CEN/TC 463/WG3 CAMPYLOBACTER INTERLABORATORY STUDY – PCR FOR CONFIRMATION AND/OR IDENTIFICATION OF CAMPYLOBACTER

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METHODS FOR MOLECULAR CONFIRMATION AND IDENTIFICATION OF THERMOTOLERANT *CAMPYLOBACTER* SPP.

- **Confirmation of thermotolerant *Campylobacter***:

- Josefsen et al., 2004 (2010) and Pacholewicz et al. 2019 (real-time PCR)

  -Targets *C. jejuni*, *C. coli* and *C. lari*

- **Identification of thermotolerant *Campylobacter*:**

- Wang et al. 2002 (conventional PCR) – but lari primers changed to Chaban 2009 (targets both subspecies).

  - Targets *C. jejuni*, *C. coli* and *C. lari* and *C. upsaliensis (also C. fetus)*

- Mayr et al., 2010 (real-time PCR)

  - Targets *C. jejuni*, *C. coli* and *C. lari*

METHODOLOGY – METHOD COMPARISON STUDY

Based on the protocol in ISO 16140-6
Work devided between EURL-Campylobacter and LGL (Ute Messelhäuser)

PCR system	Scope	Reference method
Josefsen PCR	Confirmation of thermotolerant *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari*)	Table 1
Wang PCR	Species id of *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*	Table 1 and 2
Mayr PCR	Species id of *C. jejuni*, *C. coli*, *C. lari*	Table 1 and 2

Table 1 — Characteristics of *Campylobacter*

Morphology (9.5.3)	Small curved bacilli[a]
Motility (9.5.3)	Characteristic corkscrew darting[a]
Aerobic growth at 25 °C (9.5.4)	–
Oxidase activity (9.5.5)	+

\+ Positive.
– Negative.
[a] Older cultures may rapidly lose their characteristic shape and motility and turn into less motile coccoid forms.

C. upsaliensis: 2/24 weak catalase (rest neg)
2/34 positive for Indoxyl acetate
Reference method NOT good for *C. upsaliensis*

Table 2 — Characteristics of *Campylobacter* species

Characteristic	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Catalase activity (9.6.2)	+	+	+	– or weak
Hippurate hydrolysis (9.6.3)	+[a]	–	–	–
Indoxyl acetate hydrolysis (9.6.4)	+	+	–	+

\+ Positive.
– Negative.
[a] Some hippurate-negative *C. jejuni* strains have been reported.


THANK
YOU!

STRAIN COLLECTION - SOURCE

Source	C. jejuni	C. coli	C. lari	C. upsaliensis	Other Campy	Non-Campy
Food	57	70	24	0	2	29
Primary production	32	25	10	1	22	20
Environmental (wild animals, water)	6	10	21	2	2	11
Other (pets, humans)	1	0	1	30	7	4
Not specified	8	1	1	1	0	12
Total	104	106	57	34	33	76
Reference strains	1	1	2	1	12	16

STRAIN COLLECTION – GEOGRAPHICAL ORIGIN

Origin	C. jejuni	C. coli	C. lari	C. upsaliensis	Other Campy	Non-Campy
European countries (no strains/no countries)	88/20	102/17	57/6	34/4	28/6	69/8
North America (no strains)	11	2	0	0	5	7
Asia (no strains/no countries)	2/1	2/1	0	0		
Oceania (no strains/no countries)	2/2	0	0	0		
Antarctic	2	0	0	0		

QUESTION TO WORKSHOP PARTICIPANTS FROM CEN/TC 463/WG 3 CAMPYLOBACTER

- Since *C. upsaliensis* strains are not commonly found in food nor in samples from primary production

AND

- Since biochemical tests in ISO 10272 are not efficient to identify the species

- Suggest to remove *C. upsaliensis* from table 2 (characteristics of *Campylobacter* species) of the standard
- Objections?

Table 2 — Characteristics of *Campylobacter* species

Characteristic	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Catalase activity (9.6.2)	+	+	+	− or weak
Hippurate hydrolysis (9.6.3)	+[a]	−	−	−
Indoxyl acetate hydrolysis (9.6.4)	+	+	−	+

\+ Positive.

− Negative.

[a] Some hippurate-negative *C. jejuni* strains have been reported.

RESULTS JOSEFSEN PCR

Evaluation of the method comparison study results Josefsen PCR

	Number of strains	ID ≤ AL	ED ≤ AL	Evaluation
Inclusivity	265	0 ≤ 1	Not applicable	Accepted
Exclusivity	139	Not applicable	0 ≤ 1	Accepted

Comments:

- False positive for 1 *C. upsaliensis* and 1 *C. peloridis*. But the reference confirmation method cannot distinguish between *C. jejuni*, *C. coli* and *C. lari* and other *Campylobacter* growing at 41.5 ◦C.

RESULTS WANG PCR

Evaluation of the method comparison study results Wang PCR

Target		Number of strains	ID ≤ AL	ED ≤ AL	Evaluation
C. jejuni	Inclusivity	104	2 ≤ 2	NA	Accepted
	Exclusivity	301	NA	0 ≤ 2	Accepted
C. coli	Inclusivity	105	5 ≥ 2	NA	Not accepted
	Exclusivity	300	NA	0 ≤ 2	Accepted
C. lari	Inclusivity	56	0 ≤ 2	NA	Accepted
	Exclusivity	349	NA	0 ≤ 2	Accepted
C. upsaliensis	Inclusivity	31	2 ≤ 2	NA	Accepted
	Exclusivity	374	NA	0 ≤ 2	Accepted

Comments:

- Fewer than 100 strains analysed of C. lari and C. upsaliensis
- Wang PCR for *C. upsaliensis* only approved since reference method gave very poor results (21/31 gave positive result in Wang PCR)
- Inclusivity C. coli: more than 100 strains analysed – 5 that did not give expected result for the Wang PCR when compared to ref. method. Some of those were environmental strains, but still some from poultry.

RESULTS MAYR PCR

Evaluation of the method comparison study results Wang PCR

Target		Number of strains	ID ≤ AL	ED ≤ AL	Evaluation
C. jejuni	Inclusivity	105	0 ≤ 2	NA	Accepted
	Exclusivity	301	NA	8 ≥ 2	Not accepted
C. coli	Inclusivity	105	2 ≤ 2	NA	Accepted
	Exclusivity	300	NA	0 ≤ 2	Accepted
C. lari	Inclusivity	56	1 ≤ 2	NA	Accepted
	Exclusivity	349	NA	0 ≤ 2	Accepted

Comments:

- 4 *C. coli* strains gave signal for *C. jejuni* (3 of those for both *C. coli* and *C. jejuni*) = mix/hybrids
- 4 lari gave signal for *C. jejuni* (3 of those also for *C. lari*) – mix/hybrids? Will be sequenced
- Fewer than 100 strians analysed of *C. lari*

ORGANISATION OF THE INTERLABORATORY STUDY (ILS)

- Organised by the EURL-*Campylobacter*

- Performed in December 2020

(because DIS draft needs to be provided to CEN secretariat before March 2021)

- Send off tests Dec 1

- Last date to report results Dec 22

OUTLINE OF THE ILS

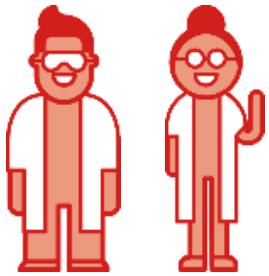
- Will be a test protocol to follow
- All strains needs to be tested for growth on mCCD agar at 41.5 ◦C and analysed with reference method (biochemical tests as described in ISO 10272:2017) and with the PCR methods

— **No of strains to analyse per participant and per method:**

Genus level confirmation method: a total of 16 different target strains (jejuni/coli/lari) and 8 non-target strains = 24 strains

Species level identification methods: 16 *C. jejuni*, 16 *C. coli*, 10 *C. lari* and 8 non-target strains strains = 50 strains per method

PARTICIPANTS OF ILS



- **Voluntary to participate**
- Need at least 10 valid datasets per method.
- Invitation will be sent to experts in WG3 Campylobacter and to all *NRL-Campylobacter* in October.
- Whenever possible, the study conditions should reflect the normal variation between laboratories.
- Will in first hand include participants that already has optimized the methods in their labs/run it routinely and that can use own PCR reagents, primes and probes.
- We will allow participants to participate in a subset of methods.
- If not enough participants we will also welcome participants that do not routinely run these PCRs. In this case we will send all primers/probes and possibly all reagents required.
- In the participant form to be sent out with the invitation in October, you will be asked to specify the terms of participation (if you have the possibility to participate/which methods are already implemented, and if participation is depended on obtaining primers/probes etc. from the EURL)

On behalf of WG3 – hope you are interested to participate!

Acknowledgement: Experts in CEN/TC 463/WG3 *Campylobacter* and especially Ute Messelhäuser