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Report of the first inter laboratory proficiency testing trail

Trail on the enumeration of *Campylobacter* in poultry products

Report : 15 January 2010

Institute of Public Health

Bacteriology : Food Pathogens

National Reference Laboratory for food Microbiology

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Table of contents

Chapter 1 : Introduction
Chapter 2 : Organization of the study
Chapter 3 : Preparation of the samples
Chapter 4 : Data analysis
Chapter 5 : Conclusions
Annexes

Chapter 1 : Introduction

This proficiency study concerned the investigation of the enumeration of *Campylobacter* in poultry products. For the moment no commercial proficiency test on the enumeration of *Campylobacter* is available on the market. In the frame of the accreditation the laboratories has to participate to a PT. Therefore different laboratories accredited for the enumeration of *Campylobacter* asked the NRL to organize a PT so that they can test their performance.

The proficiency test was organized in collaboration with the Federal Agency of the Safety of the Food Chain (FASFC) from Belgium in the framework of the tasks as NRL food Microbiology.

Chapter 2 : Organisation of the study

An invitation letter was send to the different laboratories who were accredited for this parameter the 4th of September 2009 (Annex 1)

In total we received the registration form of 8 laboratories at least the 15th of September

The samples were distributed to the different laboratories the 29th of September

The deadline for reporting of the results was the 15th of October

Final report was distributed to the participants the 15th of January 2010

Chapter 3 Contamination of the samples

Samples :

Four poultry products were bought direct after preparation at a brand that produces sometimes poultry meat products with a high initially *Campylobacter* contamination.

Sample 1 ; chicken minced meat

Sample 2 : Chicken burger

Sample 3 : minced chicken meat the so called tree trunk

Sample 4 : Sausage

Ten gram of the sample was weighted in a stomacher bag in duplicate.

We did it in duplicate so that the results can be also applicable for the calculation of the measurement uncertainty (MU).

Two of the samples were spiked with *Campylobacter*, the other two samples were tested if they are initially contaminated with *Campylobacter*.

Sample 1, the minced chicken meat, was contaminated with *Campylobacter coli* (1105-2009-2151) and sample 4 the sausage with a *Campylobacter jejuni* strain (1105-2009-2154).

Preparation of the inoculums :

The strains were grown overnight in Bolton broth and a serial dilution was made in buffered peptone water. For the contamination of sample 1 100 µl of the 10⁻³ dilution of *Campylobacter coli* was used to spike 10 g. Sample 4 was contaminated with *Campylobacter jejuni* by spiking 100 µl of the 10⁻² dilution. The pure culture was also plated out to know the level of the starting inoculums.

The initial contamination level of the samples was determined before spiking : therefore 10 g of the sample without spiking was tested in duplicate for the four different samples.

It was not necessary to determine the homogeneity of the samples because each individual sample was spiked separately with *Campylobacter* and the total sample has to be used for the determination of the *Campylobacter* counts.

The samples were placed in a cool box and brought to the dispatching of Melle so that each participating lab could pick up their samples in the afternoon.

The analysis was started the 30th of September for all the labs except one lab started the analysis the 1st of October.

Chapter 4 :Data analysis

The initial contamination level of the samples.

Sample 1, 2 and 3 were negative for *Campylobacter* spp par gram. Sample 4 was contaminated with 5.2 10² cfu/g

The inoculum level of the *Campylobacter* strains could not be determined because the jar with the plates arrived in the lab the 1st of October. During that time the plates were hold at room temperature at the dispatching. At the time of arrival at the lab the jar was incubated at 41.5°C but the counts were not correct due to a long stress of the cells.

1105-2009-2151 : 1 10⁵ cfu/ml

1105-2009-2154 : 4 10⁶ cfu/ml

For the calculation of the z-score only the lab contaminated samples were used. For the calculation of the z-score the results of the colony forming units expressed by the log₁₀ were used. The following formula was used for the calculation :

z-score = value of the lab - the average of all the labs/ standard deviation on the average

A z-score between -2 and 2 do not need actions, when the z score is higher then 3 or smaller then -3 then actions should be taken in the lab to improve the results

Results

All the labs follows the ISO 10272-2 method

The dilutions were made in buffered peptone water.

One lab did not plated out 1 ml of the initial suspension.

All the labs used mCCDA agar for the enumeration of *Campylobacter*. Some laboratories used home made plates (2) the others used commercial ready to used plates (6).

The following table expresses all the results for the different samples received from the different lab's

Results received of all the participating laboratories expressed as cfu/g

lab	1a	1b	2a	2b	3a	3b	4a	4b
1	370	630	<10	<10	<10	<10	10400	9800
2	1700	1300	<10	<10	48	46	14000	14000
3	2300	3600	<10	<10	10	10	17000	16000
4	320	1000	<10	<10	<10	<10	17000	16000
5	<100	<100	<100	<100	<100	400	3400	2100
6	200	370	20	10	<10	<10	5700	5300
7	340	110	<10	<10	<10	<10	7600	6300
8	3350	3040	<10	<10	<10	<10	21600	22000

To determine the z score only the results of the spiked samples were used for the calculations. The table below gives the z-score of the different laboratories for the four samples by using the average in the calculation.

Lab nr	Sample 1a	Sample 1b	Sample 4a	Sample 4b
1	-0.591	-0,261	0,004	0,072
2	0.749	0,334	0,473	0,534
3	1.015	1,171	0,779	0,707
4	-0.719	0,118	0,779	0,707
5	-5.793	-5,561	-1,758	-1,919
6	-1.133	-0,699	-0,943	-0,722
7	-0.666	-1,696	-0,490	-0,498

8	1.346	1,032	1,156	1,119
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Because of the big variation in the results between the laboratories it would be better to use the median instead of the average to calculate the z-score. After doing this there was only a slight difference between the two ways of calculation. The general conclusions on the laboratory performance remained unchanged. The following table presents the z-scores by using the median in the calculations.

Lab nr	Sample 1a	Sample 1b	Sample 4a	Sample 4b
1	0	-0,37991	-0,23435	-0,23072
2	1,341283	0,215727	0,234353	0,230719
3	1,607169	1,053238	0,540497	0,403471
4	-0,1277	0	0,540497	0,403471
5	-5,20151	-5,67985	-1,99726	-2,22362
6	-0,54112	-0,81752	-1,18254	-1,02594
7	-0,07438	-1,81491	-0,72893	-0,80233
8	1,937943	0,914216	0,918109	0,815461

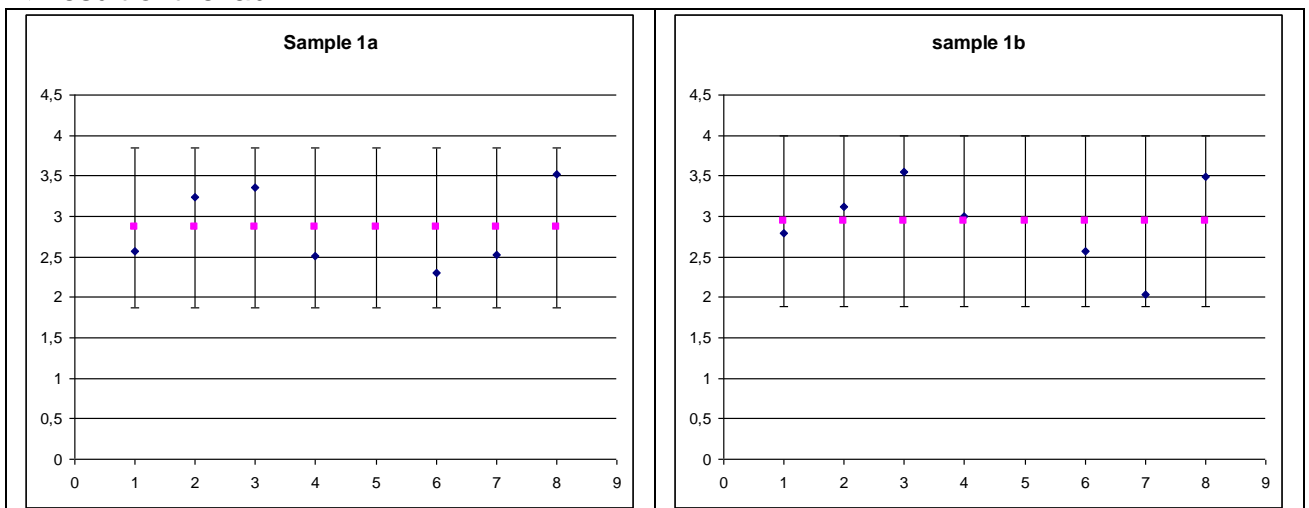
Graphical presentation of the results for the 4 samples of the different labs.by using the average in the calculation of the z-score.

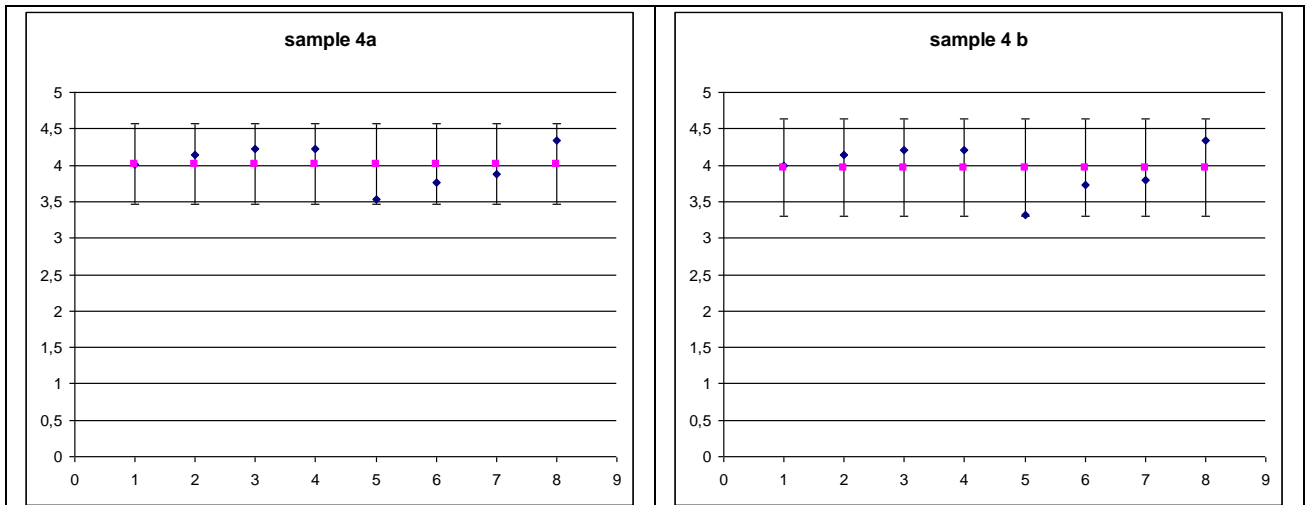
Legend for the figures

Vertical bars T: 2 times the standard deviation

■ average value

◆ result of the lab





Remarks given by the laboratories :

One lab remarked that for one sample the stomacher sac had a leak.
Another lab reported a mistake of the numbering (2 times sample 2a).

Chapter 5 :Conclusions

This was the first proficiency test organized by the NRL food microbiology for the enumeration of *Campylobacter* in poultry products.

All the labs performed well with z scores between -2 and 2.

For sample 1a and 1b one lab reported that *Campylobacter* spp was below the detection limit of 100 cfu/g. Contact was taken with the lab(5) to improve their procedure because it was also the only lab that incubated the plates first for 6 h at 37°C and afterwards at 41.5°C. They need also 72 h before the colonies could be counted whereas in all other labs the colonies were counted after 48 h of incubation.

Only one lab further identified the *Campylobacter* spp to discriminate between *Campylobacter jejuni* and *coli*.

We will organize the ring trail again at the end of September 2010.

We will thank following persons for their collaboration in the organization of this proficiency test:

- Imca Sampers (Howest)
- Lieven De Zutter (UGent)
- all the participating laboratories
- Nicole Hamers (IPH).

Annex 1 invitation in French

CONCERNE : organisation d'un ringtest pour le dénombrement de Campylobacter dans la viande de poulet

Chers Madame, Monsieur,

En tant que Laboratoire National de Référence en microbiologie alimentaire, différents laboratoires nous ont demandé s'il existe un ringtest pour le dénombrement de Campylobacter. Il n'y a pas de ringtest disponible pour tester ce paramètre ni au niveau national ni au niveau international. Seul l'institut Suédois SVA a inclus le paramètre de dénombrement de Campylobacter dans son ringtest. Mais la participation est très chère. C'est pourquoi, en tant que LNR en microbiologie alimentaire en collaboration avec l'Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (AFSCA), nous allons organiser un ringtest limité fin septembre ou début octobre.

Vous allez recevoir 4 échantillons de haché de poulet sur lesquels vous devrez réaliser un dénombrement.

Comme nous n'avons encore jamais organisé un tel ringtest et que Campylobacter n'est pas le germe le plus facile, ce premier ringtest sera gratuit.

Si vous êtes intéressés à participer, veuillez renvoyer le formulaire d'inscription ci-joint à :
Marie.Polet@iph.fgov.be

De plus amples informations suivront, avec la date exacte et le lieu d'enlèvement des échantillons (via le centre de dispatching de l'AFSCA).

Sincères salutations,

Dr. Nadine Botteldoorn
Polet

Marie

Responsable d'activité
Responsable
Unité des pathogènes alimentaires
Alimentaire

LNR Microbiologie

Annex 2 invitation in Dutch

BETREFT :Organisatie van een ringtest voor de telling van Campylobacter in kippengehakt

Beste Mevrouw, Meneer,

Als Nationaal referentielaboratorium voor levensmiddelen microbiologie werd ons door verschillende laboratoria de vraag gesteld of er een ringtest bestaat voor de telling van Campylobacter. Nationaal en ook internationaal zijn er geen ringtesten beschikbaar voor het testen van deze parameter. Enkel het Zweedse SVA instituut heeft de parameter telling van Campylobacter ingesloten. Maar deelname is heel duur. Vandaar dat we als NRL levensmiddelen microbiologie in samenwerking met het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV) een beperkte ringtest zouden organiseren tegen eind september begin oktober.

Hierbij zouden jullie 4 kippengehakt monsters ontvangen waarop de telling dient te gebeuren.

Omdat we dergelijke ringtest nog nooit hebben georganiseerd en Campylobacter ook niet de gemakkelijkste kiem is zullen we deze eerste ringtest gratis organiseren.

Wanneer jullie geïnteresseerd zijn voor deelname gelieve bijgevoegd inschrijvingsformulier terug te zenden naar : Marie.Polet@iph.fgov.be

Verdere informatie volgt met de exacte dag en de plaats van afhaling van de stalen (Via de dispatching centra van het FAVV)

Met vriendelijke groeten,

Dr. Nadine Botteldoorn

Activiteitsverantwoordelijke
Eenheid voedsel pathogenen
Microbiologie

Marie Polet

Verantwoordelijke
NRL Levensmiddelen

Annex 3 : Registration form

REGISTRATION FORM RING TEST

“Enumeration of Campylobacter in poultry products”

**Please return this form no later than September 15th, 2009
to Marie.Polet@iph.fgov.be**

Laboratory:

Contact person:

Address:

Phone number:

Fax number:

Email address:

CONFIRMS to participate to the ring test of September- October, 2009

Pick-up of samples at following locations (select)

Dispatching Melle	Dispatching Gembloux

Annex 4 Results form

To be returned to Mrs Marie Polet (Marie.polet@iph.fgov.be) before the 15th October 2009 or by fax 02 642 52 40

Laboratory name :

Date of arrival : September 2009

Condition of the samples at arrival: Good Bad

Storage condition : °C

Date of the beginning of the analyses :

Remarks :

Method used :

Diluent used for decimal dilutions

Manufacturer

Medium used for inoculation

Home made medium day of preparation
Base medium Manufacturer

Ready-to-use
date of expiry:
Manufacturer

