Detection of Salmonella and Campylobacter in Food Samples (Eva Vlková)

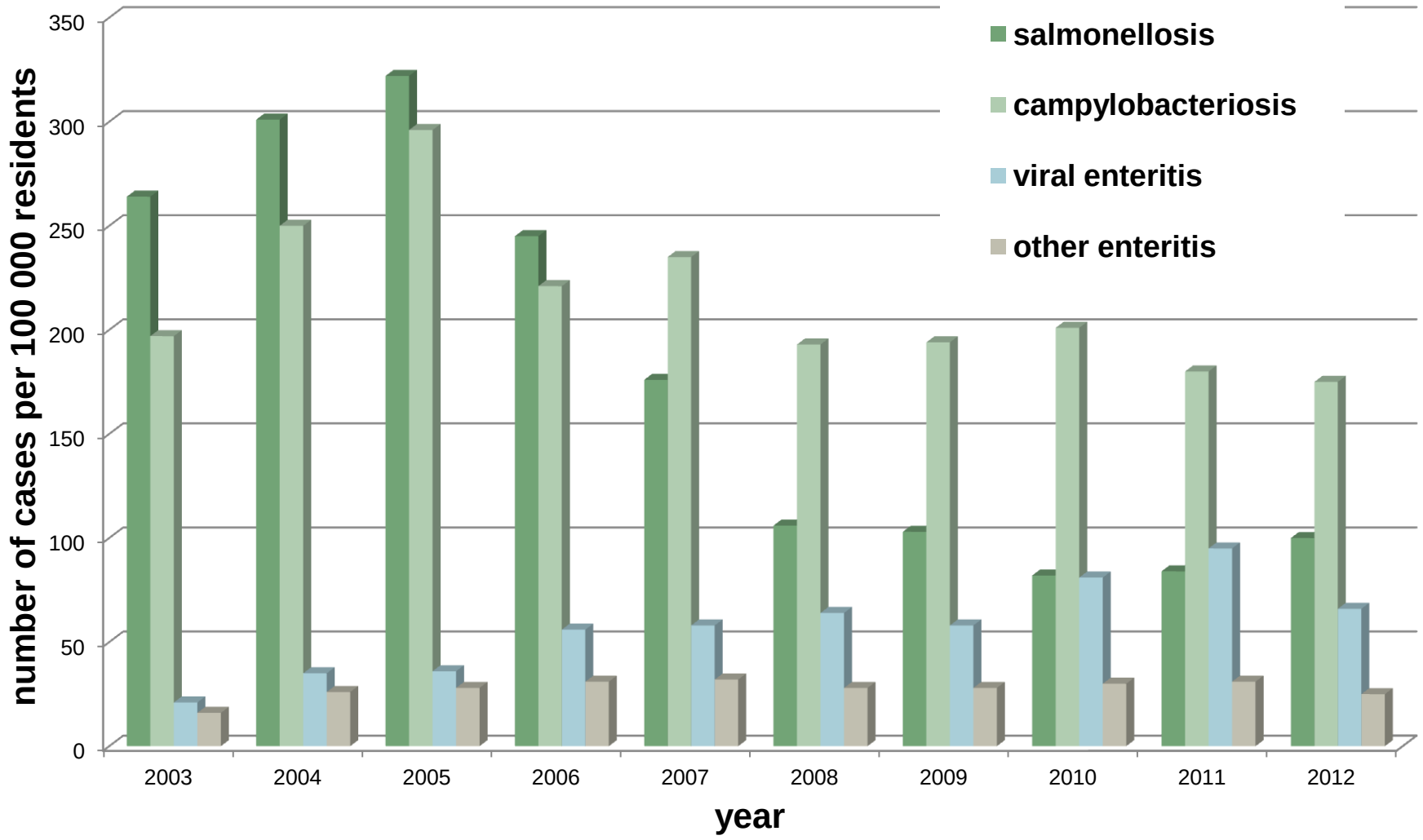
Foodborne Infections

- infections from contaminated food
- **primary contamination** – faecal-oral way (raw animal products)
- **secondary contamination** – arising during food processing
  - ✓ non-symptomatic workers
  - ✓ cross-contamination of ready to eat food with raw products (post heated contamination)
  - ✓ animal faeces as fertilizers

Most Frequent Foodborne Diseases (2002)

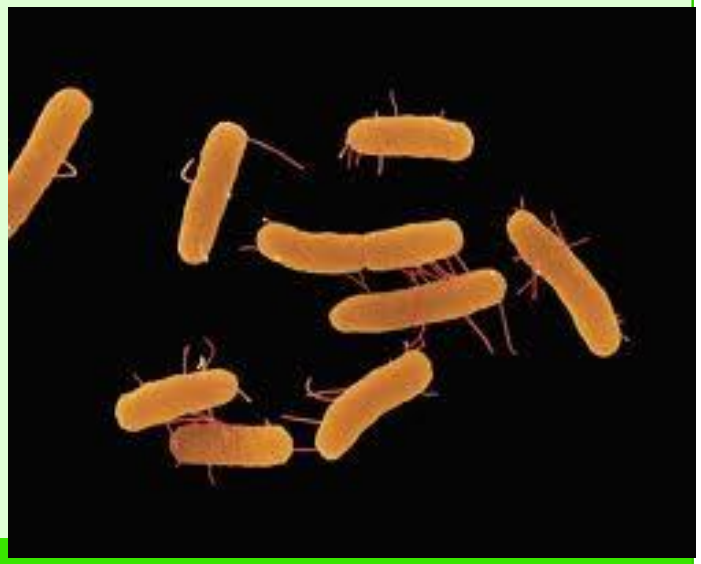
Bakteria	No. of cases
Salmonella	150 000
Campylobacter	150 000
Yersinia	10 147
Escherichia	2 664
Brucella	2 386
Listeria	860
Mycobacterium	49

Incidence of Human Enteritis (CR 2003–2012)



Characterization of Salmonellae

- Gram-negative
- non-sporing
- facultative anaerobes
- motile peritrichous rods
- diameters 0.7-1.5 μm, lengths 2-5 μm
- colonies 2-4 mm in diameter
- Enterobacteriaceae
- human and animal pathogen
- some are strictly host-adapted



Comparison with Related Genuses

Genus	Gas from glucose	Motility	H_2S production	Indole production	Citrat utilisation	% G+C
Escherichia	+	+	-	+	-	48-52
Salmonella	+	+	+	-	+	50-53
Shigella	-	-	-	-	-	49-53

- production of bacteriocins (5 % of strains) active against *E. coli*, *Shigella*, and/or other *Salmonella* strains; adsorb to the same receptor as that for colicin

Growth and Destruction of Salmonellae

- aerobic cultivation at 37°C for 24 h
- pH neutral, above 9 and below 4 bactericidal
- temperature: 5.3°C (6.2°C) - 45°C, 37°C optimum
- minimum a_w = 0.94 at neutral pH
- brine above 9% of salt is bactericidal
- nitrite is effective, more at the lower pH
- sensitive to pasteurization temperatures

$D_{70°C}$ values of *Salmonella* Enterica

Food Specimen	*Salmonella*
Chicken patties	0.32
Chicken tenders	0.32
Frankfurters	0.39
Beef patties	0.25
Beef-turkey patties	0.37

Taxonomy of Salmonellae

3 species

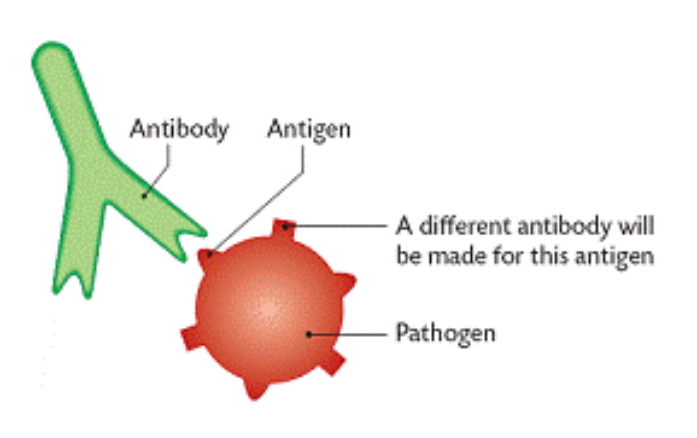
- *S. enterica, S. bongori, S. subterranea*
- more than 2 000 serovars
- *S. enterica* serovar Typhimurium or *Salmonella* Typhimurium or *S.* Typhimurium x ~~*S. typhimurium*~~

Division into Serovars

- immunological methods (agglutination tests, ELISA)

Antigens

- evoke production of antibodies
- O – somatic (composition and structure of the polysaccharides)
- Vi – capsular (when present; surface; may mask O antigens; deactivated by 100°C)
- H – flagellar



Pathogenicity

- **auxotrophic** (strictly adapted to one particular host) x ubiquitous (large number of hosts) serovars
- human adapted: Typhi, Paratyphi A, Sendai; non-pathogenic for other animal; transmission from person to person, faecal contamination of water or food
- Abortusovis – sheep, abortions
- Typhisuis – swine
- Gallinarum – poultry
- **ubiquitous** – Typhimurium, Enteritidis

Salmonella Sources

- animal (human - secondary) faecal matter
- maintained within an animal population by nonsymptomatic animals and in animal feeds
- wild animals – rats, births

S. Enteritidis – egg and poultry products

- more outbreaks in summer suggesting growth of *Salmonella* on eggs and poultry products

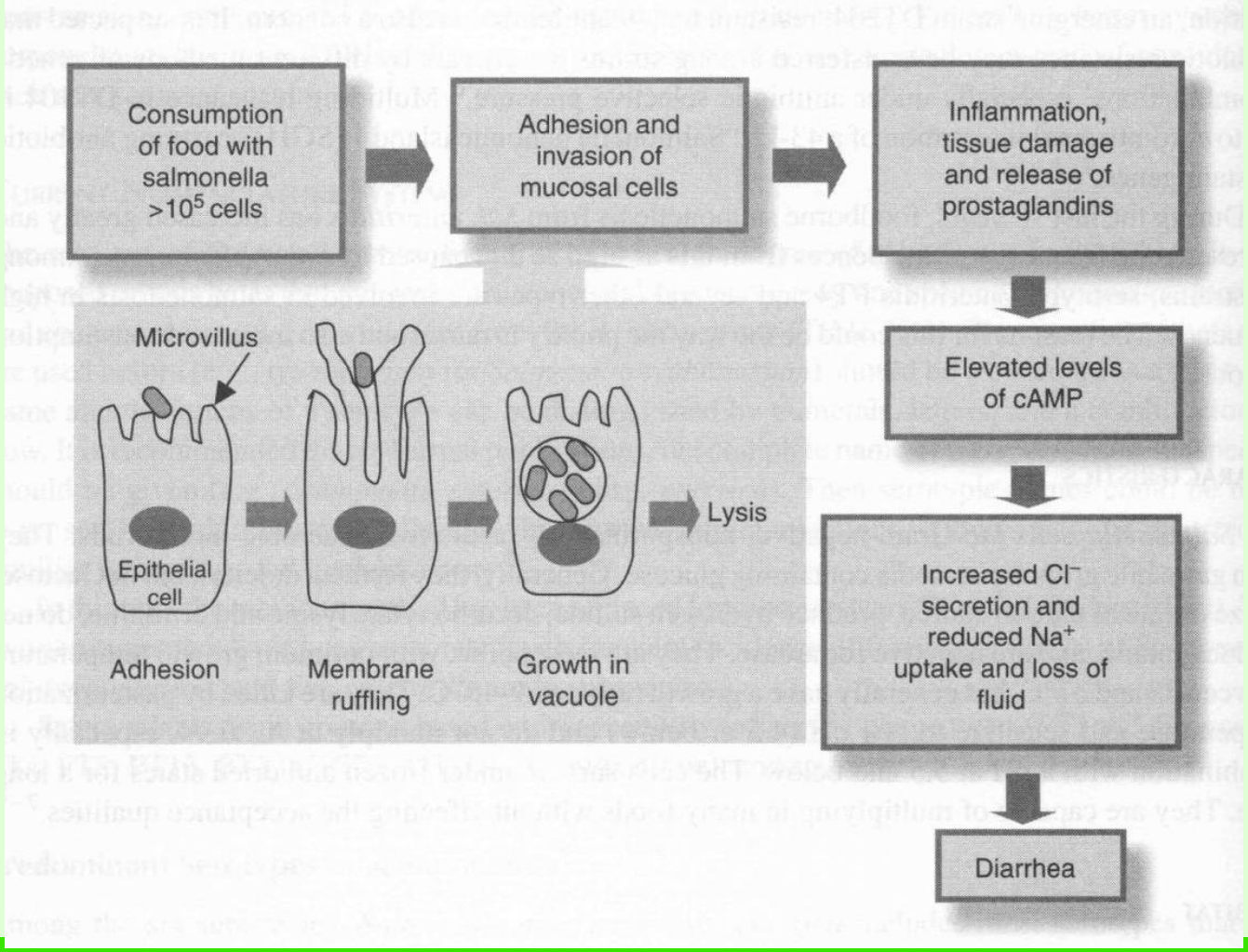
Detection of Food Spoilage

- Microorganisms must multiply and attain certain level of viable cell per g, ml or cm^2
- 10^5 : some bacterial exotoxins can be detected
- 10^{6-7}: changes in odor and color
- 10^{8-9}: changes in texture, slime formation

The Salmonella Food-Poisoning Syndrome

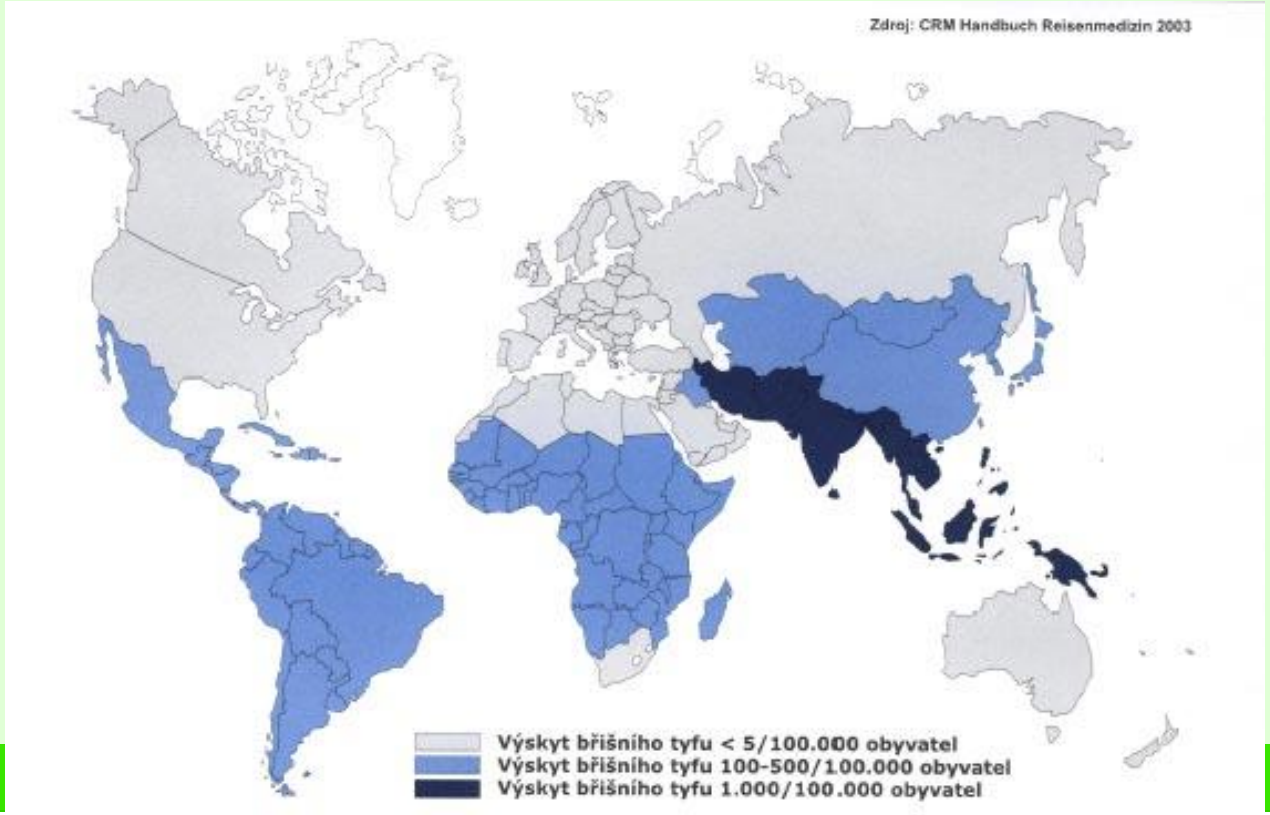
- dose 10^5-10^9 of cells
- *S.* Enteritidis, *S.* Typhimurium
- symptoms develop in 12-14 hours, persist for 2-3 days
- diarrhea, nausea, vomiting, abdominal pain, headache, chills, moderate fever, muscular weakness
- 5% of patients may become carriers

Salmonella Pathogenesis (Ray and Bhunia, 2008)



Septicemia-Typhoidic Syndrome

- human adapted *Salmonella* (typhoid fever – *S.* Typhi)
- developing countries with poor hygiene
- 16 million cases per year worldwide (600 000 deaths)



General Prevention

- proper cooking of foods (minimum pasteurization, such as 71.1°C for 15 s)
- prompt cooling (to 3-4°C or freezing, if not used in 2 h)
- properly reheating
- prevention of cross-contamination of ready-to-eat food with raw food through equipments and hands
- preventing post heat contamination
- proper sanitation
- preventing consumption of animal origin raw foods
- good personal hygiene
- not allowing sick individual to handle foods

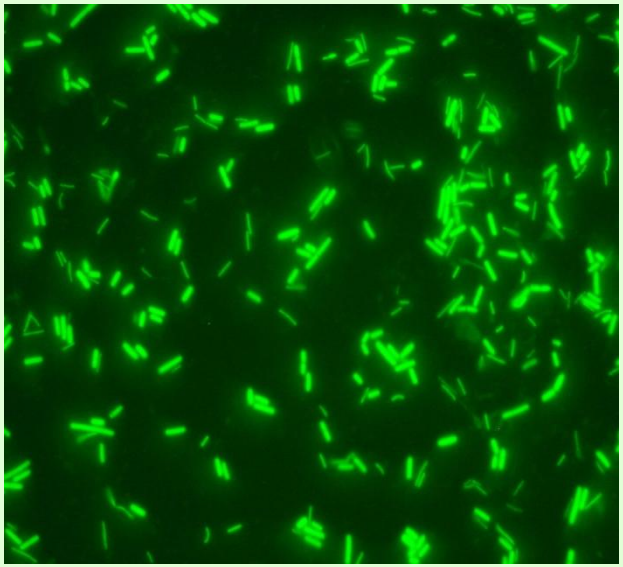
LABORATORY SAFETY

- **Do not drink, eat and smoke**
- **Protective clothing**
- **Aseptic technique**
- **Bacteriological loop, needle**
- **Bunsen burner**
- **Bacteriological stains**

Enumeration (Detection) of Bacteria

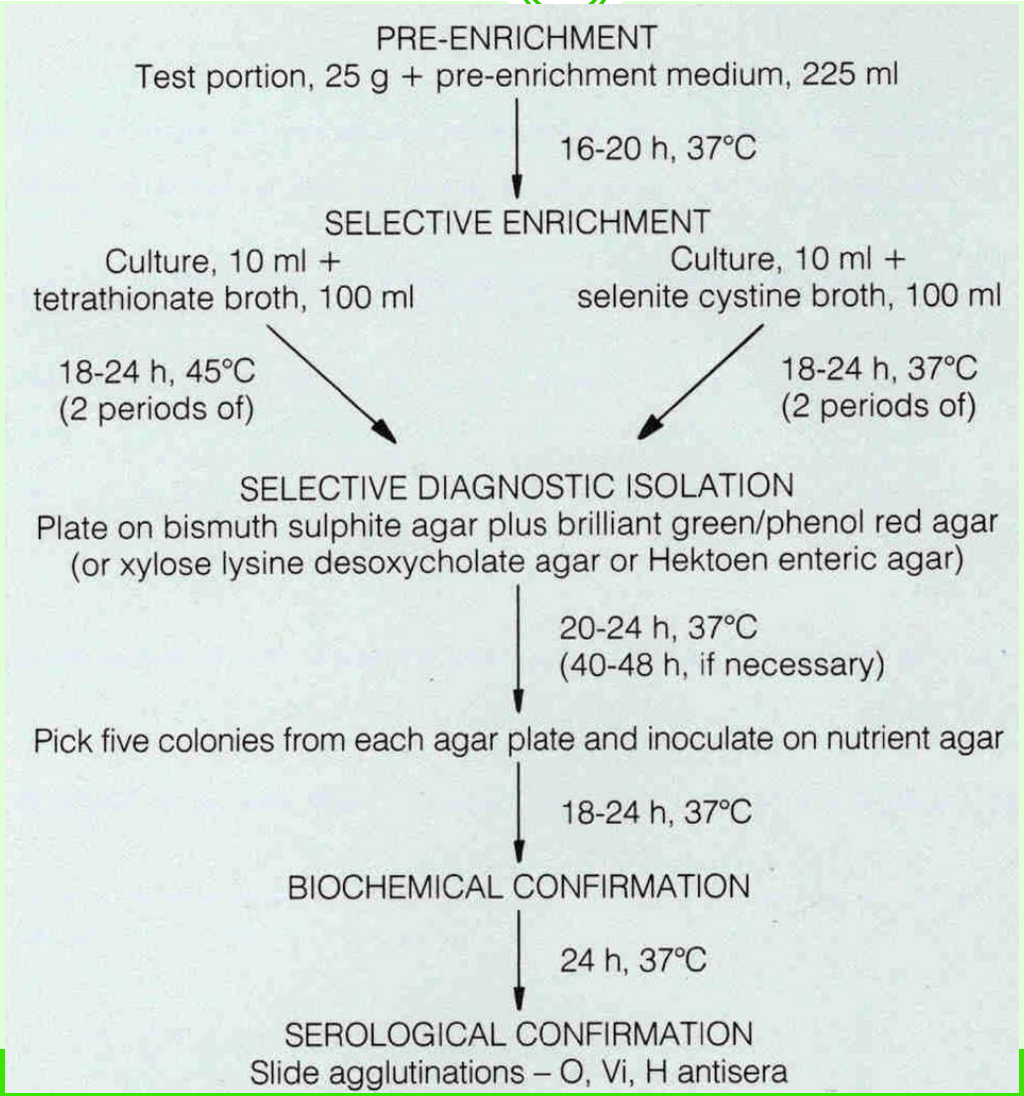
Cultivation methods

Fluoprescence in situ hybridization (FISH)



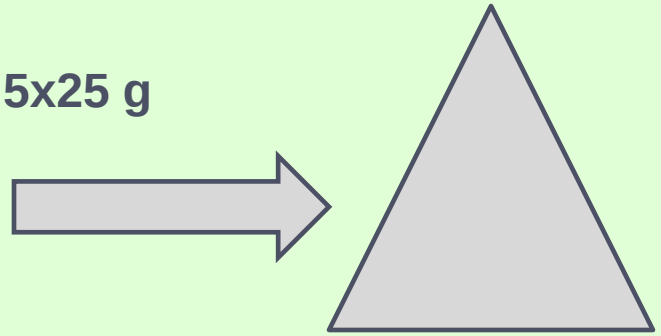
PCR methods

BSI/ISO *Salmonella* Isolation Procedure



Microbiological Evaluation of Minced Meat – Pre-enrichment

5x25 g



50 ml peptone water,
37°C/24h

Microbiological Evaluation of Minced Meat – Selective Enrichment

5x25 g

50 ml peptone water, 37°C/24h

0.1 ml

50 ml Rappaport-Vassiliadis broth, 37°C/48h

Microbiological Evaluation of Minced Meat – Selective Diagnostic Isolation

5x25 g

50 ml peptone water, 37°C/24h

0.1 ml

50 ml Rappaport-Vassiliadis broth, 37°C/48h

loopful

SS agar

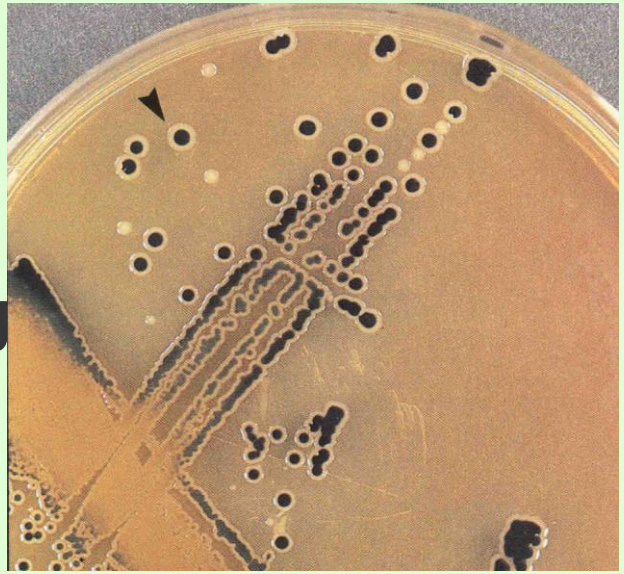
37°C/24h

Salmonella

Shigella

Salmonella Shigella (SS) Agar (Oxoid)

- **Differential, selective – *Shigella* and *Salmonella***
- **Selective factors: brilliant green, bile salts, thiosulphate, citrate**
- **Thiosulphate + iron (ferric citrate) – Indication of hydrogen sulphide production (blackening in the centres of the colonies)**



Chromogenic media

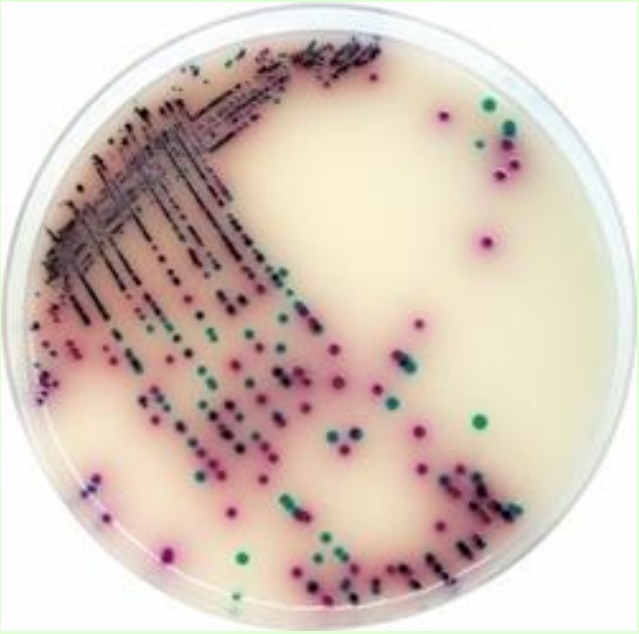
- simple and fast detection of bacteria using chromogenic substrates
- Salmon-GAL, X-Gal, X-glucuronide, etc.
- certain enzymes, produced by some bacteria, cleave these substrates, resulting in the different coloration of certain bacteria colonies
- combination with other selective factors
- other confirmation test are not necessary

TBX agar (Oxoid)

- *E. coli*
- Tryptone Bile Agar + X-Glucuronide
- β-glucuronidase

HiCrome Salmonella Agar (Sigma)

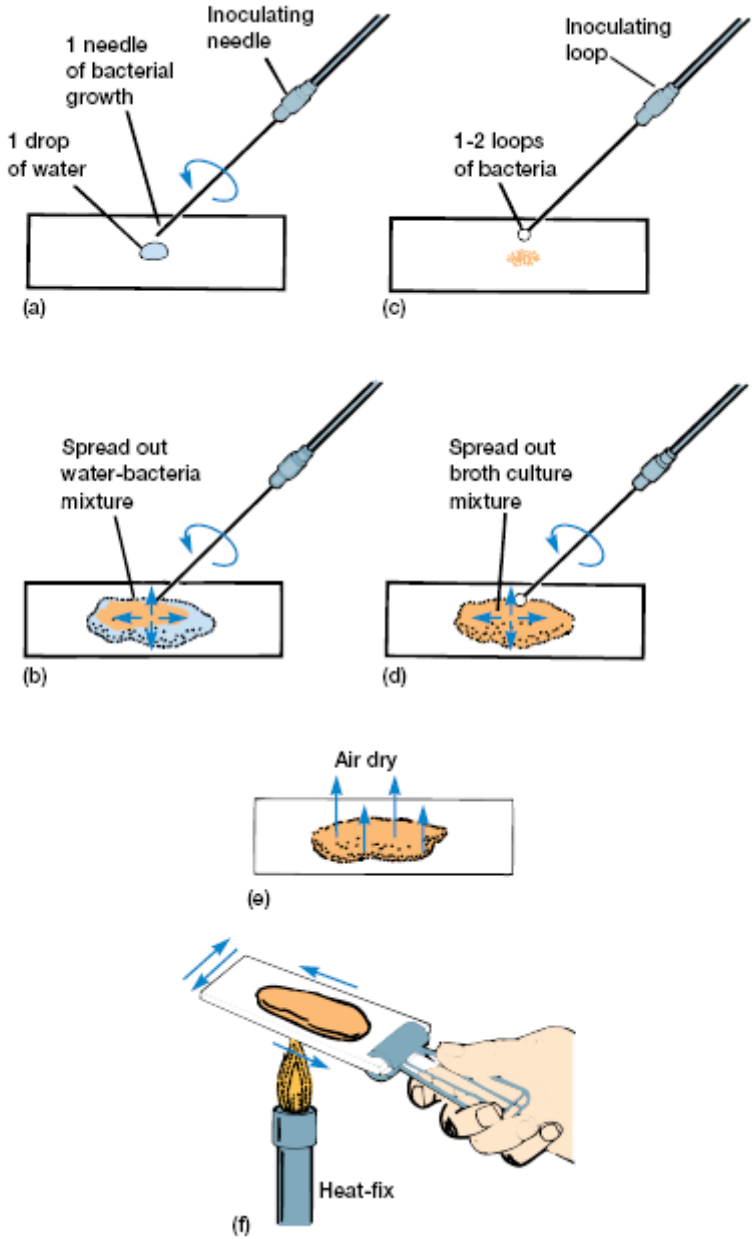
- simultaneous detection of *Escherichia coli* and *Salmonella* from food and water
- *Salmonella* give light purple colonies a halo (indoxyl-α-galactoside, indoxyl-fatty acid ester)
- *Escherichia coli* has a characteristic blue color (indoxyl-β-D-glucuronide)
- other organisms give colorless colonies



Smear preparation

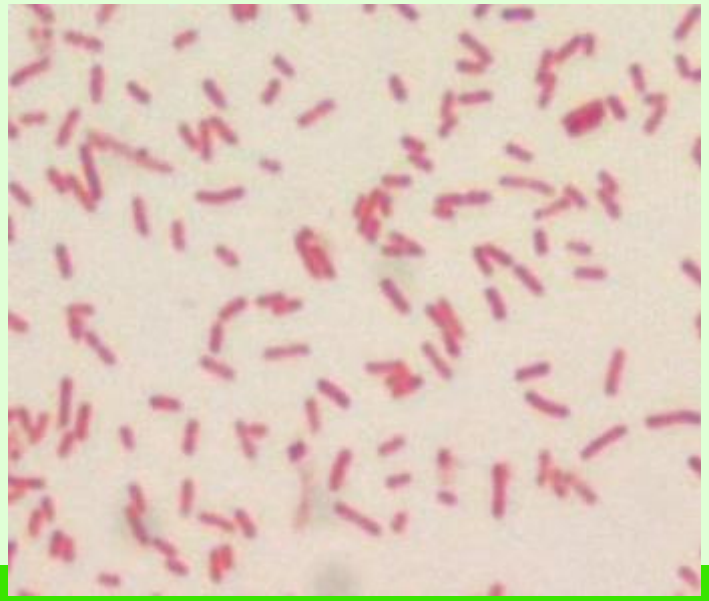
- A drop of water is placed in the centre of a slide
- One loopfuls of organisms is transferred to the centre of slide
- Spread the organisms over the slide
- The smear is allowed to dry
- Slide is passed through flame several times to heat-kill and fix organisms

Bacterial smear preparation



Gram Staining

- Smear preparation
- Stain with crystal violet 1 min
- Add Lugol solution 1 min
- Decolorize with alcohol 10-15 s
- Wash with water
- Stain with fuchsin 2 min
- Allow the slide to air-dry
- Examine with an oil immersion objective



Biochemical Confirmation – SALMtest (Lachema)

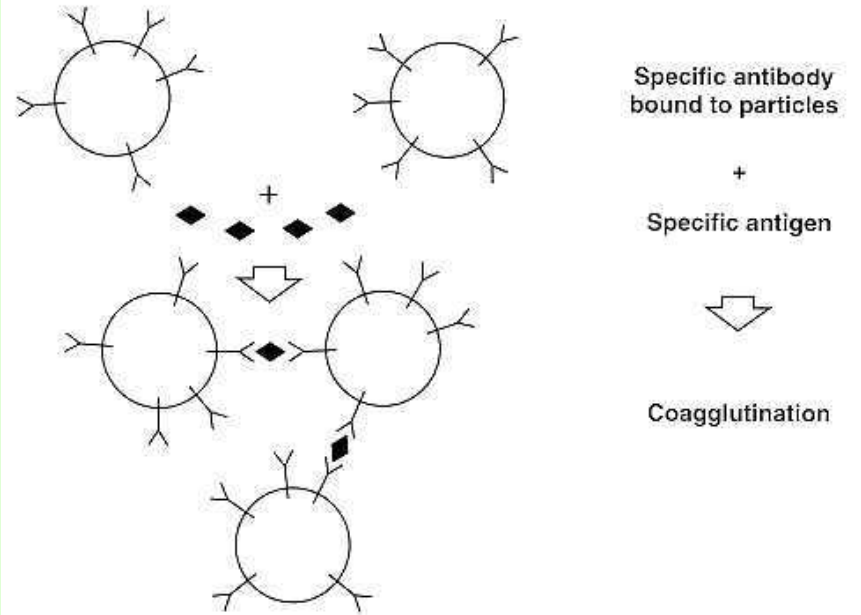
- Detection of activity of C8-esterase
- C8-esterase hydrolyzes 4-methylumbelliferyl caprylate
- 4-methylumbelliferone gives a blue fluorescence under UV light

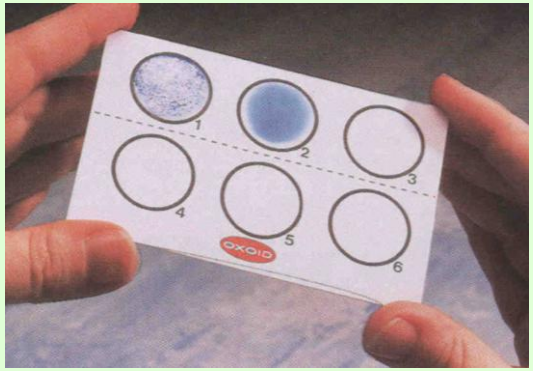
Procedure:

- Prepare suspension of bacterial culture grown on Soya-Peptone agar in saline
- Place the strip zone of SALMtest into the suspension
- Incubate for 4 hours at the room temperature
- Read the result under a UV-lamp (360 nm)

Biochemical Confirmation – Latex test (Oxoid)

- **Agglutination test**
- **Flagella antigens + Specific antibodies on latex particles**



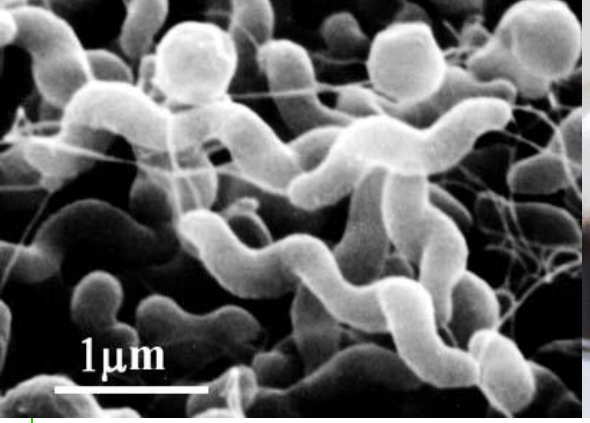


Latex test (Oxoid) - Procedure

- **Cultivation of colonies from the selective agar on non-selective plate (Soya-Peptone Agar)**
- **Drop of latex onto the test circle**
- **Mix bacteria with latex (continue mixing for 10-15 seconds) – *S. enterica* Enteritidis ATCC 13076, *Salmonella* sp. isolated from minced meat**
- **After 2 minutes observe for agglutination**

positive

negative




Campylobacter spp.

- Entrobacteria
- *C. jejuni* and *C. coli* – the most common causers of human diarrheal disease

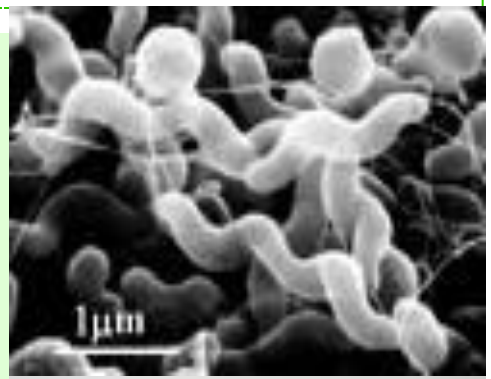
Characterization of Campylobacter

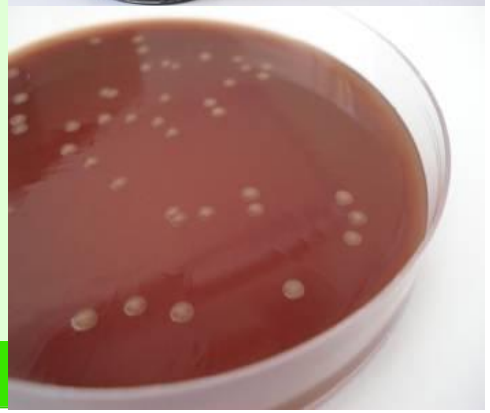
- Gram-negative
- non-sporing
- microaerophilic to anaerobic
- oxidase-positive
- spirally curved motile rods (single polar flagellum at one or both ends)
- diameters 0.2-0.6 μm, lengths 2-6 μm

Occurrence & Distribution

- Campylobacters can colonise mucosal surfaces, usually the intestinal tract of most mammalian and avian species

- The most frequently isolated species: ***C. jejuni*** & ***C. coli***

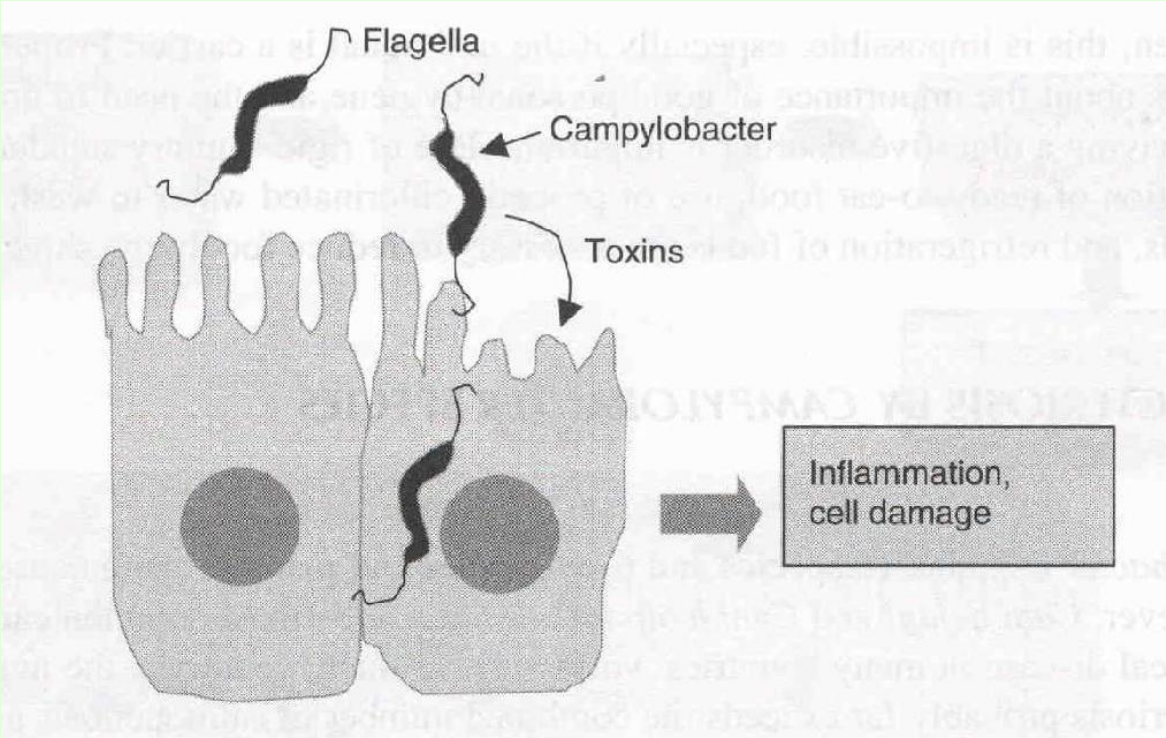




Campylobacter in Food

- contaminated with faecal material from infected individuals or sewage and water
- **raw milk**, dairy products, bakery products
- **improperly cooked chicken**, turkey products
- Chinese food, eggs
- cross-contamination in heat-processed food

Campylobacter jejuni pathogenesis (Ray and Bhunia, 2008)



Campylobacteriosis - Disease and Symptoms

- infective dose **500 cells**
- **toxins** (cytolethal distending toxins – CDT, hemolysin, phospholipase) ⟶ enteric disease symptoms (cell damage, inflammation)
- diarrhea appears in 2-5 days
- abdominal cramps, nausea, vomiting, fever, headache
- symptoms linger for 2 weeks

chronic diseases:

- Guillain-Barre syndrome – paralysis
- Reiter‘s syndrome – arthritis

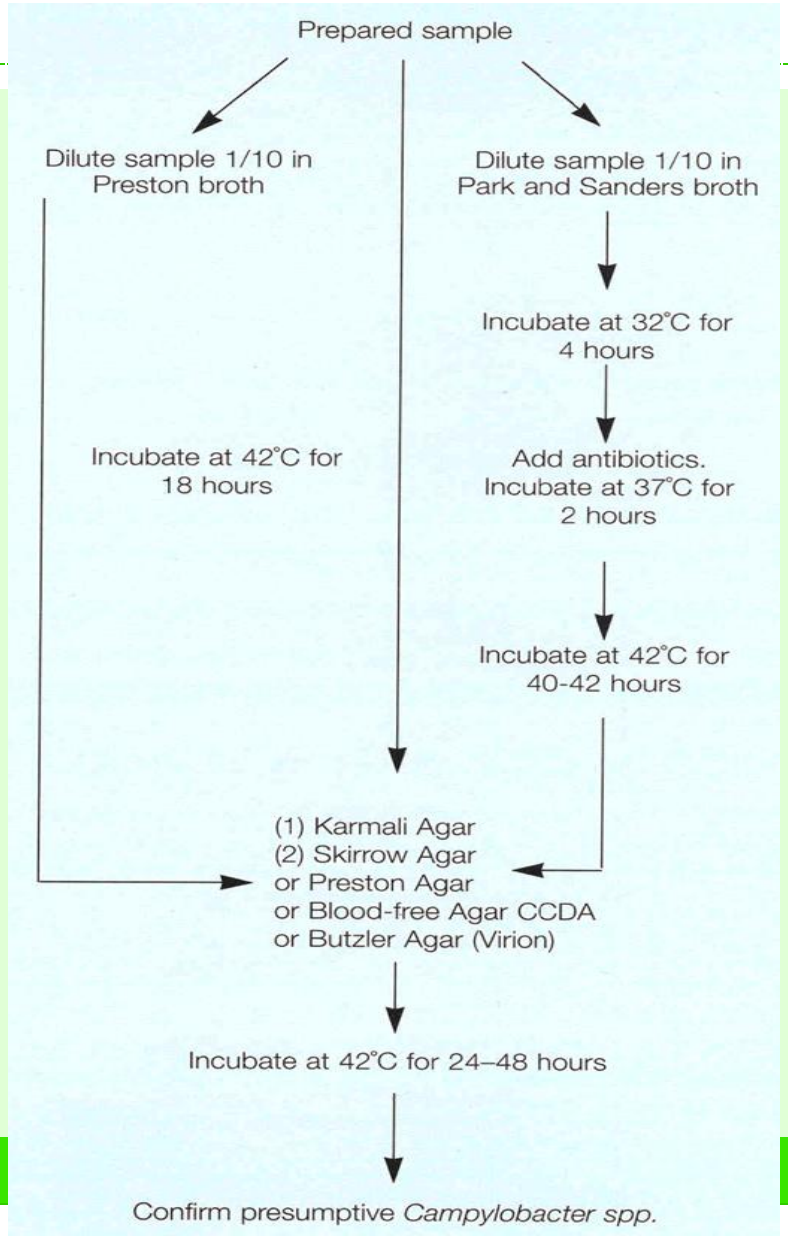
Prevention

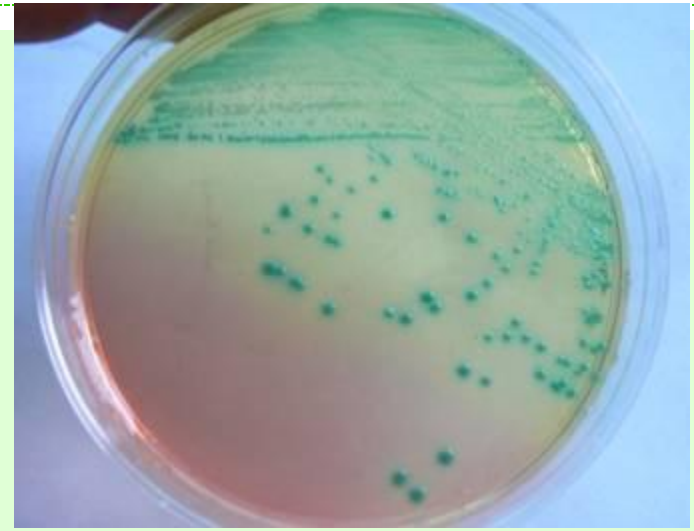
- proper sanitation during handling with raw foods
- preventing consumption of animal origin raw foods
- heat treatment
- preventing post heat contamination
- not using animal faeces as fertilizer of vegetables
- good personal hygiene
- not allowing sick individual to handle foods

Methods of detection: food and water

- Two ISO procedures:
  - 1. Horizontal method for detection of thermotolerant *Campylobacter* in food and animal feeding stuffs (ISO 10272-1:2006)
  - 2. Procedure for the isolation of *Campylobacter* from water (ISO 17995:2005)

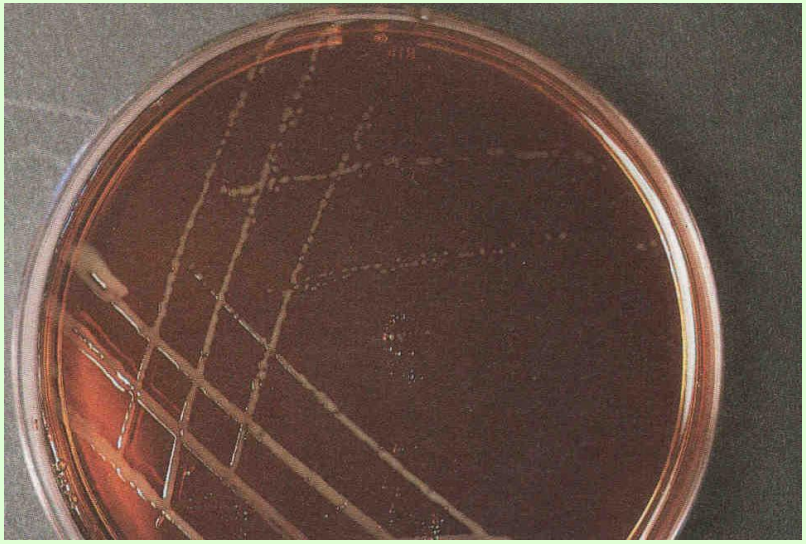
Detection of Thermotolerant Campylobacter (ISO/DIS 10272)





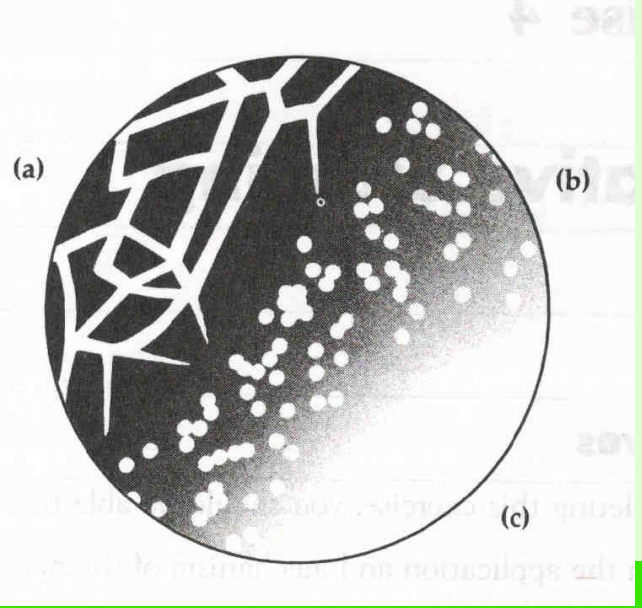
Campylobacter Agar (Preston; Oxoid)

- **Formula: Lab-Lemco powder, pepton, sodium chloride, agar**
- **Selective supplement: Polymyxin B, Rifampicin, Trimethoprim, Cycloheximede**
- **Horse blood**



Negative Staining

- **(Background staining)**
- **This method consist of mixing the microorganisms in a small amount of nigrosine and spreading the mixture over the surface of a slide.**



Negative Staining

- **Drops of water and nigrosine are placed in the centre of a microscopic slide.**
- **Place bacteria into the water with sterilised loopful.**
- **Spread the mixture of water, nigrosine and sample over the slide.**
- **Allow the slide to air-dry and examine with an oil immersion objective**

Campylobacter jejuni CCM 6189

Campylobacter jejuni CCM 6189