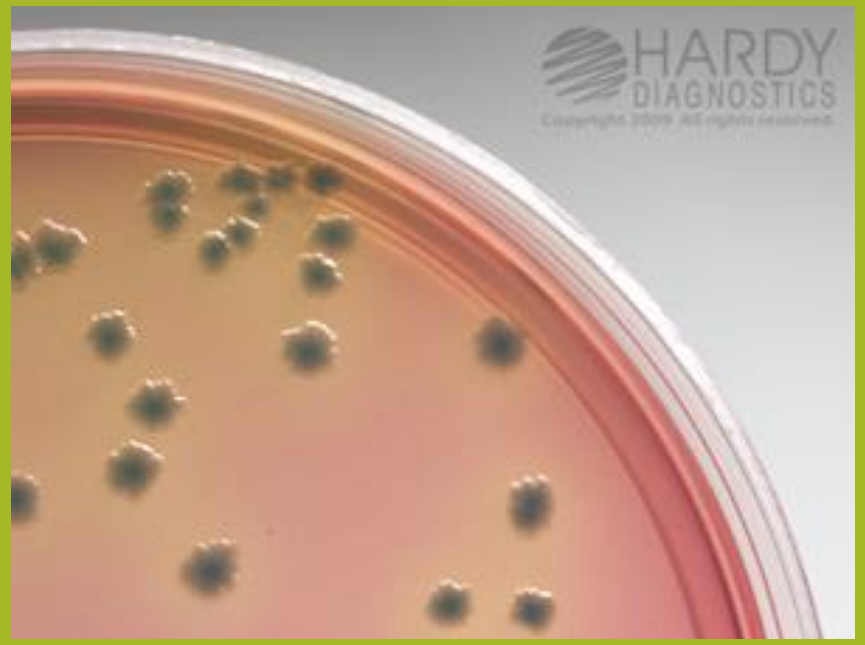
Test	Results
MAC	Colourless
SMAC	N/A
EMB	Colourless
Bismuth sulfite agar	**Brown/black** with metallic sheen
Campylobacter blood agar	No growth
HardyChrom SS NOPRO Agar	**Brown/black**
TSI	K/A, Yellow/black, +Gas, +H_2S
LIA	Purple/Back
Gram stain	Negative, Red
Oxidase	Negative, no colour change
Catalase	Positive, Bubbling seen
PCR (Stx biased)	Negative
ELISA (Stx biased)	Negative
Antibiotic sensitivity	+ ciprofloxacin
	- ampicillin



Salmonella LIA Test

SHIGELLA SONNEI

Test	Results
MAC	Colourless
SMAC	N/A
EMB	Colourless
Bismuth sulfite agar	No/minimal growth, green/brown
Campylobacter blood agar	No growth
HardyChrom SS NOPRO Agar	Teal coloured
TSI	K/A, Red/Yellow, -Gas, -H_2S
LIA	Purple/Yellow
Gram stain	Negative, Red
Oxidase	Negative, no colour change
Catalase	Positive, Bubbling seen
PCR (Stx biased)	Positive
ELISA (Stx biased)	Positive
Antibiotic sensitivity	+ ciprofloxacin and ceftriaxone
	- trimethoprim-sulphamethoxazole, ampicillin, tetracycline, chloramphenicol, nalidixic acid, cefixime



Shigella HardyChrom Result