



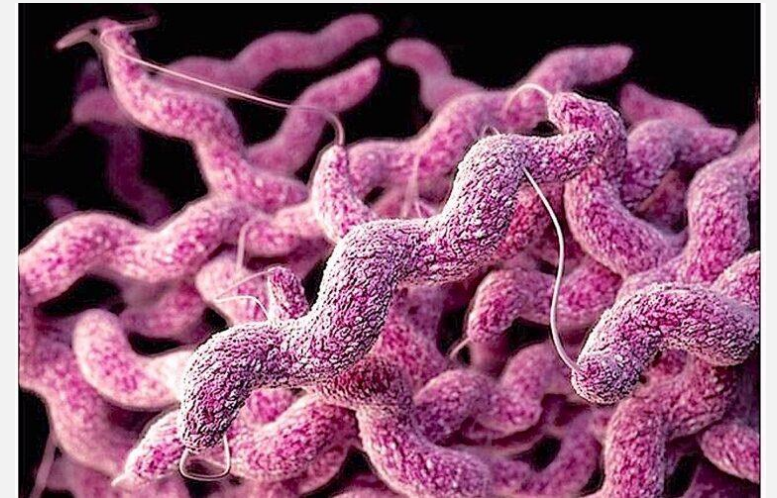
Identification of *Campylobacter jejuni* and *C. coli*,
Enterococcus faecium and *E. faecalis* by PCR.

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Campylobacter

- A leading cause of foodborne gastroenteritis in humans worldwide
- *C. jejuni* (around 85%) followed by *C. coli* and other *Campylobacter* species
- Strictly microaerophilic (5% O₂ and 10% CO₂)
- Grows well at 37 °C – 42 °C (Thermophilic *Campylobacter*)
- Commonly present in the intestinal tract of domestic and wild animals/birds, especially chickens



[\(https://about-campylobacter.com/\)](https://about-campylobacter.com/)

Standard methods for *Campylobacter* isolation

❖ OIE (WOAH)

- **Farm** samples (e.g. fresh fecal droppings, cloacal swabs or boot-swab samples) and **caecal content**

❖ ISO

- ISO 10272-1:2006(E) → Food samples
- ISO 10272-1:2017(E) → **Food** and **farm** samples

❖ FDA-BAM

- **Food** and **water** samples (e.g. shellfish; egg; milk; cheese; other dairy products; etc.)

Standard methods for *Campylobacter* isolation

❖ Similarities

- Incubation atmosphere: **microaerobic** conditions (5% O₂ and 10% CO₂)
- Incubation temperature: 37 °C or 42 °C
- Incubation time: 24 – 48 hours

❖ Differences

- **Different types** of enrichment broth and agar
- **Non-selective blood agar** for passive filtration method as recommended by OIE

Selective agents commonly used in *Campylobacter* selective enrichment broth

Table 1. Selective agents incorporated in some *Campylobacter* selective enrichment broth (concentrations in mg/l)

Medium	Cefoperazone	Vancomycin	Trimethoprim	Polymyxin B	Rifampicin	Cycloheximide	Amphotericin B
Park-Sanders broth	32	10	10			100	
Bolton broth	20	20	20				10
Hunt & Radle broth	30	10	12.5			100	
Preston broth			10	5,000 i.u.	10	100	

Media commonly used for *Campylobacter* isolation

Table 2. Agar media commonly used for *Campylobacter* isolation

Charcoal-based media	Blood-containing media
mCCDA (modified charcoal cefoperazone deoxycholate agar)	Preston agar
Karmali agar or CSM (charcoal-selective medium)	Skirrow agar
CAT agar (cefoperazone, amphotericin and teicoplanin)	Campy-cefex agar

Note: Oxygen quencher commonly used in *Campylobacter* selective agar: charcoal, blood, or FBP (ferrous sulfate, sodium metabisulfite, and sodium pyruvate)

Campylobacter colonies on media commonly used for *Campylobacter* isolation

mCCDA



Typical colonies of *Campylobacter* on mCCDA are greyish with metallic sheen, flat and moist with a tendency to spread.

Campylobacter colonies on media commonly used for *Campylobacter* isolation

Other media



Campylobacter on blood-based agar can appear as greyish with metallic sheen or brown to orange in color.

ISO protocol for *Campylobacter* isolation

- **ISO 10272-1:2017(E)**

Selective enrichment or no enrichment



Plating out on solid agar



Confirmation

Campylobacter detection by ISO 10272-1:2017(E)

Three different detection procedures depending on sample type

- ❖ **Procedure A:** Samples with **low** numbers of *Campylobacter* and **low** level of background microflora
(**selective enrichment** with **Bolton broth**)
- ❖ **Procedure B:** Samples with **low** numbers of *Campylobacter* and **high** level of background microflora
(**selective enrichment** with **Preston broth**)
- ❖ **Procedure C:** Samples with **high** numbers of *Campylobacter*
(**direct plating** method)

Campylobacter detection by ISO 10272-1:2017(E)

Procedure A

Sample 10 g or 10 ml + **Bolton broth** 90 ml (1:9 ratio)



Incubate in a microaerobic atmosphere at **37°C** for **4 to 6 h**
and then at **41.5°C** for **44 h ± 4 h**



Subculture onto **mCCDA** and **2nd medium**; incubate at
41.5°C for **44 h ± 4 h** in a microaerobic atmosphere

Campylobacter detection by ISO 10272-1:2017(E)

Procedure B

Sample 10 g or 10 ml + Preston broth 90 ml (1:9 ratio)



Incubate in a microaerobic atmosphere at 41.5°C for 24 h ± 2 h



Subculture onto mCCDA and incubate at 41.5°C for 44 h ± 4 h
in a microaerobic atmosphere

Campylobacter detection by ISO 10272-1:2017(E)

Procedure C

Direct plating on **mCCDA** and 2nd medium (optional)



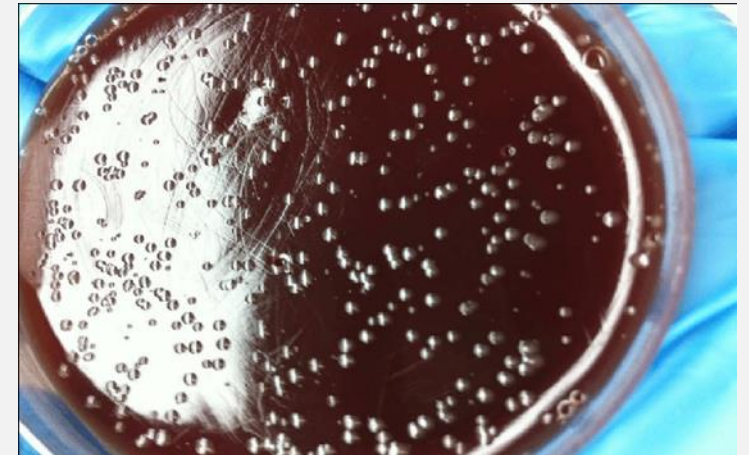
Incubate in a microaerobic atmosphere
at **41.5°C** for **44 h ± 4 h**

Campylobacter detection by ISO 10272-1:2017(E)

Confirmation of *Campylobacter*

Selection of colonies for confirmation

- Test at least 1 typical colony; if negative, test up to 4 more suspect colonies
- Subculture onto a non-selective blood agar plate, e.g. Columbia blood agar, then incubate the agar plate in a microaerobic atmosphere at 41.5°C for 24 h to 48 h



https://www.researchgate.net/figure/Colonies-of-Campylobacter-on-blood-agar_fig1_335840996

Campylobacter detection by ISO 10272-1:2017(E)

Confirmation of *Campylobacter*

Campylobacter confirmation tests:

- Examination of morphology and motility
- Absence of aerobic growth at 25°C
- Detection of oxidase activity
- Other alternative or additional tests, e.g. PCR, MALDI-TOF, serological test, etc.

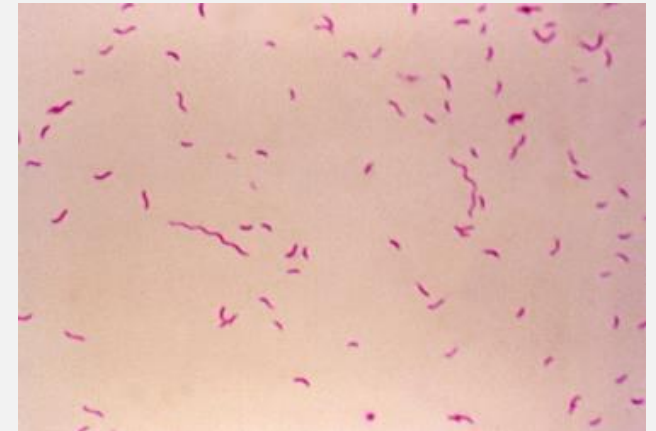


Campylobacter detection by ISO 10272-1:2017(E)

Confirmation of *Campylobacter*

Examination of morphology and motility

- Suspend one colony from the blood agar plate in 1 ml of liquid medium
- Examine for morphology and motility using a microscope
- *Campylobacter* are **curved bacilli with a corkscrew motility.**

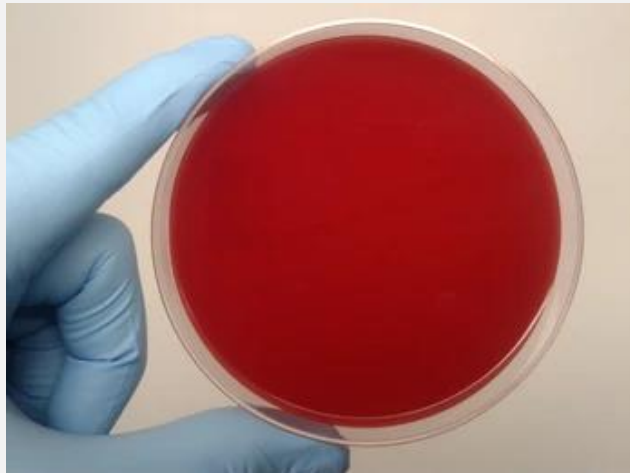


(Whitehouse et al., 2018)

Campylobacter detection by ISO 10272-1:2017(E)

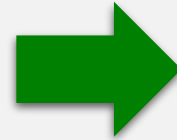
Confirmation of *Campylobacter*

Study of aerobic growth at 25°C



<https://www.shutterstock.com/search/blood-agar>

Subculture suspect *Campylobacter* colonies
on a non-selective blood agar plate



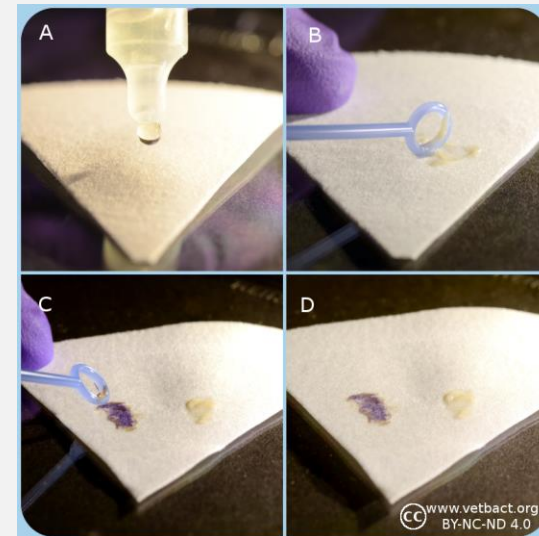
Incubate the plate aerobically at
25 °C for 44 h ± 4 h

Campylobacter detection by ISO 10272-1:2017(E)

Confirmation of *Campylobacter*

Detection of oxidase activity

- Streak a portion of a well-isolated colony onto a filter paper moistened with the oxidase reagent
- Violet or deep purple/blue color within 10 s indicates positive reaction.
- *Campylobacter* are oxidase positive.



Campylobacter detection by ISO 10272-1:2017(E)

Table 3. Confirmation tests for *Campylobacter* spp.

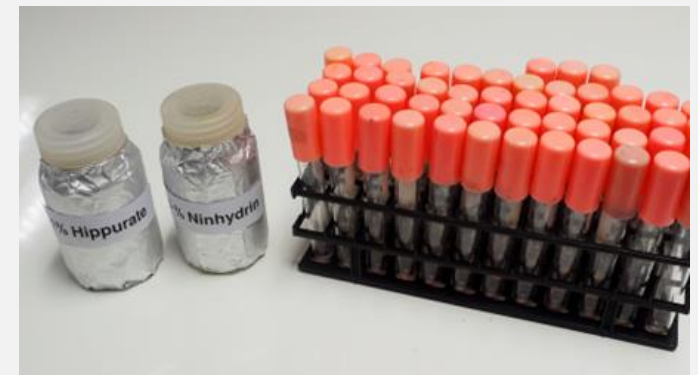
Characteristics of <i>Campylobacter</i> spp.	
Morphology	Small curved bacilli
Motility	Corkscrew motility
Aerobic growth at 25°C	-
Oxidase activity	+

Campylobacter detection by ISO 10272-1:2017(E)

Identification of *Campylobacter* species (optional)

Differentiation of *Campylobacter* species

- Detection of catalase activity
- Hippurate hydrolysis test
- Indoxyl acetate hydrolysis test
- No more nalidixic acid sensitivity test

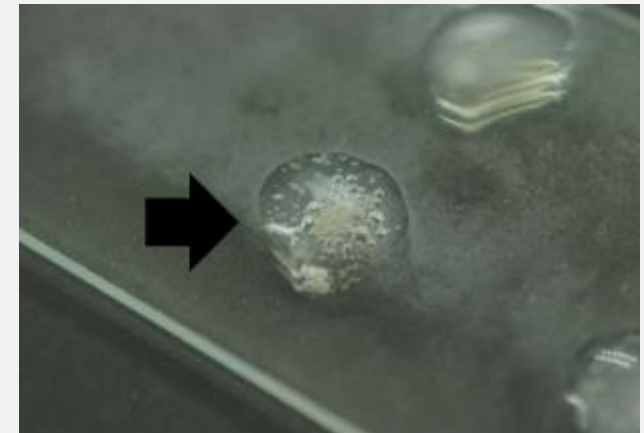


Campylobacter detection by ISO 10272-1:2017(E)

Identification of *Campylobacter* species (optional)

Catalase test

- Deposit a loopful of culture into a drop of hydrogen peroxide solution
- Bubbles within 30 s indicates positive reaction.
- Most *Campylobacter* are catalase positive.

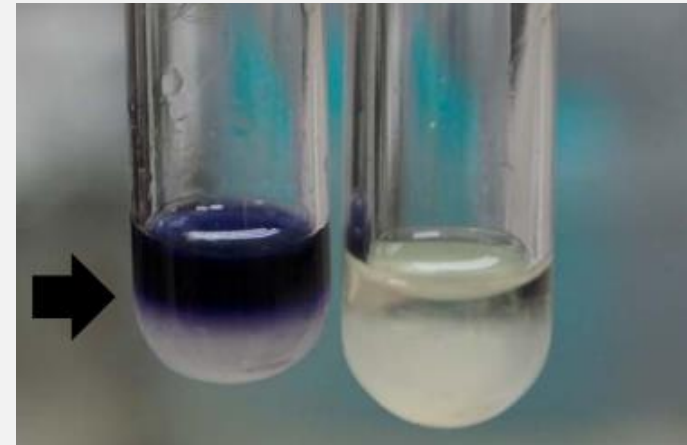


Campylobacter detection by ISO 10272-1:2017(E)

Identification of *Campylobacter* species (optional)

Hippurate hydrolysis test

- Ability to hydrolyze sodium Hippurate
- A dark purple color indicates a positive reaction.
- A pale violet color or no color change indicates a negative reaction.



Detection of hippurate hydrolysis

Inoculate a loopful of culture into a tube containing 0.4 ml of sodium hippurate solution (**heavy inoculum**)



Mix thoroughly and then incubate for 2 h in a water bath set at 37°C or 4 h in an incubator set at 37°C



Add 0.2 ml of 3.5% ninhydrin solution on top of sodium hippurate solution. Do not shake. Incubate in a water bath or an incubator set at 37°C for 10 min.



Interpret the results

Positive = Dark purple color; Negative = Pale violet color or no color change

Campylobacter detection by ISO 10272-1:2017(E)

Identification of *Campylobacter* species (optional)

Indoxyl acetate hydrolysis test

- Ability to hydrolyze indoxyl acetate
- Place a loopful of colony on an indoxyl acetate disc, then add a drop of sterile distilled water on the disc
- If indoxyl acetate is hydrolyzed, a color change to **dark blue** will occur within 5 – 10 min.



Campylobacter detection by ISO 10272-1:2017(E)

Table 4. Phenotypic characteristics of thermophilic *Campylobacter* species

Characteristics	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>
Catalase	+	+	+
Hippurate hydrolysis	+ ^a	-	-
Indoxyl acetate hydrolysis	+	+	-
^a Some hippurate-negative <i>C. jejuni</i> strains have been reported.			

OIE protocol for *Campylobacter* detection

Isolation of *Campylobacter*

- Selective media for isolation
- Passive filtration
- Incubation (atmosphere, temperature, and time)

Confirmation of *Campylobacter*

- Identification of *Campylobacter* colonies on solid medium
- Microscopic examination of morphology and motility
- Detection of oxidase
- Aerobic growth at 25 °C
- Latex agglutination test

Identification of *Campylobacter* to the species level

- Detection of hippurate hydrolysis
- Detection of indoxyl acetate hydrolysis

OIE protocol for *Campylobacter* detection

Passive filtration

Fecal sample + PBS (approximately 1/10 dilution)



Add 100 µl of the suspension onto a 0.45 or 0.65 µm filter which has been previously placed on top of a non-selective blood agar plate



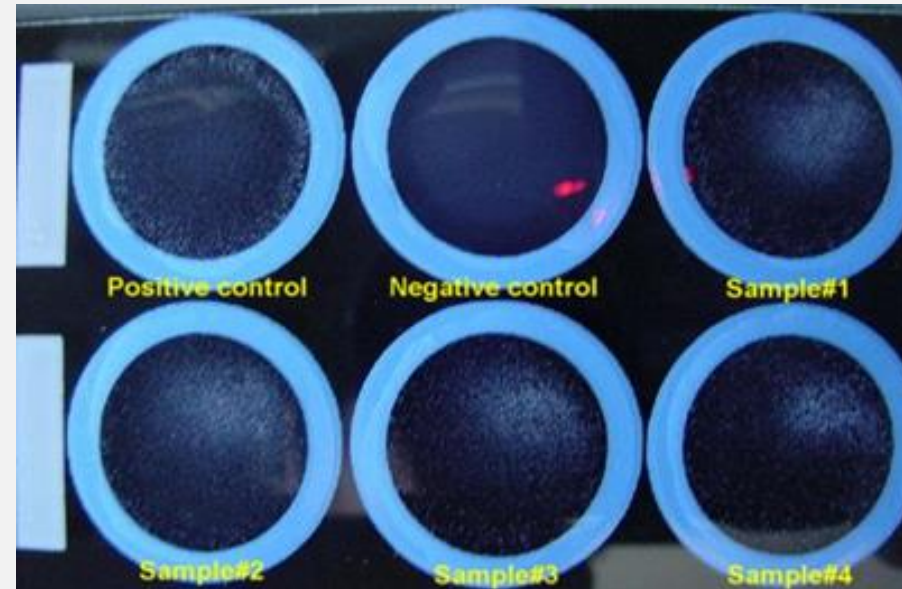
Wait for 30 - 45 minutes at 37 °C or room temperature to allow the bacteria to migrate through the filter and then remove the filter



Incubate the agar plate at 42 °C for 48 hours under a microaerobic atmosphere

OIE protocol for *Campylobacter* detection

Confirmation of *Campylobacter* using latex agglutination test

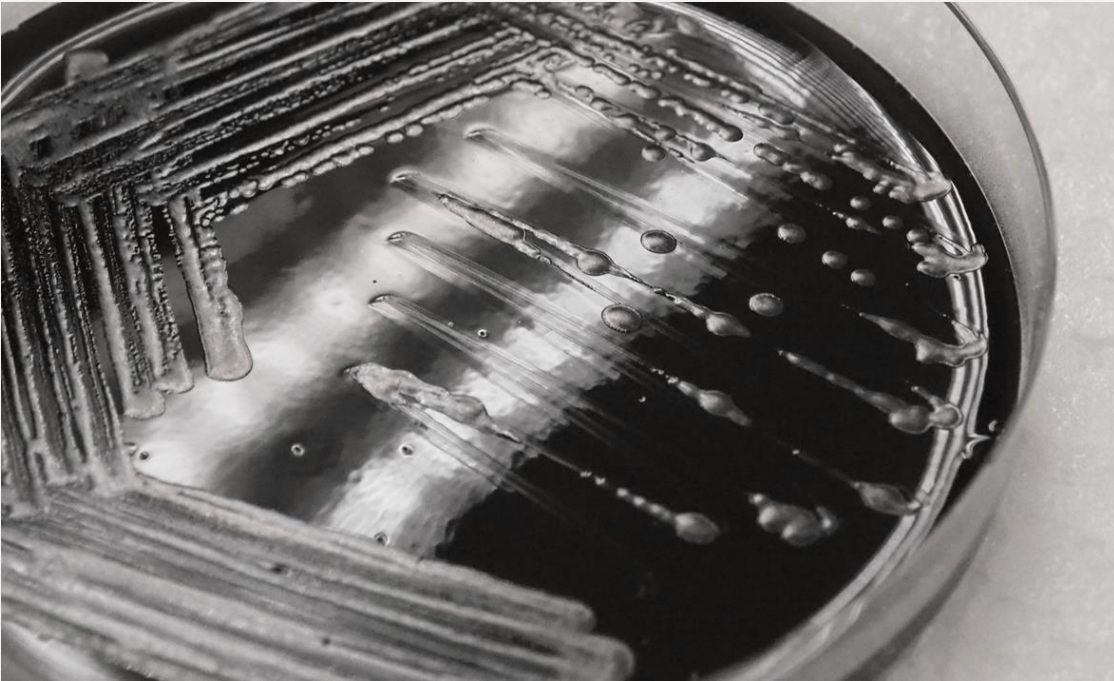


Campylobacter isolation methods for samples from broiler farms and slaughterhouses

Table 5. Recommended *Campylobacter* isolation methods for different sample types

Purpose	Target sample	Method	Media
Detect infection status of broiler farms (before slaughtering)	Boot-swab	Direct plating	mCCDA and Preston
Detect colonization status of broiler flocks (after slaughtering)	Caecal content	Direct plating	mCCDA and Preston or mCCDA
Detect contamination of broiler meat	Neck/breast skin	Selective enrichment	Preston broth and mCCDA

Campylobacter colonies on mCCDA



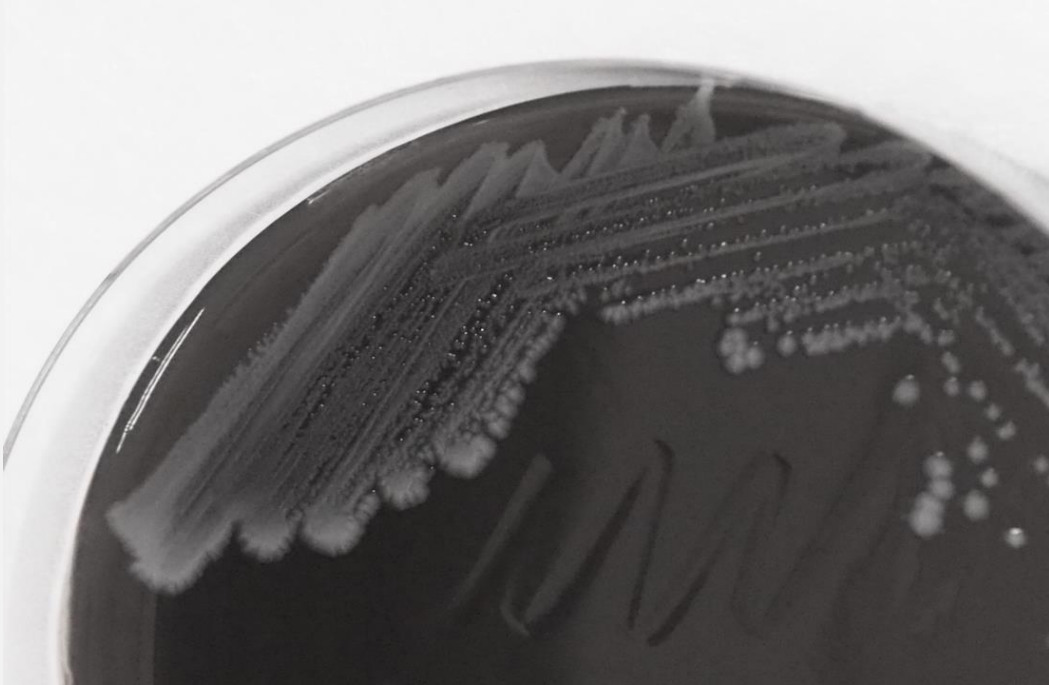
Typical colonies of *Campylobacter* on mCCDA

Campylobacter colonies on mCCDA



Sticky colonies with
pink and/or peach
in color when they
are scraped by a
loop

E. coli colonies on mCCDA



Non-sticky colonies with white/creamy in color when they are scraped by a loop

Campylobacter colonies on blood agar



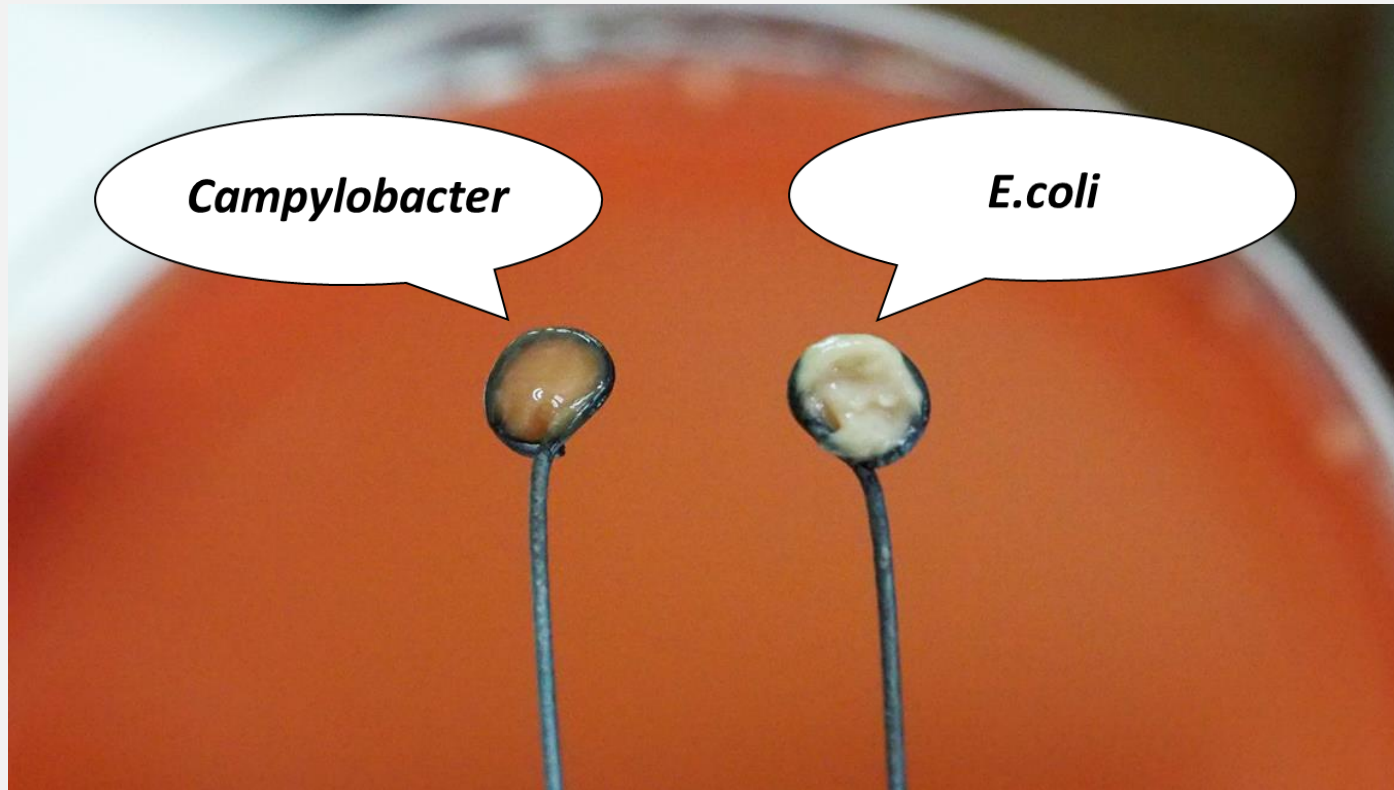
Campylobacter colonies on Preston agar



<https://microbiologyinpictures.com/bacteria-photos/campylobacter-jejuni-photos/butzler-agar.html>

Typical colonies of *Campylobacter* on blood-based agar

Campylobacter vs. *E. coli* on Preston agar



Brown to orange in color for *Campylobacter* vs. white/creamy in color for *E. coli*

Tips for *Campylobacter* isolation

- ❖ **To successfully isolate *Campylobacter* spp.**
 - Protection of samples from light, desiccation, and extreme temperature
 - Process samples as soon as possible (**same day**)
 - Refrigerate samples only when they cannot be processed on the same day
 - Leave samples at room temperature before processing
 - Recommended transport media: Amies, Cary-Blair, or Stuart



Tips for *Campylobacter* isolation

- ❖ **To successfully isolate *Campylobacter* spp. (cont.)**
 - **No enrichment** of caecal or fecal samples
 - Use gas pak or gas mix to generate microaerobic conditions (follow manufacturer's instructions)
 - Change gas pak or refill gas mix after open the jar
 - Include *Campylobacter* control strain subcultured on agar plate in the jar
 - Incubate samples at **42 °C** for **48** hrs.
 - Test pure colonies with different phenotypic morphologies



Thank you



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