

FWD-AMR-RefLabCap 1st Training Course

Isolation and phenotypic identification of *Campylobacter* incl. conventional hippurate hydrolysis and indoxyl acetate tests



FWD AMR-
RefLabCap

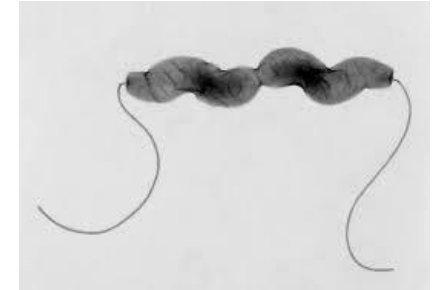
HaDEA Service Contract 20197409
Provision of EU networking and support
for public health reference laboratory
functions for antimicrobial resistance in
Salmonella species and *Campylobacter*
species in human samples

Tuesday, 19 May 2022
10:45 -11:30 CET at DTU Food



Campylobacter

- Gram negative
- Motile, thin rod, spiral shape and flagellated
- Strict microaerophilic
- Sensitive to heat, desiccation and environmental conditions
- Potentially all animal species are asymptomatic carrier of Campylobacter (poultry, swine, etc)
- 24 species but most important:
 - *C. jejuni* (92% of gastro-intestinal infections)
 - *C. coli* (5% of gastro-intestinal infections)
 - *C. lari*
 - *C. upsaliensis*
 - *C. fetus* (blood cultures and abortion- systemic)



Campylobacteriosis in humans

- Campylobacter jejuni/coli is the most common cause of bacterial intestinal disease in Europe
 - Estimated at 10 million cases per year in EU (costs: 2.4 billion)
- Largest part of the world has no data
- Difference in epidemiology between developing and industrialized countries
- Outbreaks are rare compared to Salmonella
- Even low doses has an high probability of infection
- Acute often bloody diarrhoea, with intense abdominal pain
- A few days to 1 week and usually self-limiting
- Serious outcome
 - 35-45 cases per 100,000 (EU); 3.5-4.0% hospitalized; 0.2% bacteraemia; 0.15-0.30% fatal
 - Contributing heavily to the burden of illness
 - Sequelae
 - Guillain Barré Syndrome, Irritable Bowel Syndrome, Reactive Arthritis

Detection of *Campylobacter*

- Clinical stool samples: direct plating
- Blood samples: enrichment in blood culture media
- Meat/carcass samples (food): enrichment

Trouble shooting

- For stools: has the patient been treated?
- Quality of your sample (Avoid to dry; use transport media)
- Overgrowth of contaminants
 - Increasingly a problem with ESBL producing Gram-negative bacteria
 - Is your agar of good quality?
- Experience

Culture Media Supplements

Defined substrates

- Ferrous sulphate
- Sodium meta-bi-sulphite
- Sodium pyruvate
- Haematin
- Sodium thioglycollate
- α - ketoglutamic acid

Complex substrates

- Blood
- Serum
- Charcoal

Antimicrobial Agents

Inhibitory to Gram negative organisms

- Cephalosporins*
- Trimethoprim
- Polmyxin B/Colistin
- Novobiocin

Inhibitory to Gram positive organisms

- Vancomycin/Teicoplanin
- Bacitracin
- Rifampicin*
- Sodium deoxycholate

* Activity against both Gram negative and positive organisms

Inhibitory to fungi and yeasts

- Cyclohexamide
- Amphotericin

Campylobacter Selective Agars and Methods

- Blaser - Wang agar
- Butzler agar (modified)
- Campy Cefex agar
- **Charcoal cefoperazone deoxycholate agar (CCDA)**
- Charcoal - Amphotericin - Teicoplanin agar
- Karmali agar
- Preston agar
- Skirrow agar

Campylobacter Enrichment Broths

- **Preston -1982** ISO/BS, FDA, PHLS,
- **Doyle and Roman -1982** FDA
- **Exeter -1986** FDA, PHLS
- **Park and Sanders -1989** ISO/BS, FDA

Temperature of Incubation



- 43°C
- **42°C**
- 41.5°C
- 37°C
- 37°C > 42°C
- 32°C > 37°C > 42°C

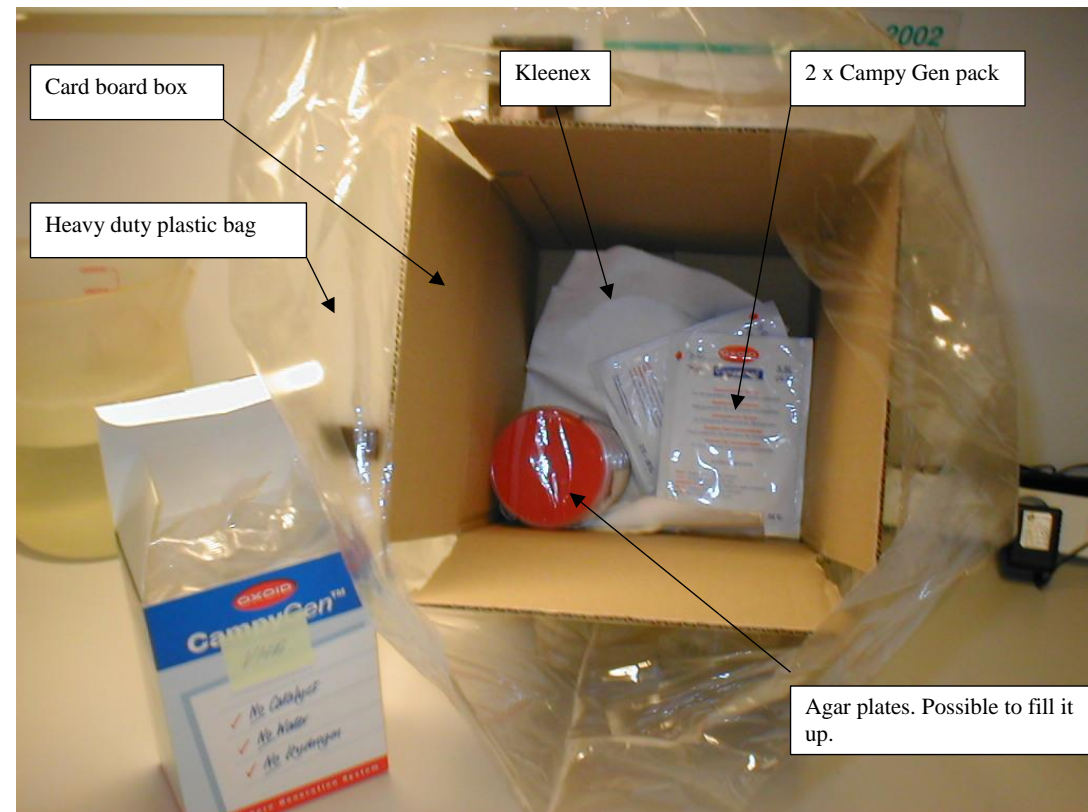
Microaerobic Requirements of Campylobacters

- **5-10% oxygen (Bolton & Coates 1983)**
- **1-10% carbon dioxide (Bolton & Coates 1983)**
- 1% oxygen (Henderson *et al.* 2000)
- 5-10% carbon dioxide (Henderson *et al.* 2000)
- 5 -9% hydrogen (Henderson *et al.* 2000)

Methods of Producing Microaerobic

- Candle jars
- Gas generating kits*
- Evacuation replacement method
- Variable Atmosphere Incubator

In the case of lack of space for incubation of Campylobacter. Please use this primitive container and place it in the 42°C incubator. It works fine if closed securely with tape.



Factors Affecting Isolation of Campylobacters

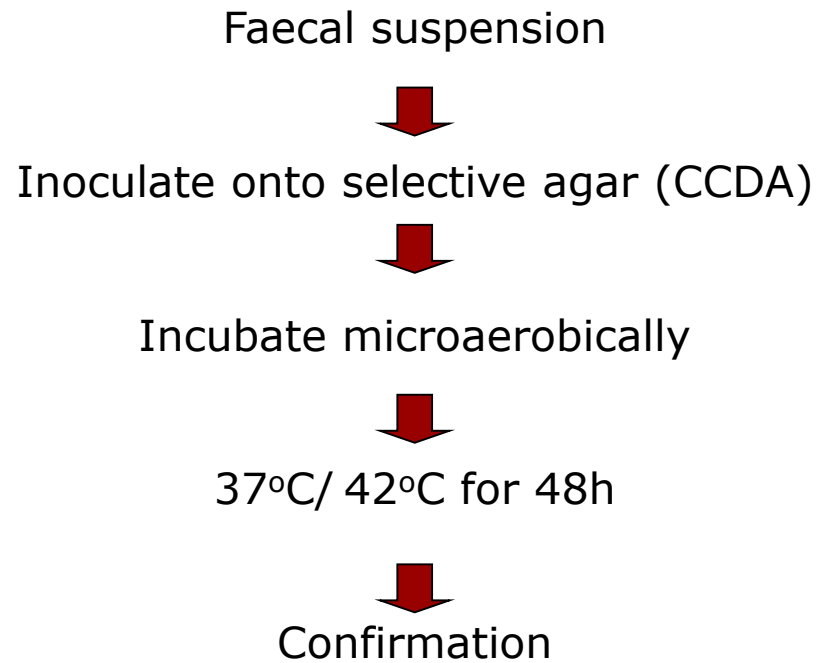
- Type of sample to be cultured
- Number and type of competing organisms
- Number of Campylobacters present
- Physiological status of campylobacters
- Culture media and isolation protocol

Isolation of *Campylobacters* from Clinical Specimens

- Faecal samples
- Blood cultures and other clinical specimens



Isolation of Campylobacters from Faecal specimens



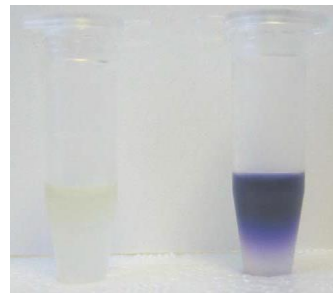
Effect of the Incubation Temperature on the Isolation of Campylobacters

- 1286 faecal specimens cultured onto mCCD agar incubated microaerobically at 37°C and 42°C for 48h
- 37°C - 173 (98%) positive
- 42°C - 152 (86%) positive
- Isolates recovered only at 37°C included: 18 *C. jejuni* and 4 *C.coli*

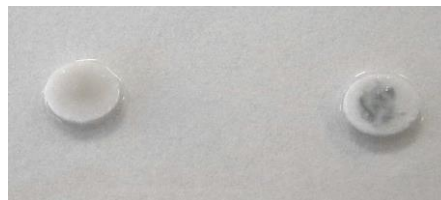
Bolton *et al.* 1988

Campylobacter identification

- Morphology: motility and Gram staining
- Oxidase
- Lactose
- Sucrose
- Gas
- Catalase
- Hippurate hydrolysis
- Hydrolysis of indoxyl acetate



Hippurate hydrolysis



Indoxyl acetate hydrolysis

	jejuni	coli	lari
Hippurate hydrolysis	+	-	-
Indoxyl acetate hydrolysis	+	+	-

Acknowledgement

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for sharing some slides

Thank you for your attention

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