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National Reference Laboratory Milk and Milk Products in the Netherlands
Combined Annual Report 2006 and 2007

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Group: Microbiology & Novel Foods

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- Qlip, Leusden (F.W. Woltjes, R.J. de Knegt), Qlip, Zutphen (dr. ir. H. van den Bijgaart)

Summary

This combined annual report of the Dutch National Reference Laboratory (NRL) Milk and Milk Products describes the activities of the NRL in 2006 and 2007.
Nowadays the NRL reference tasks for milk and milk products are based on the specific rules for raw milk and milk products as described in Commission Regulation EC 853/2004. These tasks include the parameters: Total flora (TF), Somatic cells count (SCC), and Alkaline phosphatase activity (ALP).
Currently, EC 1664/2006 states:
When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods:

- *EN/ISO 4833 for the plate count at 30 ◦C;*
- *ISO 13366-1 for the somatic cell count.*
- *When determining alkaline phosphatase activity, ISO standard 11816-1 must be applied as reference method.*

RIKILT has an accreditation for the SCC and TF reference methods and will apply for accreditation for the relatively new ALP reference method in 2008.

Activities of the NRL Milk and Milk Products during 2006 and 2007 included also:

- **Participation in the annual CRL-NRL workshops.** The 2006 annual workshop took place in Kiel, Germany. This workshop was dedicated to the use of alternative (instrumental, e.g. Bactoscan) methods for the official control on total flora counts in raw milk. The 2007 annual workshop took place at the CRL, Maisons-Alfort, France. This workshop included more general topics, like recent developments in EU food legislation, new structure of CRL/NRL networks and reports on CRL-organised interlaboratory studies.
- **Responding to CRL questionnaires.** Two CRL questionnaires were received and elaborated: Determination of total bacterial counts in raw milk in EU-member states (June 2006) and Enquiry on the Reference Materials (RMs) for Somatic Cells Counting (SCC) available in Europe (October 2007).
- **Participation in CRL-organised interlaboratory studies.** RIKILT participated in the following CRL interlaboratory studies: detection of *S. aureus* enterotoxins; enumeration of coagulase-positive staphylococci (CPS) in milk; alkaline phosphatase activity in cow, goat and ewe milk (first trial) and alkaline phosphatase activity in cows milk only (second trial); enumeration of total flora at 30◦C, using the plate count method ISO 4833.
- **Communication.** The Routine Field Laboratories MCS/Qlip in Zutphen and COKZ/Qlip in Leusden were visited in 2006 and in 2007. Various subjects, like ring trial results and methodologies used for official control on milk and milk products, were discussed. The implementation of ISO 21187 to establish the conversion relationship between routine method results (Bactoscan) and anchor method results (plate count method) for the Total Flora count was found to be completely satisfactory.

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1 Introduction

In 1992 RIKILT was designated as National Reference Laboratory (NRL) on Milk and Milk Products, according to directive 92/46/EEG laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. AFSSA-LEFHQA in Paris was assigned as the Community Reference Laboratory (CRL).
A renewal of European food safety legislation took place by the implementation per 1-1-2006 of the General Food Law (Regulation (EC) No 178/2002) and its underlying regulations (EC 852/2004, EC 853/2004, EC 854/2004, EC 882/2004, EC 2073/2005, EC2074/2005).
Generally, reference tasks on e.g. *Listeria*, *Salmonella*, *E.coli*, and *S. aureus* nowadays are horizontally (all foods) regulated instead of vertically (milk and milk products only), and specific CRL/NRLs have been designated accordingly. Formerly established CRL/NRLs were kept, but with revised tasks. Today the reference tasks for milk and milk products are based on the specific rules for raw milk and milk products as described in Annex III, section IX of EC 853/2004. These reference tasks include the parameters: Somatic cells count (SCC), Total flora (TF), and Alkaline phosphatase activity (ALP).
The responsibilities of National Reference Laboratories are describes in article 33 of EG 882/2004 (see Annex 1) and this is implemented by RIKILT through the following activities:

- Development, validation and independent quality control of official reference methods and providing information to Routine Field Laboratories
- Participation in the annual CRL-NRL workshops
- Responding to CRL questionnaires
- Participation in CRL-organised interlaboratory studies
- Participation in CRL-training courses
- NRL ring trials
- Accreditation of the reference methods
- Communication on developments in related methods and legislation matters
- Annual report of activities (from 2006 onwards)

This report describes the activities of the Dutch NRL Milk and Milk Products in the years 2006 and 2007.

2 Participation in CRL/NRLs workshops

2.1 Annual CRL/NRLs workshop 2006

The 2006 annual workshop took place on the 11th and 12th of September 2006 at the BFEL (Federal Research Centre for Nutrition and Food) in Kiel, Germany. This workshop was dedicated to the use of alternative (instrumental, e.g. Bactoscan) methods for the official control on total flora counts in raw milk. A questionnaire on this topic had been send out by the CRL earlier in the year and the results of this questionnaire were discussed. Background and implementation of EN-ISO 21187/IDF 196:2005 Milk - Quantitative determination of bacteriological quality - Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results was presented by the invited expert speaker Mr. Harrie van den Bijgaart from the Netherlands Milk Control Station (MCS), Zutphen, NL. Beside this dedicated topic, also more general items like recent developments in EU food legislation, new structure of CRL/NRL networks and the measurement uncertainty within quantitative food microbiology were discussed. The minutes of the 2006 Workshop are given in Annex 2a. Slides of the various presentations are available on request.
The workshop was attended by 18 delegates of 25 NRLs of the 25 EU Member States and 2 associated countries (Norway and Switzerland). The EC/DG SANCO “Health and Consumer Protection” representative, Thierry Chalus, was not able to attend this time. The list of attendance is given in Annex 2b.

2.2 Annual CRL/NRLs Workshop 2007

The 2007 annual workshop took place on 28th and 29th of June 2007 at the CRL AFSSA-LERQAP (AFSSA Laboratory for Study and Research on Food Quality and Food Processes), Maisons-Alfort, France. This workshop was not dedicated to a special topic, but more general topics were discussed, like recent developments in EU food legislation (Thierry Chalus, EU, DG-Sanco), new structure of CRL/NRL networks and CRL work on flow cytometry (total flora counts). Final reports were communicated on work that started during the former CRL/NRLs Milk and Milk Products, like the studies on enumeration of staphylococci, enumeration of *Enterobacteriaceae* and detection of staphylococcal enterotoxins. Roger Wood (UK) was invited to give a presentation on the (im)possibilities of the "criteria approach" to be used in legislation on reference methods. The minutes of the 2007 Workshop are given in Annex 3a. Slides of the various presentations are available on request.
The workshop was attended by 29 delegates of 27 NRLs of the now 27 EU Member States and 2 associated countries (Norway and Switzerland). The list of attendance is given in Annex 3b.

3 CRL Questionnaires

3.1 Questionnaire on Total Bacterial Counts in Raw Milk.

As a preparation for the dedicated workshop in Kiel, a CRL questionnaire was received in June 2006 on the topic: Determination of total bacterial counts in raw milk in EU-member states. A reply from The Netherlands was prepared in cooperation with Mr. Van den Bijgaart (Milk Control Station, Zutphen). The outcome of this enquiry was presented by the organisers Mrs. Suhren (BFEL-Kiel) and Mr. Lombard (CRL Milk and Milk Products, Maisons-Alfort) during the Workshop in Kiel, September 2006. After discussion and some additional remarks, the final version of the questionnaire became available in October 2006 and is given in Annex 4.

3.2 Questionnaire on Reference Materials for Somatic Cells Counting.

In October 2007, a CRL questionnaire was received on the topic: Enquiry on the Reference Materials (RMs) for Somatic Cells Counting (SCC) available in Europe. Mr. Van den Bijgaart (Qlip, Zutphen) prepared a reply on this questionnaire for the Dutch situation. The outcome of this enquiry is expected to become available in 2008.

4 Participation in CRL interlaboratory studies

4.1 History

Reference tasks for the CRL/NRLs Milk and Milk Products were adjusted because of the implementation of the General Food Law on January 1st of 2006. However, it took some time before all new horizontal CRL/NRLs were established and therefore the year 2006 has some overlap in tasks or mainly in reporting of results. Results from two CRL interlaboratory studies on *S. aureus*, conducted in 2005, became available during 2006 and therefore still are discussed in this annual report.

4.2 CRL interlaboratory study on detection of staphylococcal enterotoxins

RIKILT participated in December 2005 in the CRL interlaboratory study on detection of *S. aureus* enterotoxins according to the prescribed CRL official method. The study was intended to validate the VIDAS SET2 detection kit. The final report was received in March 2006 (available on request). All 21 participating labs had very good results with the detection method under validation.
It was concluded that at the European level, the VIDAS SET2 detection kit can be used (as the Transia Plate SET detection) for staphylococcal enterotoxins official controls in milk and milk products. The CRL accordingly modified the European Screening Method and the updated version 3 became available in May 2006 (see Annex 5). This information also was passed on to Dr. Paul in 't Veld of the VWA regio Zuid, as the (end-2006) newly established Dutch NRL on *S. aureus* and its toxins.
Two scientific papers on this topic were published during 2007:

- Hennekinne et al., Interlaboratory validation of the Vidas SET2 kit for detection of staphylococcal enterotoxins in milk products. J. AOAC Int. 90(3):756-764.
- Hennekinne et al., Intralaboratory validation according to the EN ISO 16 140 Standard of the Vidas SET2 detection kit for use in official controls of staphylococcal enterotoxins in milk products. J. Appl. Microbiol. 102(5):1261-1272.

4.3 CRL interlaboratory study on enumeration of coagulase-positive staphylococci

RIKILT participated in December 2005 in the CRL interlaboratory study on enumeration of coagulase-positive staphylococci (CPS) in milk, using both of the possible reference methods (ISO 6888 part 1 and part 2). The final report was received in May 2006 (available on request). In conclusion, the global performance of the NRLs network, both in terms of repeatability and reproducibility, was good. Moreover, most of the 21 participants (81%) showed a satisfactory individual performance, in terms of trueness (z-scores). RIKILT z-scores were all $|z| \leq 2$: satisfactory.

4.4 CRL interlaboratory studies on alkaline phosphatase activity

RIKILT participated in January 2006 in the CRL interlaboratory study on alkaline phosphatase activity in cow, goat and ewe milk, using the reference method IDF 155/ISO 11816-1. This method became officially published in May 2006. The study report was received late December 2006 and a revised version became available in June 2007 (available on request). It was the first time that the CRL organised an interlaboratory study on this subject and it turned out that the trial had been too ambitious in its experimental design. Therefore, it was not easy to have a proper interpretation of all data gathered, but this experience surely will be used in the future organisation of these types of trials.

The second CRL interlaboratory trial on alkaline phosphatase activity, this time in cow's milk only, was organised in November 2007. RIKILT also participated in this trial, using the reference method IDF 155/ISO 11816-1. The report on the results is expected to become available in 2008.

4.5 CRL interlaboratory study on enumeration of total flora at 30◦C

RIKILT participated in October 2007 in the CRL interlaboratory study on enumeration of total flora at 30◦C, using the reference method EN-ISO 4833.
During the 2006 CRL Workshop in Kiel, it was decided that also should be invited to participate in this trial: the laboratories, to be identified by the NRLs, which are in their respective countries entrusted to estimate CC values (conversion characteristics) between the reference method (EN-ISO 4833) and the instrumental methods (flow cytometry). For the Dutch situation therefore Qlip, Zutphen (formerly: Milk Control Station) also participated to this interlaboratory study.
The report on the results is expected to become available in 2008.

5 Participation to CRL training courses

No training courses were given during 2006 or 2007.

6 Analytical activities

6.1 Ring trials

Due to the limited number of Routine Field Laboratories, it is not appropriate to organize specific ring trials. However, the RFL labs as well as the NRL lab do participate in common ring trials in the dedicated topics. RFL lab ring trial results were discussed during the annual visits as referred to in section 7.1.
RIKILT participated with satisfactory-very good results to the following ring trials in 2006:

- Total flora, enumeration (4x),
- Coliforms, enumeration (4x),
- *E. coli*, enumeration (4x),
- *Enterobacteriaceae*, detection (4x),
- *Salmonella*, detection (4x),
- *Listeria monocytogenes*, detection (3x),
- *B. cereus* spores, enumeration (4x),
- *S. aureus*, detection and enumeration (4x)
- Somatic cells count, microscopy (3x)
- Alkaline phosphatase, CRL organised (1 x)

RIKILT participated with satisfactory-very good results to the following ring trials in 2007:

- Total flora, enumeration (4x),
- Somatic cells count, microscopy (1x)
- Alkaline phosphatase, CRL organised (1x)

6.2 Reference material for calibration of automated somatic cells count

The Routine Field Laboratory for somatic cells count, MCS/Qlip, uses an automated method (Fossomatic) for routine samples. The equipment for this needs to be calibrated every 3 months and RIKILT participates in this calibration by testing designated samples by the microscopic reference method for somatic cells count (ISO 13366-1). Calibration test were carried out in March, May, August, and November 2006; and in February, April, July, and October 2007.

6.3 Alkaline phosphatase

The reference method for alkaline phosphatase (ISO 11816-1) was recently elaborated to produce the validation data and the method (RSV N0335) will be submitted for accreditation in 2008. Since no official standard reference material is available, reference material was produced by spiking ALP-free milk with raw milk to a expected level of ca. 500 mU/l. COKZ/Qlip also uses this type of in-house reference material. No common ring trials are available and therefore a secondary control system is used by always repeating one sample from the previous series of samples during the current one.

In 2007 was started with a system to compare results on blind samples that were already tested by COKZ/Qlip before. Three series of 5 samples each were tested this way in 2007 to get started and this way of comparing results will be continued in the future by testing ca. 6 times per year a set of 5 samples each time by both labs.

7 Communication

7.1 Contacts with MCS and COKZ

In combination with RIKILT project 71.313.51, "Government supervision COKZ", RIKLT visited COKZ/Qlip in Leusden on 15 December 2006 and on 15 November 2007. Various subjects, like ring trial results and methodologies used for official control on milk and milk products, were discussed. Detailed visit reports are given in the annual reports on the Government supervision COKZ (Van Ruth et al., 2007, Van Ruth et al., 2008).

MCS/Qlip in Zutphen was visited on 13 February 2007 and on 5 December 2007. The former visit was used to exchange information on methods of examination and on the implementation of ISO 21187 to establish the conversion relationship between routine method results (Bactoscan) and anchor method results (plate count method) for the Total Flora count. The latter visit was in combination with a visit of a group of Brazilian managers from LANAGRO laboratories. The general presentation on the Dutch NRL Milk and Milk Products activities, as a part of their training course, is given in Annex 6.

During the 2007 annual meeting, the CRL promised to work out a structured check-list for visiting the laboratories establishing CC values (and others). This check-list will be used in future visits to the Routine Field Laboratories.

7.2 Legislation

During the 2006 Workshop in Kiel already some Regulation amendments were announced and these became official by the publication on 6 November 2006 of COMMISSION REGULATION (EC) No 1664/2006, amending Regulation (EC) No 2074/2005 as regards implementing measures for certain products of animal origin intended for human consumption and repealing certain implementing measures.
Consequently, current legislation is given below per topic.

7.2.1 Official method Total Flora count

1. *When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods:EN/ISO 4833 for the plate count at 30∘C;*

2. *The use of alternative analytical methods is acceptable:(a) For the plate count at 30∘C, when the methods are validated against the reference method mentioned in point 1(a) in accordance with the protocol set out in EN/ISO standard 16140 or other similar internationally accepted protocols.In particular the conversion relationship between an alternative method and the reference method mentioned in point 1(a) is established according to ISO standard 21187.*

RIKILT has an accreditation (www.rva.nl L014) for this reference method, based on RIKILT standard operating procedure RSV A0016.

7.2.2 Official method Somatic Cells Count

1. *When checking against the criteria laid down in Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004, the following standards must be applied as reference methods: (b) ISO 13366-1 for the somatic cell count.*

2. *The use of alternative analytical methods is acceptable: (b) For the somatic cell count, when the methods are validated against the reference method mentioned in point 1(b) in accordance with the protocol set out in ISO 8196 and when operated in accordance with ISO standard 13366-2 or other similar internationally accepted protocols.*

RIKILT has an accreditation (www.rva.nl L014) for this reference method, based on RIKILT standard operating procedure RSV A0716. The draft version of the revised ISO/DIS13366-1/IDF 148-1 was published in July 2006. The final version of this ISO standard is expected early 2008 and will be implemented accordingly.

7.2.3 Official method Alkaline Phosphatase activity

1. *When determining alkaline phosphatase activity, ISO standard 11816-1 must be applied as reference method.*

2. *The alkaline phosphatase activity is expressed as milli units of enzyme activity per litre (mU/l). A unit of alkaline phosphatase activity is the amount of alkaline phosphatase enzyme that catalyses the transformation of 1 micromole of substrate per minute.*

3. *An alkaline phosphatase test is considered to give a negative result if the measured activity in cow's milk is not higher than 350 mU/l.*

4. *The use of alternative analytical methods is acceptable when the methods are validated against the reference method mentioned in point 1 in accordance with internationally accepted protocols.*

RIKILT will apply for accreditation for this reference method in 2008, based on RIKILT standard operating procedure RSV N0335.

8 References

EU legislation

(EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

(EC) No 852/2004 on the hygiene of foodstuffs

(EC) No 853/2004 laying down specific hygiene rules for food of animal origin

(EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption

(EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

(EC) No 2073/2005 on microbiological criteria for foodstuffs

Reg 2074/2005

Reg 1664/2006

ISO documents:

NEN-EN-ISO 4833:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 degrees C

NEN-ISO 8196-1:2000 Milk - Definition and evaluation of the overall accuracy of indirect methods of milk analysis - Part 1: Analytical attributes of indirect methods

NEN-ISO 8196-2:2000 Milk - Definition and evaluation of the overall accuracy of indirect methods of milk analysis - Part 2: Calibration and quality control in the dairy laboratory

NEN-EN-ISO 11816-1:2006 Milk and milk products - Determination of alkaline phosphatase activity - Part 1: Fluorimetric method for milk and milk-based drinks

NEN-EN-ISO 11816-2: Milk and milk products - Determination of alkaline phosphatase activity - Part 2: Fluorimetric method for cheese

NEN-EN-ISO 13366-1/IDF 148. Milk - Enumeration of somatic cells - Part 1: Microscopic method (Reference method)

NEN-EN-ISO 13366-2:2006 Milk - Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-opto-electronic counters

NEN-EN-ISO 16140:2003 Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.

NEN-EN-ISO 21187:2005 Milk - Quantitative determination of bacteriological quality - Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

RIKILT standard operating procedures (in Dutch):

RSV A0016 Voedingsmiddelen en diervoeders - Bepalen van het aantal aeroob kweekbare micro-organismen bij 30˚C; plaatmethode.

RSV A0716 Melk - Bepaling van het aantal somatische cellen; fluorescentie microscopie (telling).

RSV N0335 Milk and milk products-Determination of alkaline phosphatase activity-Fluorimetric method for milk and milk-based drinks.

Other references:

Hennekinne JA, Ostyn A, Guillier F, Gohier M, Messio S, Dragacci S, Krys S, Lombard B., 2007. Interlaboratory validation of the Vidas SET2 kit for detection of staphylococcal enterotoxins in milk products. J. AOAC Int. 90(3):756-764.

Hennekinne JA, Guillier F, Perelle S, De Buyser ML, Dragacci S, Krys S, Lombard B., 2007. Intralaboratory validation according to the EN ISO 16 140 Standard of the Vidas SET2 detection kit for use in official controls of staphylococcal enterotoxins in milk products. J. Appl. Microbiol. 102(5):1261-1272.

Van Ruth, S.M., S.E. Brouwer, W.J. de Boer en W.F. Jacobs-Reitsma. 2007. Kwaliteitsbewaking van onderzoek uitgevoerd door het COKZ t.b.v. de Landbouwkwaliteitsregeling en de afgifte van exportcertificaten (Jaarverslag 2006). RIKILT Rapport 2007.509. In Dutch.

Van Ruth, S.M., S.E. Brouwer, W.J. de Boer en W.F. Jacobs-Reitsma. 2008. Kwaliteitsbewaking van onderzoek uitgevoerd door het COKZ t.b.v. de Landbouwkwaliteitsregeling en de afgifte van exportcertificaten (Jaarverslag 2007). RIKILT Rapport 2008xx. In preparation. In Dutch.

Annex 1 Responsibilities of NRLs as described in EC 882/2004.

Article 33 (of EC 882/2004): National reference laboratories

1. Member States shall arrange for the designation of one or more national reference laboratories for each Community reference laboratory referred to in Article 32. A Member State may designate a laboratory situated in another Member State or European Free Trade Association (EFTA) Member and a single laboratory may be the national reference laboratory for more than one Member State.
2. These national reference laboratories shall:
   (a) collaborate with the Community reference laboratory in their area ofcompetence;
   (b) coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11;
   (c) where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;
   (d) ensure the dissemination to the competent authority and official national laboratories of information that the Community reference laboratory supplies;
   (e) provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans adopted in accordance with Article 53;
   (f) be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.
3. Article 12(2) and (3) shall apply to national reference laboratories.
4. Member States shall communicate the name and address of each national reference laboratory to the Commission, the relevant Community reference laboratory and other Member States.
5. Member States that have more than one national reference laboratory for a Community reference laboratory must ensure that these laboratories work closely together, so as to ensure efficient coordination between them, with other national laboratories and with the Community reference laboratory.
6. Additional responsibilities and tasks for national reference laboratories may be laid down in accordance with the procedure referred to in Article 62(3).
7. Paragraphs 1 to 5 shall apply without prejudice to more specific rules and in particular Chapter VI of Regulation (EC) No 999/2001 and Article 14 of Directive 96/23/EC.

Annex 2a. Report of the CRL/NRLs Workshop 2006.

Site de Maisons-Alfort

EU COMMUNITY REFERENCE LABORATORY FOR MILK & MILK PRODUCTS

LABORATOIRE D'ETUDES ET DE RECHERCHES
SUR LA QUALITE DES ALIMENTS
ET SUR LES PROCEDES AGROALIMENTAIRES

Workshop of the National Reference Laboratories "Milk & Milk Products"
"Total flora in raw milk: use of alternative methods"

Report

11 & 12 September 2006, BFEL, Kiel, Germany

23 Avenue du
General De Gaulle
F – 94 706
Maisons-Alfort

www.afssa.fr

Opening, 11 September 2 pm

Paul TEUFEL, Director of the Institute for Hygiene and Food Safety of BFEL (Federal Research Centre for Nutrition and Food), welcomed the participants. He presented BFEL, a federal structure with several locations in Germany (see his slides). The location in Kiel includes the one that he leads and that he presented in more details. In particular, the laboratory is NRL for the control of raw milk, including total flora (TF). He also introduced the topic of the workshop.

Gertraud SUHREN, in charge of TF in raw milk in the institute and for the NRL Milk, welcomed also the participants, and gave some details on the programme.

Bertrand LOMBARD, on behalf of the CRL "Milk", thanked both of them for their kind invitation to host the workshop. The location of the workshop in Kiel had been envisaged given the leading role and expertise of the Kiel institute on this topic, as well as the possibility to have practical demonstrations of apparatus on this site.

A roll-call of delegates took place. B. Lombard transmitted in particular the excuses of Thierry CHALUS (EC/ DG SANCO) who could not attend at the last minute due to urgent matters requesting his presence at office. The list of attendance, with excuses received, is provided separately.

B. LOMBARD presented the background of the activities of the CRL "Milk" and of the network of NRLs "Milk" in this field. The use of alternative/instrumental methods for official controls of TFin raw milk, justified by the difficulty to use in routine for large-scale controls the reference method (total plate counting-TPC, EN ISO 4833), is a difficult topic and has been on the agenda of several workshops of the NRLs Milk. The difficulty lies in particular on how to make the conversion of the units of the instrumental methods' results into the unit of the reference method, cfu's, in which the legislative limits are expressed. It was therefore decided at the 2005 general workshop (30 June-1 July) to have a workshop dedicated to that topic. He recalled that it was admitted to use instrumental methods for official controls, under a certain number of conditions that would be detailed during the workshop: mainly the use of the IDF/ISO Standard for the establishment of the conversion relationship.

In this context, the objective of the workshop was to give more guidance and details on how to implement instrumental methods for routine TF analysis of raw milk in Europe in the frame of official controls of milk hygiene, with a focus on the establishment of conversion characteristics with the reference method.

Recent developments in EU-Food Hygiene legislation and new structure of CRLs/NRLs - Responsibilities of CRLs and NRLs with special emphasis on "Milk"

Both aspects were presented by B. LOMBARD, in the absence of T. CHALUS.

He first recalled the legislative frame for the control of raw milk: Regulations 852/2004 (Annex II, Chapter XI), 853/2004 (Annex III, Section IX) –separately enclosed.

He also presented a draft Regulation amending Regulations 853/2004, 854/2004, 2074/2005, 2075/2005 and 2076/2005 (document SANCO/644/2006 rev. 5, enclosed). In this draft, a section is devoted to the definition of reference methods and to the conditions to use alternative/instrumental methods for the control of raw milk (somatic cells count, total flora) and for the determination of phosphatase activity (Amendments to Regulation 2074/2005, Annex VIa). It had been prepared by T. CHALUS in collaboration with the CRL Milk further to the will of the NRLs Milk to define these aspects in a legislative text (see in particular the 2005 workshop).
Some comments were raised about the analysis of colostrums, introduced in this text.

> ➔ The CRL and NRLs drew the attention of DG SANCO that the applicability of the reference method for TF (EN ISO 4833) had not been tested, and that the use of instrumental methods could be even more problematic.

B. LOMBARD presented also the new structure of CRLs in the field of biological contaminants, and the need for each country to identify new corresponding NRLs (see Regulation 776/2006, enclosed).

Measurability and measurement of the parameter "total bacterial count" in raw milk

G. SUHREN introduced the scientific background of the workshop (see her slides enclosed), the factors determining the hygienic status of milk and milk products, the different types of methods for TF determination in raw milk, and the difference of measuring principle between the reference method (TPC) and alternative/instrumental methods, inducing a major difference in terms of analytical target between these methods.

Use of alternative methods within the EU – Outcome of the questionnaire

As scheduled at the 2005 workshop in order to prepare this workshop, a questionnaire had been prepared by G. SUHREN and B. LOMBARD to get an overview of national practices in European countries regarding the TF control in raw milk and the use of instrumental methods. This questionnaire had been dispatched to the NRLs by circular letter dated 19/06, replies had been requested by 01/08.

G. SUHREN and B. LOMBARD had prepared the outcome of the questionnaire, based on replies received (17), dispatched by e-mail of 07/09. B. LOMBARD orally presented the outcome for sections 1 & 2, G. SUHREN for section 3 (see slides, enclosed).

Briefly:

- 16 countries apply a conversion procedure between the instrumental methods and the reference method, as to express the results in the unit of the reference method (except CH and N where the results of the alternative methods are not converted and expressed in number of alternative method units).
- 11 countries have defined one conversion characteristic (CC) for the whole country, 2 others have different CC depending on regions, 2 depending on animal species and 2 depending on the type of alternative method.
- 11 countries follow ISO 21187 for establishing the CC.
- 9 countries use a linear regression equation with log-transformed values.

It was noted that extreme CC values were found in France, depending on the region. An important point was noticed: if ISO 21187 was followed, the variability range between different CCs was much narrower !

Several participants made some comments, additions and mentioned some factors significant for the CC formula .

➔ Further additions/modifications for the outcome were requested by 22/09, as to finalize the outcome of the questionnaire.

Demonstration of an automated instrument for the determination of "total bacterial count in milk" in a research laboratory

G. SUHREN introduced the demonstration of the BactoScan FC (see her slides) that took place in the BfEL laboratory, which has been involved in the development and the validation of this apparatus.

Visit of Milk Control Laboratory of Schleswig Holstein

BfEL organized our transportation to the Milk Control Laboratory (MCL) of Schleswig Holstein, located in Kiel The participants were received by the MCL Head, Henning ROWEHL, who introduced us in a large-scale laboratory for routine raw milk analysis, with several BactoScan apparatus.

Present status of revision of Standard EN ISO 16140

B. LOMBARD gave a short presentation (see his slides, enclosed) on the Standard EN ISO 16140 devoted to the validation of alternative methods in food microbiology, and its revision recently started, within WG 3 "Method Validation" of ISO/TC 34/SC 9.

He emphasized that one of the reason to revise the standard was to cover the case of quantitative alternative/instrumental methods for TF in raw milk, further to an IDF request. In WG 3, a liaison is ensured with IDF through Inger ANDERSSON.

Revision of EN ISO 16140 with special emphasis on the parameter "total bacterial count"

G. SUHREN gave a presentation, prepared with I. ANDERSSON, to explain why the case of instrumental methods for TF in raw milk needed to be covered in a specific way, within the larger topic of validation of alternative methods in food microbiology. She then introduced these specificities, including:

- attributes of the routine method (measuring range, carry-over effect, precision, evaluation of factors affecting the results),
- alternative method as an estimate of the reference method,
- rating of the elaborated attributes.

These specific aspects would be covered in a given part or in an annex of the future revised EN ISO 16140. A cross-reference to ISO 21187 would also be made.

Establishing a conversion relationship between routine method results and anchor method results and its verification

Harry van den BIJGAART had been invited to take part to the workshop, on behalf of IDF/ISO, to present the standardization works on the topic, especially to develop the joint Standard ISO 21187 IDF 196 on CC. He gave a presentation (see his slides appended) on the history of the standard, and on its content. He developed the factors influencing the CC value, as well as the guidance to establish CC. He finally suggested some key points that should specifically concern the CRL and the NRLs regarding that topic: responsibilities, pre-requisites for applying alternative methods, sound protocol for applying CC, conformity of actual practice with written protocol.

Measurement uncertainty

B. LOMBARD gave a presentation on measurement uncertainty (MU) in quantitative food microbiology (see his slides, enclosed): both MU estimation, according to ISO/TS 19036, and the interpretation of results in terms of conformity with legal limits. In the EC Regulation 2073/2005 on microbiological criteria for operators, MU is not taken into account in an harmonized way, and for official controls, the decision rules are left up to the competent authorities in each country.

It was commented that ISO/TS 19036 did not cover the case of MU of CC, whereas it should not be avoided in our case.

➔ It was agreed that CC uncertainty should be addressed in the case of TF control in raw milk by alternative methods, and that its estimation should be further investigated, based on a paper to be provided by Thomas BERGER (CH).

Conclusions

A series of conclusions were drawn on the following aspects from the presentations and discussions during the workshop.

<u>Factors influencing CC value</u>

➔ According to the replies to the NRL questionnaire, the presentation of Hvd Bijgaart and the discussions, the following factors were considered as having a significant influence on the CC value:

- Type of measuring apparatus.
- Nature of the milk: species, colostrum.
- Extreme geographical differences: islands/continent, non European locations (such as Guyanne), different climates but not mountains/flat land.
- Sample handling: storage (time/temperature), preservation, transportation (time/temperature).

Requirements for CC determination

➔ Requirements for CC determination:
- to determine CC according to the guidance of ISO 21187;
- to estimate CC formula over 1 year (as to average seasonal variations, collection frequency).

Requirements on alternative methods

➔ Given the amount of evaluation studies performed on the Bactoscan series and available in the scientific literature, Carole MICMACHER, the DG SANCO representative at the 2001 workshop of the NRLs Milk (11&12/10) had clarified that this apparatus could be used for official control of raw milk, as a temporary measure awaiting the implementation of the MicroVal certification of alternative methods in microbiology according to EN ISO 16140. This temporary approval had been reassessed by T. CHALUS at the 2003 workshop (3&4/07), but he had clarified that a trade name of an instrument could not be written in the European legislation to avoid a commercial exclusivity. This was recalled as still valid.

➔ Regarding other and new apparatus, it was clarified that they could be used for TF official control in raw milk, if at least validated according to EN ISO 16140 and/or IDF 161. A certification was not yet required, because not yet implemented to date.

Responsibilities

➔ Each NRL is responsible for the CC determination in its country:
- 1 CC per country as a general rule, or more if justified,
- Implementation of ISO 21187 when establishing CC.
- Some special tasks can be outsourced (e.g.: statistical calculations, analyses) to, for example, companies producing/selling instruments.
- The NRL needs to have access to details (information of samples analysed, analytical data used for CC establishment, for deriving the different factors in the formula for conversion if needed. In case of availability problems, the NRL shall refer to the national competent authority.
- The NRL should visit laboratories establishing CC.

Work programme

➔ It was agreed that an inter-laboratory trial should be organized in 2007 on the reference method for TF (EN ISO 4833):
- for NRLs,
- for the laboratories identified by the NRLs, using the reference method to estimate CC value.

Closure, 12 September, 3 pm

B. LOMBARD, on behalf of the CRL Milk and of the participants, thanked P. TEUFEL, G. SUHREN and all their colleagues for their warm welcome, the excellent organization of the workshop, the visit of the laboratory and of the milk control station, and the nice dinner the day before.

B. LOMBARD gave special thanks to Gertraud, who, unfortunately for us, had announced that she would retire in the coming months. He highlighted her great input into our works, especially for the milk hygiene (TF, somatic cell count), she has been clearly our reference on these topics. He also stressed her human qualities of kindness.

B. LOMBARD, 24 November 2006

Annex 2b List of attendance CRL/NRLs Workshop 2006

9th Workshop of the NRLs "Milk & Milk Products"

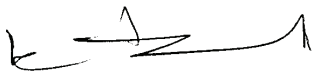
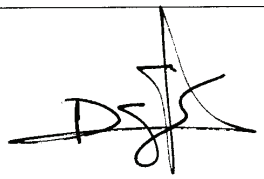
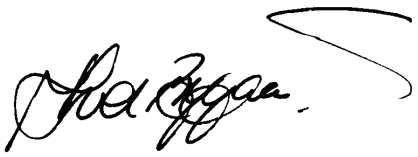
BFEL, Kiel – 11 & 12 September 2006

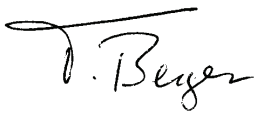
List of attendance

NAME, ADDRESS	SIGNATURE
Mrs Paula ALVES **Senior Researcher** **LNIV** **Estrada de Benfica, 701** **PT – 1500 LISBOA**	
Mrs Anna-Maria FERRINI **Researcher-Coordinator NRL Milk** **Istituto Superiore di Sanita (ISS)** **Viala Regina Elena, 299** **IT - 00161 – ROMA**	**EXCUSED**
Mrs Tuula PIRHONEN laboratory **Microbiologist, quality manager** **Food Safety Authority (EVIRA)** **Mustialankatu 3** **FIN - 00790 – HELSINKI**	Tuula Pirhonen
Mr Theophanis SAGRIS **Head of Microbiology Departement** **Veterinary Laboratory of Larisa** **Ad National Road of Larisa-Trikala** **6 km** **GR - 41110 – LARISA**	EXCUSED

Mrs Klytemnestra VELETA **Head of Laboratory** **Veterinary Laboratory of Patras** **15, Notara street** **GR - 264 42 – PATRAS**	
Mr Miguel RODRIGUEZ **Director** **Laboratorio Agroalimentario de Santander** **Ministerio de Agricultura, Pesca y Alimentacion** **Prolongacion Marqués de la Hermida s/n** **SP - 39011 - SANTANDER - CANTABRIA**	
Mr John EARLY **Scientist** AGRIC Food & Biosciences Institute ~~**Dept of Agriculture for Northern Ireland**~~ **AFBI-Food Microbiology** **Newforge Lane** **UK - BELFAST, BT9 5PX** N. Ireland.	
Mrs Bernadette HICKEY **Agriculture Inspector** **Dairy Science Laboratory** **Backweston Campus** **Young's Cross** **Celbridge - Co. Kildare** **Ireland**	
Mrs Wilma JACOBS-REITSMA **Coordinating Microbiologist** **RIKILT- Institue of Food Safety** **Bornesesteeg 45** **NL - 6700 AE - WAGENINGEN**	
Mrs Claudia KRALIK **Coordinator NRL Milk** **Autrian Agency for Health and Food Safety (AGES)** **Spargelfeldstraße 191** **A - 1220 WIEN**	

Mrs Gertraud SUHREN **Scientist** **Federal Reserach Center for Nutrition and Food (BFEL)** **Hermann - Weigmann - Straβe 1** **D - 24103 – KIEL**	
Mr Geert VAN ROYEN TIM DE COCK **Scientist** **ILVO** **Brusselsesteenweg 370** **B- 9090 – MELLE**	
Mr Peter ZAJAC **Expert milk & milk products** **State Veterinary and Food Institute Nitra -NRLM** **Akademicka 3** **SK - 949 01 NITRA**	
Mrs Jolanta ROLA **Responsible of reference activities** milk and milk products **PIWET - National Veterinary Research Institute** **Al. Partyzantow 57** **PL - 24 100 PULAWY**	
Mrs Liga JANKEVICA **Microbiologist** **Dairy Laboratory** **Instituta Street 1** **Stopinu District** **LV – 2169 ULBROKA**	EXCUSED
Mr Toomas KRAMARENKO **Chief specialist** **Estonian Veterinary and Food Laboratory** **Kreutzwaldi, 30** **EE - 51006 TARTU**	

Mrs Ruta BUBULIENE **Head of Food Microbiology Departement** **National Veterinary Laboratory** **J.Kairiukscio, 10** **LT - 08409 VILNIUS**	**EXCUSED**
Mrs Krisztina FIAS-KISS **Food Microbiologist** **National Food Investigation Institute** **Mester Street 81** **H – 1095 BUDAPEST**	
Mr Constantinos ECONOMIDES **Veterinary Officer** **LCFAO** **Departement of Veterinary Services** **CY – 1417 ATHALASSA**	
Mr Karl ECKNER **Research Manager** **Norsk Matanalyse** **Postboks 6166 Etterstad** **NO - 0602 OSLO**	
Mr Véronique LAFARGE **AFSSA-LERQAP** **Unit CHPL**	**EXCUSED**
Mr Harrie VAN DEN BIJGAART **C/O IDF-ISO/TC 34/SC 5** **Netherlands Milk Control Station** **P.O. Box 119** **NL-7200 AC Zutphen**	

Mr Thierry CHALUS **EC/DG SANCO** **Rue de la Loi 200** **B – 1049 BRUXELLES**	**EXCUSED**
Mr Bertrand LOMBARD **Co-ordinator CRL Milk** **AFSSA-LERQAP**	
Mr Thomas Berger Agroscope Liebefeld - Posieux Research Station Schwarzenburgstr. 161 CH - 3003 Bern	

Annex 3a. Report of the CRN/NRLs Workshop 2007.

AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

Site de
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LABORATOIRE D'ÉTUDES
ET DE RECHERCHES SUR
LA QUALITÉ ALIMENTAIRE
ET SUR LES PROCÉDÉS
AGROALIMENTAIRES

EU COMMUNITY REFERENCE
LABORATORY FOR
MILK AND MILK PRODUCTS

Report of the 10th Workshop of the National Reference Laboratories for Milk and Milk Products

28th & 29th June 2007
AFSSA-LERQAP, Maisons-Alfort, France

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RÉPUBLIQUE FRANÇAISE

Opening : Thursday 28th June, 9.30 am

Laurent LALOUX, Acting Head of the AFSSA Laboratory for Study & Research on Food Quality & Food Processes (LERQAP), Community Reference Laboratory for Milk and milk products (CRL Milk), opened the meeting and welcomed the participants.

Bertrand LOMBARD, CRL Co-ordinator, introduced the meeting that he would chair. He also introduced Adrien ASSÉRÉ, CRL Deputy co-ordinator, who brings him assistance for the management of the CRL and the coordination of the NRLs network.

B. LOMBARD reminded that the scope of the CRL/NRLs Milk had been restricted in 2006 to the hygiene of raw milk (total floral –TF, somatic cells count –SCC) and to the heat-treatment characterization of milk and milk products (phosphatase). The pathogenic criteria for milk and milk products (processing and end-products) were now covered by other CRLs/NRLs.

Roll-call of delegates

Each delegate introduced itself (the list of attendance is available on http://crl.lerqap.free.fr/espace/?key=16de27e06eb166657d0f651c6ea19502). 29 delegates from 27 NRLs of 27 EU Member States and 2 associated countries (NO, CH) were represented, as well as EC/ DG SANCO "Health & Consumer Protection" (Thierry CHALUS).

Roger WOOD, FSA (Food Standard Agency, UK) was also invited in order to present the criteria approach for the phosphatase topic.

Additional documents (i.e. agenda and presentations) are available on the following URL: http://crl.lerqap.free.fr/espace/?key=5e75b2c7441d8c407b7964bf2b4ca228

1. General information

1.1. Testing methods for raw milk and heat-treated milk of the Commission Regulation (EC) No 1664/2006

T. CHALUS presented the new Regulation 1664/2006 that establishes for the analysis of milk and milk products the methods to be used for own checks and official controls for total flora, somatic cells and alkaline phosphatase activity. This regulation specifies the reference methods and the conditions of use of alternative methods (see his slides). Criteria for heat-treatment of goat and ewe's milk would be established as soon as a limit for phosphatase would be defined.

T. CHALUS also informed that the Hygiene Package regulations would follow a revision process by the European Commission (EC) in 2009. Regarding milk, EC did not intend to modify the criteria for raw milk (TF, SCC). EC intended to define criteria for colostrums, similar to the ones for milk, but with no exception for TB and brucellosis. The definition of raw milk would not be modified, but enlarged to all "farm animals".

➔ Regarding colostrums, upon request of T. CHALUS, NRLs were invited to send to the CRL any information available on TF & SCC levels in colostrums in their respective countries.

T. CHALUS presented the different regulatory texts concerning milk and milk products and their connections. T. CHALUS promised to prepare a table presenting these different texts that would be distributed to the NRLs. The consolidated versions of the different regulations can be found on the Eur-Lex website (see http://eur-lex.europa.eu/RECH_consolidated.do).

B. LOMBARD recommended Constantinos ECONOMIDES (CY) (and other NRLs) to contact the CRL for antimicrobial residues in food (AFSSA Fougères, http://crl.fougeres.afssa.fr/) for information about the reference methods for antimicrobial substances in milk.

B. LOMBARD mentioned that the scope of the CRL Milk was restricted to hygiene. Therefore casein analyses were out of scope. He suggested to refer to the legislation or to contact Marina NICOLAS (AFSSA-LERQAP, Unit CALAS) or R. WOOD, participating to the EC expert group on that topic.

2. Work undertaken as former CRL Milk and Milk Products

2.1. Enumeration of staphylococci

Alexandra CAUQUIL (AFSSA-LERQAP, Unit HMPA) presented the work performed by her unit in the former mission of the laboratory as CRL Milk & Milk Products. The HMPA Unit had organized a stability study of staphylococci in liquid pasteurised milk for proficiency testing (PT) trials. This study had concluded to the suitability of either boric acid mixture for low contamination levels or boric acid alone for medium and high contamination levels. See her slides.

The stability had not yet been tested for TF, it would be done in the future.

A. CAUQUIL presented also the outcome of the December 2005 PT trial on staphylococci enumeration. See her slides. The report can be found on the CRL website http://crl.lerqap.free.fr/espace/?key=8a6cc0620d00a9eadb9980938fdb5cf1.

From a more general point of view, T. CHALUS highlighted that the participation of NRLs to ring trials organized by the CRL was an important duty for the NRLs. He detailed the new requirements of DG SANCO on this aspect: DG SANCO asked to be informed by the CRLs of non-participating NRLs or of NRLs having deviating results with no satisfactory follow-up actions. B. LOMBARD added that PT was a important tool to improve the quality of analysis.

2.2. Enumeration of *Enterobacteriaceae*

A. CAUQUIL presented the results of a study on the applicability of ISO 21528-Part 1 (Most Probable Number-MPN) and Part 2 (Colony Counting Technique-CCT) for the enumeration of *Enterobacteriaceae* (see her slide). The report of this study is available on http://crl.lerqap.free.fr/espace/?key=c15823fe960f5daa9ff5b36e1b7c7be2.

The MPN technique showed a large variability but no other reference method was available for low numbers (below 100 cfu/ml). CCT gives more precise results over 100 cfu/ml.

B. LOMBARD informed that ISO/TC 34/SC 9 was preparing an amendment of the standard for both parts (CCT and MPN) replacing glucose agar by OF medium for confirmation. Moreover the part 1 (MPN) could be simplified by omitting the EE enrichment .

Some NRLs (BE, PT, UK) reported that ready-to-use media seemed to be more reliable than powdered media. With stressed strains and with some PT samples, differences were found between manufacturers.

Bernadette HICKEY (IE) pointed out that the MPN technique was too time-consuming for own checks for an indicator flora (ISO 21528-1 is prescribed as the reference method in some *Enterobacteriaceae* criteria of Regulation 2073/2005 on microbiological criteria for food).
B. LOMBARD fully supported this view for a laboratory point of view but as CRL was not able to give opinion on criteria in the DG SANCO working group in charge of Reg. 2073. Moreover, she mentioned that the criterion 2.2.7 of this regulation (*Enterobacteriaceae* in milk powder and whey powder) states a limit of 10 cfu/g, and CCT instead of MPN could be used to check this limit.

➔ It was agreed that the following comments would be transmitted
- by the CRL to DG SANCO,
- by the NRLs to their Competent Authorities, that could forward them to DG SANCO:
1/ The criterion 2.2.1 (*Enterobacteriaceae* in liquid pasteurised milk and other liquid dairy products), specifying limits of m< 1 cfu/ml and M=5 cfu/ml, requires the use of a MPN technique as ISO 21528-1, which is not practicable for routine own checks. The setting-up of higher limits would be needed from an analytical point of view.
2/ In the criterion 2.2.7 (*Enterobacteriaceae* in milk powder and whey powder), ISO 21528-2 should replace ISO 21528-1 as the reference method.

2.3. Detection of staphylococcal enterotoxins

Jacques-Antoine HENNEKINE (see his slides) summarized the 2 major activities of the laboratory as former CRL "Milk and Milk Products":

(1) the management of the NRLs' network (training & confirmatory analysis),

(2) the development and validation of analytical methods (intra- and inter-laboratory studies, development and improvement of the European screening method (ESM)). ESM v3 is available on http://crl.lerqap.free.fr/espace/?key=e4a086af5a6ac8344fb75509c4690144.

3. Main working areas

3.1. Milk hygiene and microbiology

a. Total flora in raw milk

- Work on flow cytometry at the CRL

A. CAUQUIL presented the flow cytometer (BactoCount IBCm) purchased by the CRL at the beginning of the year and the study in progress (see her slides).

Karin KNAPPSTEIN (DE) and Koen DE REU (BE) advised to trace each sample to the farm of origin, and to register also the age of the samples from milking, as to study the influence of these factors on the conversion characteristic (CC). The TF complete range should also be covered.

- Outcome of the 2006 NRLs workshop

B. LOMBARD presented the main topics covered at the 2006 NRL Workshop (Kiel, 11&12 September) dedicated to TF determination by alternative (instrumental) methods. He thanked again Gertraud SUHREN who had been the main organizer of the workshop ad who retired in the meantime: he paid tribute to her outstanding contribution to the works on this topic.

The conclusions of the 2006 workshop (see the report) were endorsed.

It was mentioned that instrumental methods had not been evaluated for colostrums.

➔ The CRL would include colostrums in its study using Bactocount.

- PT trial on total flora

A.CAUQUIL presented the PT trial organised in 2007 that would take place on week 40, exceptionally opened to non-NRL labs (the ones in charge of establishing CC). Given the limited number of participation replies received, the CRL would send a reminder the following Monday for an answer by the following Friday.

Some aspects of the PT trial were clarified:
- the results of the non-NRL participants would be transferred to the CRL via their respective NRLs;
- for the reference method, 1 plate/dilution could be used, in accordance with the new version of ISO 7218, to be released soon.

- Discussion

Hans-Georg WALTE (DE) presented an inter-comparison study with Bactoscan FC in Germany in 2007 (see his slides).

Discussion arose about the audit/visit by the NRLs of labs in charge of establishing CC.

A round table was performed about how CC is handled (one or several value(s), calculation, NRL involvement, …) in each Member States.

➔ In conclusion, the following actions were planned:

1. To re-dispatch the 2006 questionnaire to NRLs about CC values (to update replies and to get replies from new NRLs).

2. To prepare a check list for the NRLs to conduct their visit of labs establishing CC value (CRL, in collaboration with Karin KNAPPSTEIN & Koen de REU).

3. To work on instrumental methods other than Bactoscan (such as Bactocount), once the CRL study would have sufficiently progressed.

b. Somatic cell count

- Follow-up to the 2005 NRL Workshop

B. LOMBARD presented the actions undertaken on the topic of somatic cell count (SCC) since the 2004 NRL workshop dedicated to SCC (Kiel, 9&10 Sept.) and the 2005 general workshop (30/06-01/07).

BfeL had investigated the feasibility of manufacturing the template to implement the microscopic method and the outcome was positive. The NRLs could therefore contact directly BfeL (K. KNAPPSTEIN) to get it.

- Update on IDF/ISO works

➢ Reference method ISO 13366-1

B. LOMBARD reported on the revision of the Standard microscopic method ISO 13366-1. See documents sent prior to the workshop available on the following URL http://crl.lerqap.free.fr/espace/?key=d86285e7442dfbda871bfec2525385bb.

9 NRLs took part to the collaborative study organized for IDF/ISO by Silvia ORLANDINI (IT) in October 2005 for deriving the precision data of the revised Standard method. The report is available in the IDF Bulletin.

Cecildina PIRES GOMES (PT) wondered why, for the dying solution, tri-chlorethane had been replaced by tetra-chlorethane in the new version of the standard. B. LOMBARD recommended to use the end of the stock before shifting to the other solvent, but he would check with Silvia ORLANDINI.

➢ Reference system

Thomas BERGER (CH) presented a new concept introduced within IDF/ISO by Christian BAUMGARTNER (D). See his slides. The idea was globally to add to the definition of a reference method, not really satisfactory for SCC since based on microscopy, its implementation by a network of expert labs also involved in the development of reference materials that could be used to calibrate instrumental methods used by routine labs. In this scheme, the NRLs could play an important role as members of the expert network.

B. LOMBARD found the idea interesting and well adapted to the particular situation of SCC. It was clarified that some but not all NRLs could take part to this network.

➔ The CRL would follow closely the progress of this project within IDF/ISO.

3.2. Alkaline phosphatase activity

- 2006 PT trial

Anne-Cécile BOITELLE (AFSSA-LERQAP, Unit CALAS) presented the outcome of last year's PT testing on Alkaline Phosphatase (AP) activity in milk (see her slides).

For the PT trial, the changes in AP concentration with time were evaluated. No change was determined within 3 days.

R. WOOD recommended that for the statistics of PT trial (evaluation of laboratory performance), z-scores could be calculated on single results and not on duplicate means, and also on all data, including the ones of labs being considered as outliers.

➔ The CRL would recalculate z-scores according to R. WOOD's recommendations.

Discussions arose on how to derive the PT target standard deviation (σ_p): either from precision data obtained for the Standard method to be applied (current option), or from participants' results.

➔ A WG was set up to investigate (i) how to derive σ_p and (ii) how to evaluate participants' performance, in the particular case of AP activity. WG to be convened by the CRL and comprising R. WOOD, Miguel RODRIGUEZ (SP), T. BERGER (CH), Karl ECKNER (NO) and Jan DE BLOCK (CVUO, BE).

The next PT trial was planned in December 2007, on a reduced scale (whole and semi-skimmed cow's milk). It was clarified that it would be possible to use another method (Charm test) during the PT trial but the results could not be included in the data statistical processing for assessment of lab performance, since only Fluorophos is currently prescribed as the reference method for official controls.

- Update on works on goat's and ewe's milk

Marina NICOLAS (AFSSA-LERQAP, Unit CALAS) presented the update of the CRL works on that topic (see her slides).

➢ Goat's milk

Based on the trials performed on milk from several countries, the same limit than for cow's milk (350 mU/l) could have been settled at the 2005 workshop. But Theophanis SAGRIS (GR, Larissa) had reported the existence of values clearly above the intended limit, he was therefore to send milk samples to the CRL to further investigate the situation, but had not done so.

➔ The NRLs from EI, UK, NL, GR & CY were to send milk samples or results to the CRL as to settle the limit at the 2008 workshop.

➢ Ewe's milk

Data from SP and FR were not conclusive, there was a need for further data, with in particular a follow-up of the decrease in microbial load during heating.

> ➔ The CRL would send to the NRLs a reminder to get more data, especially from GR.

- State of the art on cheeses

M. NICOLAS presented the current information available on that topic (see her slides).

She pointed out the need of revision of the IDF/ISO Standard for thermized/raw milk cheeses, as well as the need to open the composition of the cheese buffer.

The determination of AP in soft cheese was a need from DG SANCO. Contributions from NRLs were requested.

> ➔ The NL, BE(CLO), PT and possibly D NRLs promised to collaborate on that topic.

- Comparison of the alternative method against the reference method

AC BOITELLE presented the results of a CRL study comparing Novalum and Fluorophos for AP activity in milk. (see her slides). For the time being, a bias between the 2 methods had been found. This was under investigation by the manufacturer of Novalum.

- Pertinence of the criteria approach

R. WOOD presented the criteria approach (see his pdf document and slides).

B. LOMBARD considered that in principle, for a determination which is based on an instrumental and which depends on manufacturers, the criteria approach would be preferable (is to state in the regulation performance criteria to be met by any methods), instead of referring to a given reference method depending on a proprietary instrument.

The criteria approach is applicable only to rationale methods (the results are not method-dependent, the analyte is fully chemically defined). The problem is that it does not seem to be the case for the analyte "AP activity".

> ➔ The topic needed further investigation and would be reconsidered at the 2008 workshop (possibility to implement the criteria approach for AP activity).

- Future activities

M. NICOLAS presented the foreseen activity (see her slides) and the work programme was defined (see item 4).

4. Setting up the work program for the following years

According to the needs identified in the former items of the agenda, and under the condition that necessary resources would be allocated to the CRL, a tentative work programme for the years 2007 to 2009 was defined (see Annex 1). In addition, the 2010 workshop would be devoted to SCC/alternative methods.

5. Closure of the workshop

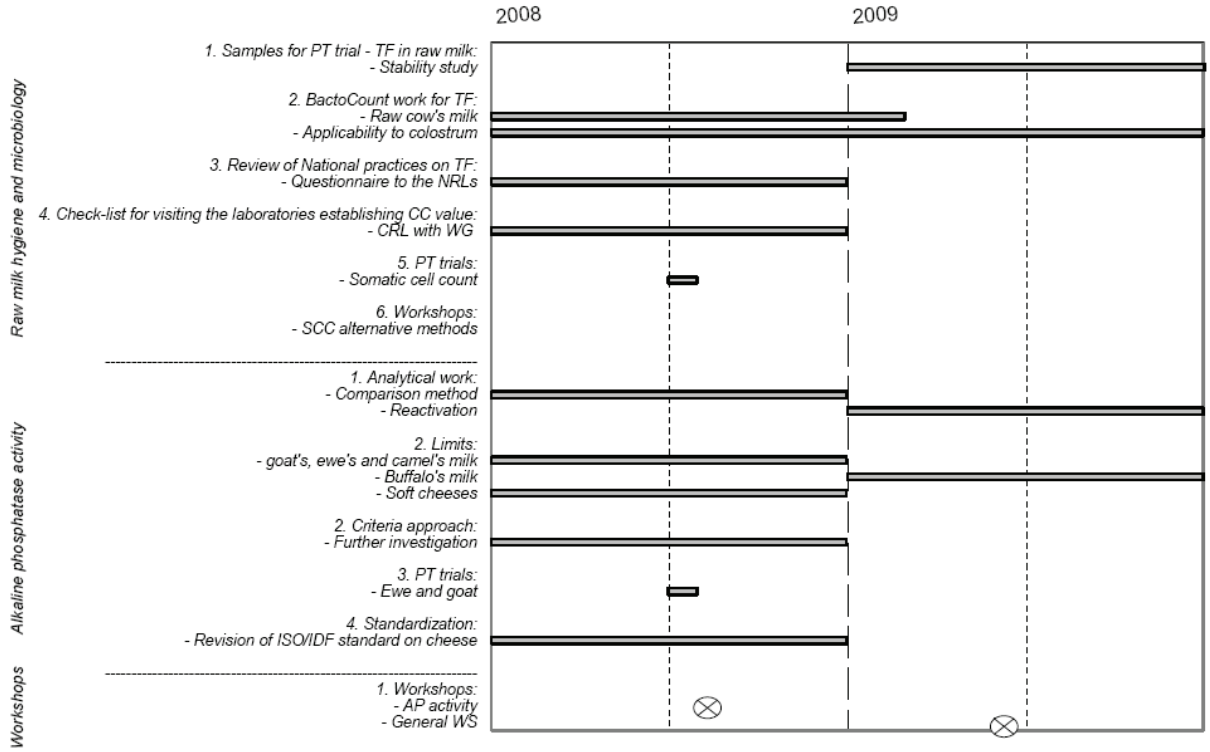
B. LOMBARD closed the meeting on Tuesday at 1:30 pm. He thanked all the attendees for their participation to that meeting, hoping that it had met their expectations.

The next workshop would probably take place in Vienna (AT), at the kind invitation of AGES (to be confirmed), during the 2nd semester of 2008. It would be a targeted workshop, dedicated to the Alkaline Phosphatase Activity.

6. Visit of the laboratory

B. LOMBARD and A. ASSERE lead 2 groups to visit the premises, to present the teams and works of the CRL activities.

EU CRL Milk and Milk Products - Work program



Annex 3b List of attendance 2007 CRL/NRLs Workshop

afssa
AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS

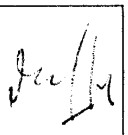
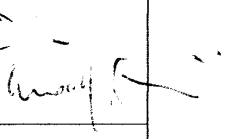
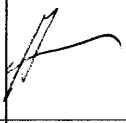
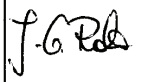
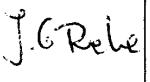
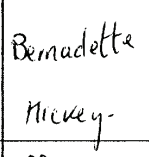
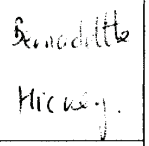
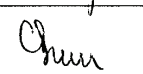
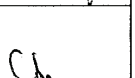
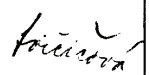
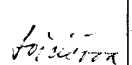
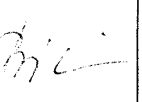
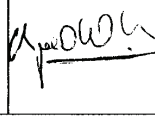
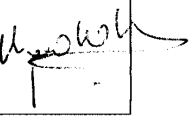
List of attendance

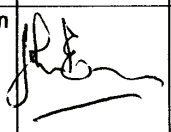
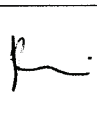
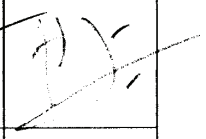
Eligible attendees

10th Workshop of the NRLs for Milk and Milk Products

June 28 & 29, 2007
AFSSA – LERQAP, Maisons-Alfort

Name and Surname	Address	June 28, 2007	June 29, 2007
Claudia Kralik	Austria **NRL** Institut für Lebensmitteluntersuchung Wien, Spargelfeldstrasse 191, 1226 Wien claudia.kralik@ages.at		
Koen de Reu	Belgium **NRL** Brusselsesteenweg 370, B-9090 Melle Koen.Dereu@ilvo.vlaanderen.be		
Véronique Ninane	Belgium **NRL** Département Qualité des productions agricoles - Chaussée de Namur, 24 - B-5030 Gembloux ninane@cra.wallonie.be		
Constantinos Economides	Cyprus **NRL** Cyprus Veterinary Services, 1417 Nicosia neconomides@vs.moa.gov.cy		
Katerina Bousova	Czech Republic **NRL** Sidlistni 24, Praha 6 - Lysolaje 165 03 katerina.bousova@svupraha.cz		
Heli Reinet	Estonia **NRL** Kreutzwaldi 30, Tartu 51006 heli.reinet@vetlab.ee		
Tuula Pirhonen	Finland **NRL** Mustialankatu 3, FI-00790 Helsinki, Finland tuula.pirhonen@evira.fi		
Karin Knappstein	Germany **NRL** Hermann Weigmann-Str. 1, 24103 Kiel, Germany karin.knappstein@bfel.de		
Klytaimnistra Veleta	Greece **NRL** 15, Notara Str - Patras, 26442 kepatra@otenet.gr		

Theofanis Sagris	Greece **NRL** Larisa-Trikala (National Road) - 41110 Larisa theosag@mail.gr		
A. Maria Ferrini	Italy **NRL** Istituto Superiore di Sanità - CNQRA- Viale Regina Elena, 299, 00162 Roma, Italy annamaria.ferrini@iss.it		
Inese Ozollapa	Latvia **NRL** 3 Lejupes str., Riga, LV-1076 inese.ozollapa@ndc.gov.lv		
Rūta Bubulienė	Lithuania **NRL** J. KAIRIŪKŠČIO 10, LT-08409, VILNIUS rbubuliene@nvl.lt		
Wilma Jacobs-Reitsma	Netherlands **NRL** PO box 230, 6700 AE Wageningen wilma.jacobs@wur.nl		
Jolanta G. Rola	Poland **NRL** 24 - 100 Pulawy, Al. Partyzantów 57 jolarola@piwet.pulawy.pl		
Celcidina Pires M. Gomes	Portugal **NRL** ESTRADA DE BENFICA 701, 1549-011 LISBOA celcidina.pires@lniv.min-agricultura.pt		
Bernadette Hickey	Republic of Ireland **NRL** Department of Agriculture and Food, Backweston Campus, Young's Cross, Co. Kildare bernadette.hickey@agriculture.gov.ie		
Cristina Gabriela Dumitrescu	Romania **NRL** Campul Mosilor Street, no.5, 021201, Bucharest iispv@b.astral.ro		
Ivičičová Adriana	Slovakia **NRL** State veterinary and food institute Nitra , Akademická 3, 949 01 Nitra nrlm@svunitra.sk		
Majda Biasizzo	Slovenia **NRL** Gerbeceva 60, 1000 Ljubijna majda.biasizzo@vf.uni-lj.si		
Miguel Ángel Rodríguez	Spain **NRL** Concejo s/n. 39011-Santander mrodriguezl@mapya.es		

Åsa Rosengren	Sweden **NRL** Box 622, SE-751 26 UPPSALA asro@slv.se		
John Early	United Kingdom **NRL** Newforge Lane, Belfast BT9 5PX - Northen Ireland john.early@afbini.gov.uk		
Adrien Asséré	France **CRL** DIR - 23, avenue du Gal de Gaulle - 94700 Maisons-Alfort a.assere@afssa.fr		
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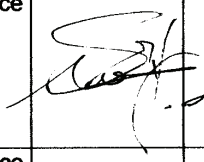
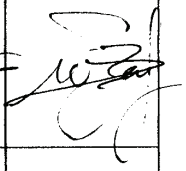
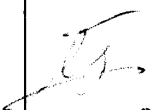
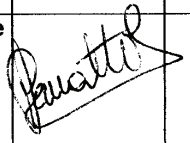
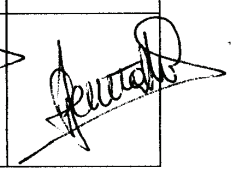
Foulo Basse	France **CRL** PAFT - 23, avenue du Gal de Gaulle - 94700 Maisons-Alfort f.basse@afssa.fr		
Jacques-Antoine Hennekinne	France **CRL** TOP-BAC - 23, avenue du Gal de Gaulle - 94700 Maisons-Alfort ja.hennekinne@afssa.fr		
Marina Nicolas	France **CRL** CALAS - 23, avenue du Gal de Gaulle - 94700 Maisons-Alfort m.nicolas@afssa.fr		
Nathalie Pfauwathel	France **CRL** CALAS - 23, avenue du Gal de Gaulle - 94700 Maisons-Alfort n.pfauwathel@afssa.fr		

Annex 4 Outcome of the 2006 questionnaire to the NRLs Milk

Determination of total bacterial count in raw milk in EU-member states

Outcome of the 2006 questionnaire to the NRLs "Milk"

Authors: Gertraud SUHREN (BfEL-Kiel)
& Bertrand LOMBARD (CRL "Milk", AFSSA-LERQAP)

Version 2, 26 October 2006

Background

At the 8th Workshop of the National Reference Laboratories "Milk and Milk Products" (30 June & 1 July 2005, AFSSA, Maisons-Alfort, France), it was decided to organize a workshop dedicated to alternative methods for total flora in raw milk. This workshop took place on 11-12 September 2006 at BfEL (Kiel, Germany).

In order to prepare this workshop, it was decided to circulate a questionnaire to the NRLs "Milk" to review national practices on that topic. The questionnaire was circulated on 19 June by the CRL "Milk" to the NRLs "Milk". The replies were requested by 1 August.

According to Regulation (EC) No.853/2004[1], raw milk has to be tested at least twice per month for the parameter "total bacterial count" –TBC- (plate count at 30°C per ml) in order to check whether the rolling geometric mean over a two-month period with at least two samples per month meets the criterion

≤ 100 000/ml for raw cow's milk or

≤ 1 500 000l (or 500 000/ml[2]) for raw milk from other species.

The analytical reference method for the determination of total bacterial count is the Standard EN ISO 4833 *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of micro-organisms – Colony count technique at 30°C*.

In many countries raw milk is tested by alternative methods for the purpose of

(i) controls according to Regulation 853/2004 and/or

(ii) milk quality payment.

[1] Annex II, Section IX "Milk & Milk Products", Chapter I "Raw Milk – Primary Production", III "Criteria for raw milk".

[2] If the raw milk is intended for the manufacture of products made with raw milk by a process that does not involve any heat treatment.

The controls performed according to Regulation 853/2004 can be carried out, according to the text of this regulation[3]:

1. by/on behalf of milk producers, operators collecting/processing the milk,
2. in the context of national/regional control schemes.

Well known examples of instrumental alternative methods are Bactocount™, Bactoscan™, plate loop method.

The questionnaire prepared in collaboration by Gertraud SUHREN (BfEL-Kiel) and Bertrand LOMBARD (AFSSA-LERQAP) comprised 3 sections:

1. the situation of milk production,
2. the situation of testing TBC in ex-farm raw milk
3. the use of results from alternative methods within Regulation 853/2004.

Each NRL was to describe the situation in its country, or in some cases in a given part of the country (region).

The NRLs were encouraged to use the national network of milk control laboratories, breeders associations or comparable organisations.

Replies received

On 1 September, 17 replies were received from the following NRLs (or replying on behalf):

BE/Belgium (Geert VAN ROYEN, ILVO)	CH/Switzerland (Thomas BERGER, ALP)
CY/Cyprus (Charalambos KAKOYIANNIS, LCFAO)	D/Germany (Gertraud SUHREN, BfEL)
EE/Estonia (Toomas KRAMARENKO, VFL)	
ES/Spain (Javier LAGUNILLA, LIGAL-Galicia for CAN)	ES (Miguel RODRIGUEZ, LA Santander)
FI/Finland (Tuula PIRHONEN, EVIRA)	FR/France (Sabine PICARD, CNIEL for AFSSA-LERQAP),
IE/Ireland (Bernadette HICKEY, DSL)	LU/Luxemburg (Robert KOETZ, ASTA for LNS)
LV/Latvia (Inese OZOLLAPA, NDC)	MT/Malta (Angele CASHA, MRAE)
NL/Netherlands (Wilma JACOBS, RIKILT)	NO/Norway (Karl ECKNER, Matanalyse)
PL/Poland (Jolanta ROLA, PIWET)	PT/Portugal (Celcidina PIRES-GOMES, LNIV)
SK/Slovakia (Peter ZAJAC, SVU-Nitra)	

[3] Annex II, Section IX "Milk & Milk Products", Chapter I "Raw Milk – Primary Production", III "Criteria for raw milk"

Summary of the replies

Questions 1: Situation of milk production

A summary of the replies received is given in Table 1[4].

Question 1.1: The milk of how many dairy farms need to be tested in your country according to Regulation (EC) No. 853/2004?

The number of dairy farms to be tested received vary widely from one country to the other, from 350 (MT) to 310 000 (PL). It may be questioned whether in some cases, the number would correspond to the farms both controlled according to Regulation 853 and for milk payment. For France, it has been specified in that sense.

Question 1.2: How many farms produce

Cow's milk ……
Goat's milk ……
Sheep's milk ……
Milk of other species – please specify: ……

Logically, the vast majority of farms produce cow's milk, with a number varying largely, from 140 (MT) to 730 300 (PL). For the other species, in some cases the numbers are not known. Only 2 countries indicated other species than cow, sheep and goat: buffalo in CH and NL, horse in NL.

Question 1.3: What is your average herd size?

Cows ……
Goats ……
Sheep ……
Milk of other species – please specify: ……

The average herd size most often given is for cows, and is always below 100 (except in CY: 250), from 4 (PL). It is generally smaller than for the 2 other species.

Question 1.3: How often is raw milk delivered to the dairies?

Twice a day ……*% of dairy farms*
Once a day ……*% of dairy farms*
Every second day ……*% of dairy farms*
Every third day ……*% of dairy farms*
Others – please specify ……

For most of the countries, the milk is most often delivered to the dairies once a day. In CY, it depends on the species: twice per day for sheep and goat, every second day for cow. In LV, it depends on the season: once a day in summer/autumn, and 70 % every 2nd day in winter/spring.

[4] The tables and figures are gathered at the end of the document, in annexes I & II.

Questions 2: Situation of testing TBC in ex-farm milk

A summary of the replies is given in Table 2.

Question 2.1: Which type of laboratory(ies) analyse(s) milk samples (e.g. food control laboratories, milk control laboratories, private/public laboratories)?

Milk control laboratories (MCL) perform analyses of milk samples in all countries, at least by alternative methods and sometimes by the reference method also. In some countries, private laboratories are also involved. In SK, the official controls are performed only by the NRL.

Question 2.2: Do you use the results of total bacterial count in ex-farm milk:

Only with respect to food hygiene legislation (853/2005) ? *yes* □ */ no* □

Both – for quality payment and hood hygiene legislation ? *yes* □ */ no* □

Most of the countries use TBC results for both food hygiene legislation and milk quality payment, except in PL where the results are used only for food hygiene.

Question 2.3: Which analytical method(s) is/are applied?
Please specify the reference method at national level (e.g. national standard) and the version of the alternative methods (e.g. Bactoscan FC).

EN ISO 4833 or comparable national standard ……..
Bactocount ……..
Bactoscan ……..
COBRA ……..
Petrifilm ……..
Plate loop method ……..
Simplified colony count method ……..
Others ……..

Except LIGAL/Galicia in SP, all countries/regions (the NRL often) use a reference method: the EN ISO 4833 Standard or equivalent.

Regarding the alternative methods:

- the Bactoscan series 8000/FC is used in most countries: 16,
- the Bactocount is used in several countries: 6,
- the plate loop technique: 4 countries,
- COBRA :1 country,
- a simplified CCT: 5 countries,
- Petrifilm: 1 country.

Most of the countries use several types of alternative methods, depending in particular of the species (NL, NO) or the size of the labs (PL).

Sample handling

Samples are taken
Manually
yes □ / *no* □
automatically
yes □ / *no* □

Samples are
not preserved
yes □ / *no* □
refrigerated at ……°C
chemically preserved
yes □ / *no* □
by (e.g. azidiol)……

Time/temperature conditions between sampling and analysis
…….hours at ……°C

In most of the countries, samples are taken automatically or both automatically/manually. In EE and NL, they are taken only manually, and mostly manually in ES (Santander) and LV.

Samples are preserved in most of the countries, except in CH. The most frequent preservation mean is refrigeration, combined sometimes with a chemical (azidiol), or azidiol alone (ES, LU, MT).
The storage conditions vary in time (up to a maximum of 24 to 72 h), at a refrigeration temperature.

Question 3: Use of alternative method results within Regulation (EC) 853/2004

A summary of the replies is given in **Table 3.**

Question 3.1: Is there in your country a conversion procedure for converting alternative method results onto the scale of the reference method, in which the limits of Regulation (EC) No. 853/2004 are fixed?

With the exception of one country (IRL), all NRLs replying indicated that a conversion procedure is applied. Another country (NL) did not specify the conversion applied and made the remark that the farmers are paid on the basis of the results of the alternative method; in this case it is no clear how the results have to be interpreted with respect to Regulation (EC) No. 853/2004.

Question 3.2: Do you use only one conversion characteristic in your country or different ones with respect to regions and/or species?

In 11 countries one conversion is applied for the whole country.
Different conversions are applied in 2 countries (ES and FR) with respect to regions, in 2 countries (FR and SK) with respect to animal species and in 2 countries (CH, LV) with respect to the alternative methods applied. As summarized in **table 2,** different alternative methods are applied in several countries, but answering question 3 only in these 2 cases the respective conversion characteristics are specified.

Question 3.3: Did you follow *ISO 21187/IDF 196 Milk – Quantitative determination of bacteriological quality – Guidance establishing a conversion relationship between routine method results and anchor method results and its verification* when establishing the conversion characteristic(s)?

11 NRLs answered this question with "yes", one with "no" and 4 did not give an answer. It is assumed that the latter did not follow ISO 211687 when establishing their conversion characteristics.

Question 3.4 asked for the specified conversion(s) for example as an equation for the linear regression.

9 NRLs specified their conversion characteristic(s) by a linear regression equation of log-transformed values.

Of these with the exception of one region (ES-1), all used the counts of the alternative method as the independent variable x and the transformed value on the scale of the reference method as the dependent variable y.
In one country (EE), two equations are indicated, depending on the cfu-number.
Two countries (BE and FR) apply conversion tables and 3 countries (LU, MA and PL) the conversion of a neighbour country.
One country relies on conversion factors established by the manufacturer of the instrument (IRL).
In 3 counties (CH, DE and ES), the conversions are actually under progress and discussion respectively.

The conversions reported are displayed in **fig.1** for the Bactoscan FC method and cow's milk or not further specified animal species and in **fig 2** only those conversions where ISO 21187 was

followed. **Fig. 3** gives the conversions for sheep's and goat's milk with Bactoscan FC method. **Fig. 4** displays the conversions applied with the Bactoscan 8000 method for cow`s milk or not further specified species. . In all graphs only the spectrum between 10 and 1 000 Bactoscan counts are considered. The conversions which were established without considering ISO 21187 are represented by dotted lines. As for the Bactocount method only one conversion was specified it is not reported in a graph.
The graphs show great differences between the conversions indicated. But as it becomes obvious from fig. 1 and 2, the differences are lower when ISO 21187 is followed.

Annex I: Tables

Table 1: Situation of milk production (C): for cows, (S+G): for sheep & goat NA: non available A: Algarve, no information for other regions

Question Nr		***1.1***	***1.2 (number farms)***				***1.3 (herd size)***				***1.4 (delivery frequency, per day)***			
Country		***(tot. nb farms)***	***Cows***	***Goats***	***Sheep***	***Other***	***Cows***	***Goats***	***Sheep***	***Other***	***2x***	***1x***	***2nd***	***3rd***
Belgium	BE	15 253	15181	62	10		34	150	100		0	0	10	90
Switzerland	CH	31 673	31 673	3 125	781	7 (buffalo)	18	11	10	Small	25	8	66	0
Cyprus	CY	?	230	4100			250	200			100 (S+G)		100 (C)	
Germany	DE	114 000	113 500	277	111		40	NA	NA		0	25	75	0
Estonia	EE	1 620 (C)	1732	272			11-100	1-5			0	90	10	
Spain 1[5]	ES	15 300	15 300				20-25				0	15	85	0
Spain 2[6]	ES	see[7]	28 786	20 635	24 727		35	69	107		3 (C)	47 (C)	40 (C)	10 (C)
Finland	FI	14 800	14 800	NA			20				0	0	100	0
France	FR	105 120[8]	97 507	3 070	4 543		38	116			NA			
Ireland	IE	22 386	22 386	NA	NA		49	NA	NA				Majority	
Luxembourg	LU	920	920				40				0	0	100	0
Latvia	LV	58 664	56 357	2307			6	4			0	100[9]; 30[10]	70[11]	0
Malta	MT	350	140	200			60	10			0	100	0	0
Netherlands	NL	21 500	21 150	350	10	10 (horse) 5 (buffalo)	65	500	NA	NA	0	<1	20	80
Norway	NO	17 860	17 300	500			17	83			0	0.3	14	61[12]
Poland	PL	310 000	730 300	50 700	16 100		4	3	20			60	40	
Portugal	PT	16 000	16 000	288 A	NA		19	42 A	NA	NA	0.1(C), 48.6(S)	0.5(C), 0 (G)	99.5(C), 0 (G)	0(C), 95.2 (G)
Slovakia	SK	1299	933	15	351		NA				0	90	10	0

[5] LIGAL (Galicia) for NRL-CNA (Majadahonda). Replies for the Galicia region.
[6] NRL-LA Santander
[7] 2 % random and 100 % if non compliance detected for inhibitors, SCC and TBC
[8] for milk payment
[9] in summer/autumn
[10] in winter/spring
[11] in winter/spring
[12] + 25 % > every 3rd day

Table 2 : Situation of testing TBC in ex-farm milk

Question Nr		***2.1***	***2.2 (Use of results)***	***2.3 (Methods)***		***2.4 (Sample handling)***		
Country		***Type Lab (incl. milk control labs –MCL)***	***Reg. 853:*** - ***only*** - ***+quality payment***	***Reference method EN ISO 4833 ?***	***Alternative methods***	***Sampling (manually/ automatically)***	***Preservation***	***Storage***
Belgium	BE	MCL (private)	Both	Yes or AFNOR NF 08-051	BSC FC	Auto	0-4°C	< 36 h/0-4°C
Switzerland	CH	MCL	Both	MDSA, ch. 56, E1 (eq. EN ISO 4833)	BSC 8000 then FC (2007)	Man/auto	No	< 30 h/2,5°C or < 12 h/< 8°C
Cyprus	CY	Governmental (NRL), ½ gov.(MCL) and private labs	Only (NRL) and both (MCL)	Yes (NRL)	Bactocount (MCL)	Man (NRL) /auto (MCL)	4°C	< 24h/4°C
Germany	DE	MCL (private)	Both	Yes (German Food Law)	BSC FC	Auto	Azidiol, Kathon, boric acid mixture	24-48h/≤8°C
Estonia	EE	Official, MCL and private	Both	Yes	BSC 8000/SPC	Man	0-4°C	< 36h/0-4°C
Spain 1	ES	MCL	Both	No	BSC FC	Man/auto	4-6°C + azidiol	24-48h/4-6°C
Spain 2	ES	MCL (mostly private)	Both	Yes	Bactocount: 1 BSC FC: 12 BSC 8000: 2	Man (mostly)	Azidiol (mostly)	< 72h/<6°C
Finland	FI	Private dairy labs	Both	Yes	Bactoscan FC: 2 Plate loop	Auto	6°C	36-72h/<6°C
France	FR	MCL	Both	Yes	Bactocount: 1 BSC 8000/FC: 13 COBRA: 3 Plate loop: 3	Man/auto	2°C	24-48h/2°C
Ireland	IE	Private (15)	Both	Yes	BSC 8000/FC: 5 Plate loop: 7 Simplified CCT: 2	Auto	<4°C	< 24h/<4°C
Luxembourg	LU	Governmental MCL	Both	Yes	SPC, BSC FC	Auto	Azidiol	<36h/<8°C

Question Nr		*2.1*	*2.2 (Use of results)*	*2.3 (Methods)*		*2.4 (Sample handling)*		
Country		*Type Lab (incl. milk control labs –MCL)*	*Reg. 853:* - *only* - *+quality payment*	*Reference method EN ISO 4833 ?*	*Alternative methods*	*Sampling (manually/ automatically)*	*Preservation*	*Storage*
Latvia	LV	Governmental (NRL), private MCL, dairy labs	Both	Yes (NRL)	Bactocount-70: 1 IBC-50: 1 BSC-FC: 1 CCT	Man (mostly)	4°C + azidiol	36h/0-4°C
Malta	MT	MCL	Both	Yes	Bactoscan FC	Auto	Azidiol	24h/4°C
Netherlands	NL	MCL (private)	Both	Yes	SPC (S), BSC-FC (C+G)	Man	0-4°C	1-36h/0-4°C
Norway	NO	MCL	Both	Yes	Bactocount (C) Plate loop (G)	Auto	0-4°C	36h/0-4°C
Poland	PL	Public, private MCL	Only	Yes	Bactocount 70: 7 BSC 8000: 30 Petrifilm (small labs) SPC (small labs)	Man/auto	0-4°C	24-47 h/0-4°C
Portugal	PT	NRL and official food lab, private (milk payment)	Only/both	Yes and comparable national standard	Bactoscan (at least 10 labs), simplified CCT: 1	Man/auto	2-5°C 4°C+Azidiol	4-24 h
Slovakia	SK	NRL (official control), private (milk payment)	Only/both	Yes (NRL)	BSC	Man/auto	4-8°C Azidiol, Heeschen r.	24 h/4-8°C

Table 3: Use of alternative method results

Question No.		***3.1***	***3.2***	***3.3***	***3.4***	
Country		***Conversion procedure existing***	***Number of conversions applied***	***Based on ISO 21187***	***Specified conversion(s)***	***Remarks***
Belgium	BE	yes	1	yes	table	
Switzerland	CH	yes	1	yes	BSC-FC: log (cfu/ml)=0.860*log(IBC/ml)-0.008 BSC-8000: log (cfu/ml)=0.838*log(imp/ml)+0.178	BSC-FC: tentatively, available by the end of 2006; recently experiments with Bactocount started
Cyprus	CY	Yes	1	Yes	No data	
Germany	DE	yes	1	yes	log_{10}cfu/ml=0.923 log_{10} BC-FC+2.767 Range 10-70 000BC	Still under discussion; actually a preliminary conversion is applied
Estonia	EE	Yes	1	Yes	< 500 00 cfu/ml: y=0.9923BC-8000-0.3156 ; $s_{y,x}$=0.22 >500 000cfu/ml: y=0.9765 BC-8000-02914 ; $s_{y,x}$=0.21	
Spain 1	ES	yes	Different region	Yes	log_{10}BC-FC=0.9265 log_{10} cfu/ml+0.7435	
Spain 2	ES	Yes	Different-region	-	log_{10}cfu/ml=0.933 log_{10} BC-FC-0.5747	Common conversion is being carried out
Finland	FI	Yes	1	No	log_{10}cfu/ml=0.9254 log_{10} BC-FC-0.0586	
France	FR	yes	Regions, species	-	Several tables	- MCL have defined their own tables, depending on the region and species. The initial conversion was based on large series of comparisons with the SPC. - the routine method is compared to the reference method at least 1/week with at least 10 samples. Then on-going follow-up cumulated over 6 months. - participation to PTs 6 times/year on the routine and the reference methods.
Ireland	IE	no	Different - labs	-		500-600 samples tested by plate count and Bactoscan and relationship established by manufacturer. Each processor has a different conversion
Latvia	LV	Yes	Different methods	yes	BSC-FC: y=0.9044x-0268 Bactocount: y=1.057x-3.981	
Luxembourg	LU	yes	1	Yes		Preliminary conversion of DE is applied
Malta	MA	yes	1	-		Modified version of Italian Foss conversion
Norway	N	Yes	1	Yes		Producer is paid on basis of Bactocount units

Question No.		*3.1*	*3.2*	*3.3*	*3.4*	
Country		*Conversion procedure existing*	*Number of conversions applied*	*Based on ISO 21187*	*Specified conversion(s)*	*Remarks*
Netherlands	NL	yes	1	yes	Log cfu=07837log BF+2.7734	
Poland	PL	yes	1	-		German conversion is used
Portugal	PT	Yes (1 lab)	1 (1 lab)	yes	Y=0.9x+0.2	Common conversion is being carried out and under discussion
Slovakia	SK	Yes	Different-species	yes	Cows: $\log_{10}$cfu/ml=0.914 $\log_{10}$ BC-FC+2.842; $s_{y,x}$=0.210 Sheep: $\log_{10}$ cfu/ml=1.088$\log_{10}$ BC-FC+2.292; $s_{y,x}$=0.305	

Annex II: Figures

Fig 1 Bactoscan FC: Conversion raw milk in EU-member states

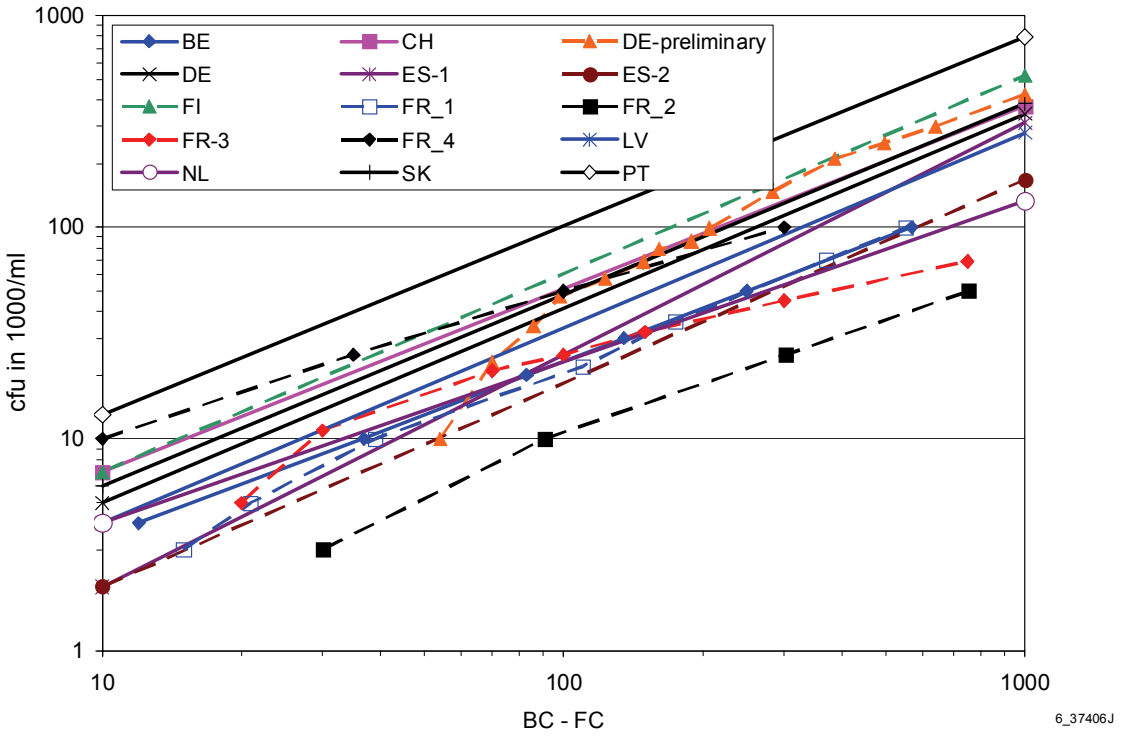


Fig 2 Bactoscan FC: Conversion raw cow's milk based on ISO 21187

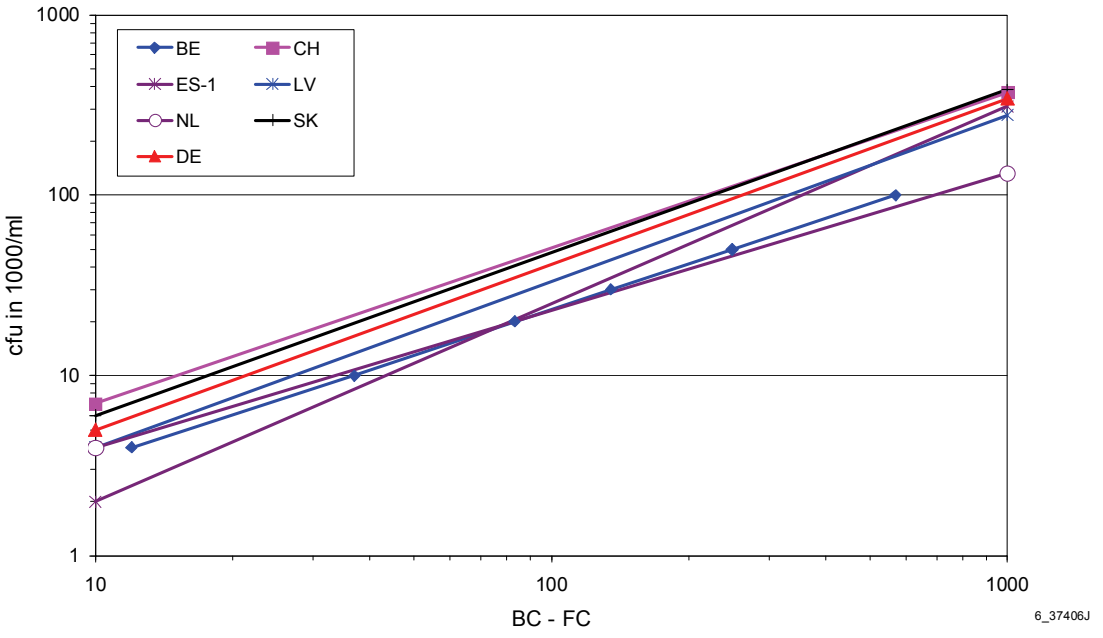


Fig 3 Bactoscan FC: Conversion for sheep's and goat's milk

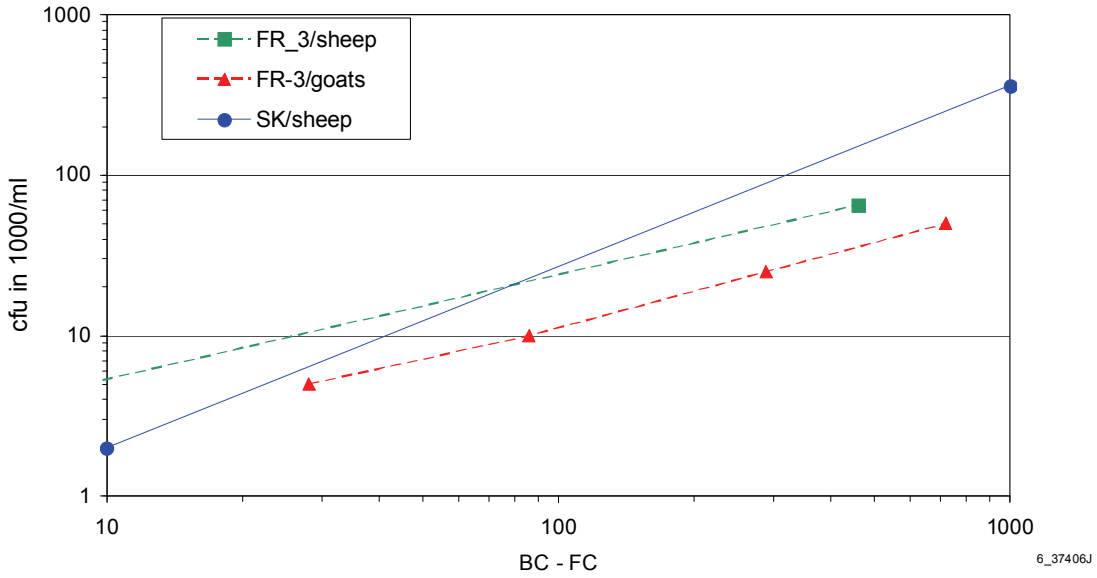
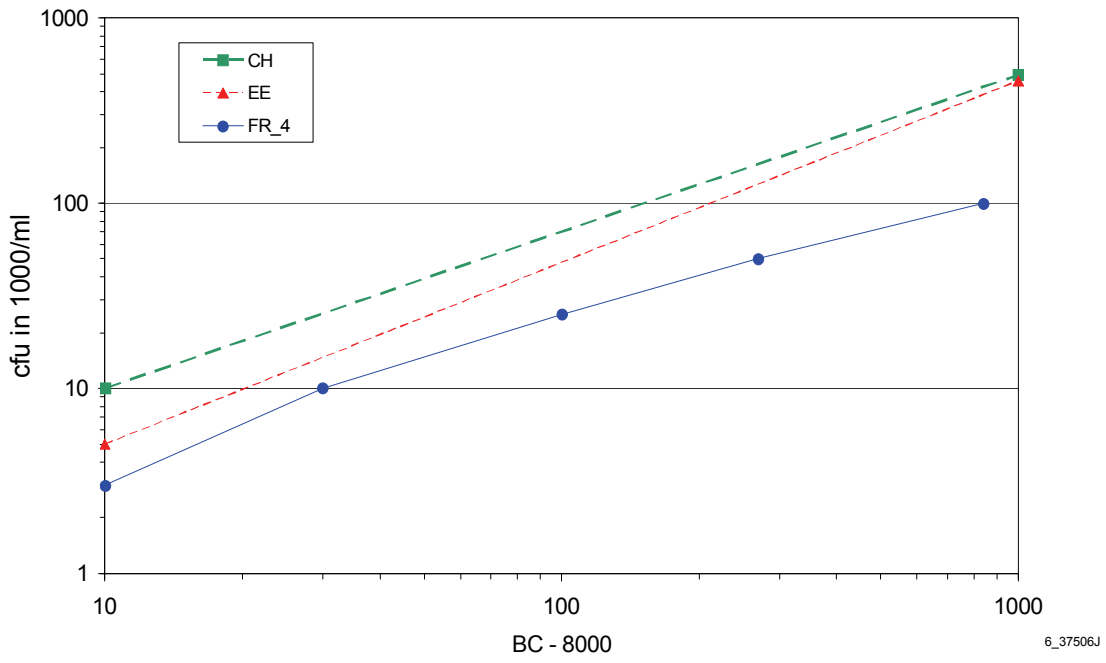


Fig 4 Bactoscan 8000: Conversion raw cow's milk in EU-member states



Annex 5. Detection of staphylococcal enterotoxins in milk and milk products.

European screening method of the CRL Milk and Milk products. Version 3, May 2006.

Site de Maisons-Alfort

EU COMMUNITY REFERENCE LABORATORY FOR MILK & MILK PRODUCTS

LABORATOIRE D'ETUDES ET DE RECHERCHES SUR LA
QUALITE DES ALIMENTS ET SUR LES PROCEDES AGRO- ALIMENTAIRES

Detection of staphylococcal enterotoxins in milk & milk products

European screening method of the CRL "Milk & Milk Products"

Version 3, May 2006

Martine GOHIER, Annick OSTYN, Jacques-Antoine HENNEKINNE, Sophie KRYS
Toxins, organic pollutants and pesticides Unit

Bertrand LOMBARD
Co-ordinator of the CRL

Foreword

The Commission Regulation (EC) N° 2073/2005 of 15 November 2005 [1] on microbiological criteria for foodstuffs prescribes the detection of staphylococcal enterotoxins in cheeses made from raw milk or thermized milk, when the criteria M for *S. aureus,* applied at the time during the manufacturing process when the number of staphylococci is expected to be the highest, is higher than 10^5 ufc/g .

1 Scope

This document specifies a method for the extraction, and two methods at choice for the detection of staphylococcal enterotoxins in milk and milk products, preceded or not by a treatment of the extract:

- extraction using dialysis/concentration followed by a detection step using the Vidas SET2 kit (bioMérieux)

Or

- extraction using dialysis/concentration followed by a pre-treatment of the extract using rabbit immunoglobulins (IgG) (Diffchamb) and then a detection step using Transia Plate SET kit (Diffchamb)

The method described in this document is to be used as the screening method for implementing the Regulation 2073/2005.

2 References

[1] Anonymous (2005), Commission Regulation (EC) N° 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of European Union, L 338, 22.12.2005 pp. 01 – 26.

[2] Hennekinne, J-A., Gohier, M., Maire, T., Lapeyre, C., Lombard, B., Dragacci, S., (2003). First proficiency testing to evaluate the ability of National Reference Laboratories of the European Union to detect staphylococcal enterotoxins in milk products. J. AOAC Int. 86, 2, 332-339.

[3] Point 3.4 page 7 of the Report of the 8th Workshop of the National Reference Laboratories on Milk & Milk Products, June 30 – July 1, 2005, Maisons-Alfort.

[4] Hennekinne, J-A., Guillier, F., Perelle, S . De Buyser, M-L., Dragacci, S., Krys.S, Lombard, B. Intralaboratory validation according to the EN ISO 16140 standard of the Vidas SET2 detection kit for a use in official controls of staphylococcal enterotoxins in milk products. Submitted in Journal of Applied Microbiology (JAM-2006-0181).

[5] Hennekinne, J-A., Ostyn, A., Gohier, M., Guillier, F., Krys. S, Lombard, B. Detection of Staphylococcal enter toxins in milk and milk products, interlaboratory validation of the Vidas SET 2 detection kit, March 2006, report SE/IV 2006-1, AFSSA.

3 General principle

The detection of staphylococcal enterotoxins mainly consists of two or three steps, depending on the detection kit used :

1. Extraction/concentration
   The sample is mixed and homogenised with distilled water. The toxins diffuse in water and are recovered, after two centrifugations, in the supernatant.
   This aqueous phase is concentrated overnight by dialysis.
2. Pre-treatment using rabbit IgG ONLY if the detection step is performed using Transia plate SET detection kit. This pre-treatment is applied in order to decrease false positive results due to cross reaction by protein A.
3. Immuno-enzymatic detection: Transia plate SET or Vidas SET2.

4 Reagents

4.1 Distilled water

4.2 Hydrochloric acid (5 M and 1 M), e.g. MERCK (ref: 1.09911)

4.3 Sodium hydroxide (5 M and 1 M), e.g. MERCK (ref: 1.09913)

4.4 Polyethylene glycol 20 000 (PEG), quality for synthesis, e.g. MERCK (ref.: 8.18897)

4.5 Sodium chloride, quality for analysis ACS ISO, e.g. MERCK (ref: 1.06404)

4.6 Di-sodium hydrogen phosphate dodecahydrate, GR ISO, e.g. MERCK (ref: 1.06579)

4.7 Phosphate Buffered Saline solution (PBS solution) ($NaCl/NH_2PO_4$: 145 mM/10 mM)

4.9 Dialysis membrane, MWCO: 6 000-8 000 daltons; flat width: 23 ± 2 mm, e.g. Spectra/Por (ref.: 132 650)

4.12 Glass-wool

4.13 Decomplemented normal rabbit serum. Diffchamb, ref : AK0224

4.14 Transia Plate SET detection kit. Diffchamb, ref : ST0796

4.15 Vidas SET2 detection kit. bioMérieux, ref : 30 705

5 Apparatus

5.1 Mixer

5.2 Turrax, blender or stomacher

5.3 pH-meter

5.4 Spinner, 3 100 to 10 000 g, capable of being refrigerated at 4°C.

5.5 Incubator/Shaker for microplates, capable of being heated at 25°C or at room temperature.

5.6 Closures (l = 35 mm), e.g. Spectra/Por (ref. : 132 736)

5.7 Laboratory glass-ware (funnel, beaker, vial …)

5.8 Microplates reader, filter 450 nm

5.9 Vidas or miniVidas reader

6 Procedure

6.1 Preparation

If the samples have to be kept more than one night before beginning the extraction, they shall be stored at –18°C.

Mix the whole sample if possible, or a representative part of it (comprising about 10% of rind for cheese), with a mixer (5.1).

Weight 25 ± 0.1 g of the mixed sample and transfer this portion into a beaker (5.7).

6.2 Extraction step

Add 40 mL of warm distilled water (4.1) (38 ± 2°C) to the test portion (6.1) and homogenise the mixture by using a turrax, a blender or a stomacher (5.2). Rinse the system with warm distilled water.

Allow the toxin to diffuse by shaking the sample, at room temperature for about 30 min.

Acidify the mixture with a few drops of hydrochloric acid (5M or 1M) (4.2) until pH 3.5 ± 0.5.

Centrifuge the mixture at least 3 100 g for 15 min at 4°C if possible (5.4).

Transfer the supernatant in a beaker and neutralise it at pH 7.3 ± 0.3 with sodium hydroxide (5M or 1M) (4.3).

Centrifuge again as described above.

Recover the aqueous phase and proceed in accordance with 6.3.

NOTE 1: If the supernatant is not clear enough, centrifuge again as described above.

NOTE 2: The pH of the supernatant after the first centrifugation has to be < 4.5. If it is not the case, acidify until pH 3.5 ± 0.5 and centrifuge again as described above.

6.3 Dialysis - concentration step

For each extract, cut about 50 cm of dialysis membrane (4.9). Soak the membrane in hot distilled water (85 ± 5°C) for at least 5 min before use.

Rinse the membrane with distilled water (outside and inside) (4.1).

Lock one end of the membrane with a closure (5.6), fill it up with the final extract solution as prepared in 6.2 by using a funnel (5.7). Lock the other end with a second closure.

NOTE : If the extract obtained in 6.2 is not clear enough, filter it through a small piece of glass-wool (4.12) before filling up the membrane.

Lay down the filled dialysis membrane in a tray containing the 30% PEG solution. To obtain such a solution, dissolve, for each sample, 30 g of polyethylene glycol (4.4) in distilled water to obtain a final weight of 100 g.

Allow the extracts to concentrate overnight at 4 ± 4°C.

Take the dialysis membrane out of the PEG solution and rinse the outer-part of the membrane with distilled water to remove all traces of PEG.

Recover the concentrated extract using the PBS solution (see below) to rinse the inner-part of the dialysis membrane to obtain a 5 mL (or 5 g) concentrated extract. Transfer carefully the concentrated extract into a glass vial (5.7).

Dissolve 9 g of sodium chloride (4.5) and 3.58 g of di-sodium hydrogen phosphate dodecahydrate (4.6) in 1L of distilled water. Adjust pH at 7.4 using HCl.

NOTE 1: If the extract is not concentrated enough, lay down it in the PEG solution for some time or by adding some PEG in powder.

NOTE 2: If the extract (6.3) analysed within 48 h, store it at 4 ± 4 °C. If the extract cannot be analysed within 48 h, store it in a freezer at – 18 °C.

6.4 Pre-treatment using rabbit IgG (ONLY in the case of a detection step using Transia plate SET kit)

As studies performed recently showed that a treatment of the concentrated extract using rabbit IgG before performing the detection step decreased the rate of positive deviations, it has been decided in accordance with the NRLs at the 2005 Workshop [3] to include a new step using rabbit IgG treatment.

Before testing the concentrated extract using Transia Plate SET, it is necessary to treat it with addition of rabbit serum (reference AK 0224) according to the following procedure :
- Place 900 µL of concentrated extract (6.3) in a tube and add 100 µL of decomplemented normal rabbit serum (4.13).
- Incubate for 1 hour at room temperature (18-25°C) with agitation.
- Perform the Transia plate SET kit (6.5.1) with 100 µL of treated extract .

6.5 Detection

6.5.1 Detection using Transia Plate SET kit

The Transia Plate SET kit (4.14) has been proved by the EU CRL for Milk to be one of the most suitable commercialised kits for performing this detection [2].

This detection method is based on a sandwich-type ELISA. The solid support of the reaction is a microtiter plate coated with antibodies specific for staphylococcal enterotoxins.

An immune complex is formed by the coated Ab, the toxins present in the sample and anti-SE antibodies conjugated with peroxidase.

The presence of enterotoxins is revealed by using a mixture of peroxidase substrate/chromogen and determined by a colorimetric measure at a wavelength of 450 nm.

Perform the Transia plate SET test on 100 µL of the treated extract (6.4).

6.5.2 Detection using Vidas SET2 kit

Preliminary studies in 2004 and 2005 followed by an intra-laboratory validation according to the protocol based on the Standard EN ISO 16140 showed that the Vidas SET2 detection kit could be used as a screening method to detect staphylococcal enterotoxins in milk & milk products [4]. Further to the inter-laboratory study performed on December 2005, the Vidas SET2 detection has been fully validated for the detection of staphylococcal enterotoxins in milk products [5].

The Vidas SET2 is a rapid and fully automated kit detecting without differentiation the staphylococcal enterotoxins types A to E, using a cone coated with antibodies specific for SEA, SEB, SECs, SED and SEE. An immune complex is formed between the coated antibodies, the toxins in the concentrated extract and the anti-SE antibodies conjugated with alkaline phosphatase. All reagents are included in the wells of the strip used.

Briefly, the concentrated protein extract or the controls are distributed in the strips and incubated in the automate miniVidas. Two fluorescence measures (sample, blank) are performed for each test by the automate. The ratio (relative fluorescence value or RFV) between these two measures is interpreted to declare or not a sample as positive.

Perform the Vidas SET2 test on 500 µL of concentrated extract (6.3).

6.6 Interpretation of results

6.6.1 Transia plate SET kit

Quality control

Absorbance of the positive control shall be higher than or equal to 0.50.
Absorbance of the negative control shall be lower than or equal to 0.30.
If the controls do not meet these requirements, invalidate the results.

Determination of the positive threshold

Perform two negative controls and measure their absorbance NC_1 et NC_2 at 450 nm.

Calculate the positive threshold (T) as following :

$$T = \frac{NC_1 + NC_2}{2} + 0.20$$

Interpretation

Consider that staphylococcal enterotoxins are detected in a 25 g portion if the absorbance of the extract is higher or equal to the threshold (T).

Consider that staphylococcal enterotoxins are not detected in a 25 g portion if the absorbance of the extract is lower than the threshold (T).

6.6.2 Vidas SET2 kit

Determination of the Test value (TV)

TV = sample RFV/standard RFV

Interpretation

Consider that staphylococcal enterotoxins are detected in a 25 g portion if the TV of the extract is higher or equal to 0.13.

Consider that staphylococcal enterotoxins are not detected in a 25 g portion if the TV of the extract is lower than 0.13.

7 Handling precautions and decontamination

Staphylococcal enterotoxins are harmful if swallowed, inhaled or absorbed trough skin (wear gloves).
Contaminated single use reagents are eliminated in a clinical waste which will be incinerated.
Other contaminated materials are decontaminated during 1 hour at least in a sodium hypochlorite 5° solution.

Annex 6. Presentation for Brazilian visitors on 3 December 2007 as part of their training course.

Management of the NRL milk ...

Wilma Jacobs-Reitsma

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Cluster Microbiology & Novel Foods (MNF)

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Cluster Microbiology & Novel Foods (MNF)

- General microbiology
- Food microbiology
- Antibiotic residue detection
- Molecular biology
- Genomics
- Novel foods
- GMOs
- Species identification

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

National Reference Laboratory (NRL) "milk"

- Projectleader: Wilma Jacobs-Reitsma
- RIKILT staff involved:
  - El Bouw, Hannie Vastenburg, Rob Bakker, Wendy van Overbeek, Sabrina Oostra, Maria Baltussen, Wilma Jacobs-Reitsma
- Financial situation 2007: 37,8 k€
  - 25 days analists, 25 days scientists, labmaterials, travel costs

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

European Union

- As a member state of the European Union the Netherlands must comply with EU-legislation.
- This legislation consists of
  - Regulations (acts directly)
  - Directives (to be implemented somehow in legislation of each Member State)
  - Decisions (only applies to its particular addressee of the decision)

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European Union in 2007

General info:

htpp://europa.eu

Legislation:

htpp://eur-lex.europa.eu

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European Union

- Topics like “milk and milk products”, “bivalve mollusks”, “Salmonella”, etc.
- Community Reference Laboratories
  - 1 EU CRL per topic
- National Reference Laboratories
  - 1 NRL per Member State (in general)
- Routine Field Laboratories
  - 1 or (much) more per Member State

NRL milk and milk products, History

- Directive 92/46/EEG laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products
- Topic: milk and milk products
  - CRL: AFSSA -LERHQA in Paris (Bertrand Lombard)
  - NRL NL: RIKILT (Henk Stegeman)
  - RFL: MCS, COKZ

Directive 92/46/EEG

- In The Netherlands implemented in the “Landbouwkwaliteitswet” (Law on agricultural quality)
- Parameters (in milk and milk products):
  - Somatic cell count
  - Total aerobic count, coliforms count
  - Pathogens like Salmonella, Listeria, *E. coli* 0157, *Staphylococcus aureus* (enterotoxins)

NEW: General Food Law (2002)

- Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
- And Regulations:
  - (EC) No 852/2004 on the hygiene of foodstuffs
  - (EC) No 853/2004 laying down specific hygiene rules for food of animal origin
  - (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption
  - (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
  - (EC) No 2073/2005 on microbiological criteria for foodstuffs

General Food Law

- Effects of this new legislation on CRL/ NRLs:
  - Horizontalisation of topics
    - Salmonella as a zoonosis -> Salmonella in food and feed
    - Milk and milk products -> no pathogens anymore
  - Former established CRL/NRL’s were kept, but with revised tasks
  - New CRL/NRL’s appointed (separately for eg Listeria, S. aureus (toxins), E. coli 0157, etc.

NRL milk

- Reference Tasks per 1-1-2006:
- Hygiene of raw milk:
  - Somatic cells count (SCC)
  - Total flora (TF)
- Heat treatment characterization of milk and mlik products:
  - Alkaline phosphatase activity

NRL (raw) milk

- Specifieke rules for raw milk
  - Annex III, section IX, Chapter I, Part III of EU Reg. 853/2004.
- Testing methods for raw milk (and heat-treated milk)
- Annex VIa, Chapter 1 of Reg 1664/2006 (in addition to Reg 2074/2005)
  - EN-ISO 4833 for plate count at 30°C, conversion relationship established according to ISO 21187
  - ISO 13366-1 for somatic cell count (or 13366-2 as validated according to ISO 8196)
- Annex VIa chapter II of Reg 1664/2006
  - ISO 11816-1 for alkaline phosphatase activity

NRL tasks according to EG 882/2004, art. 33

Artikel 33. Nationale referentielaboratoria

1. De lidstaten zorgen ervoor dat voor elk communautair referentielaboratorium zoals bedoeld in artikel 32 één of meer nationale referentielaboratoria worden aangewezen. Een lidstaat kan een laboratorium in een andere lidstaat of in een lid van de Europese Vrijhandelsassociatie (EVA) aanwijzen en één laboratorium kan voor meer dan één lidstaat als nationaal referentielaboratorium fungeren.
2. Deze nationale referentielaboratoria moeten:
   a) op hun bevoegdheidsterrein samenwerken met het communautaire referentielaboratorium;
   b) voor hun bevoegdheidsterrein de werkzaamheden van de officiële laboratoria die overeenkomstig artikel 11 belast zijn met de analyse van monsters coördineren;
   c) indien nodig, vergelijkende tests tussen de officiële nationale laboratoria organiseren en zorgen voor een passend vervolgtraject voor de vergelijkende tests;
   d) ervoor zorgen dat de door het communautaire referentielaboratorium verstrekte informatie aan de bevoegde autoriteit en de officiële nationale laboratoria wordt doorgegeven;
   e) wetenschappelijke en technische steun verlenen aan de bevoegde autoriteit voor de uitvoering van de gecoördineerde controleplannen die overeenkomstig artikel 53 zijn aangenomen;
   f) zorgen voor de uitvoering van andere specifieke taken waartoe is besloten volgens de procedure van artikel 62, lid 3, onverminderd bestaande aanvullende verplichtingen op nationaal niveau.
3. Artikel 12, leden 2 en 3, is van toepassing op de nationale referentielaboratoria.
4. De lidstaten brengen naam en adres van elk nationaal referentielaboratorium ter kennis van de Commissie, het bevoegde communautaire referentielaboratorium en de overige lidstaten.
5. De lidstaten met meer dan één nationaal referentielaboratorium per communautair referentielaboratorium moeten ervoor zorgen dat deze laboratoria nauw samenwerken met het oog op een doeltreffende coördinatie, zowel onderling als met de andere nationale laboratoria en met het communautaire referentielaboratorium.
6. Aanvullende verantwoordelijkheden en taken voor de nationale referentielaboratoria kunnen worden vastgesteld volgens de procedure van artikel 62, lid 3.
7. De leden 1 tot en met 5 zijn van toepassing onverminderd meer specifieke voorschriften, en in het bijzonder hoofdstuk VI van Verordening (EG) nr. 999/2001 en artikel 14 van Richtlijn 96/23/EG.

Implementation of NRL milk task by RIKILT

- Development, validation and independant quality control of official reference methods and providing information to Routine Field Laboratories
  - Participation in the annual CRL-NRL workshops
  - Participation in CRL-organised ring trials
  - Participation in CRL-training courses
  - Organisation of NRL ring trials (when appropriate)
  - Annual report of activities (from 2006 onwards)
  - Accreditation of the reference methods

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Activities of RIKILT NRL milk in 2007

- Annual report on 2006
- Visits at COKZ and MCS
- Testing of somatic cell count samples for calibration of Fossomatics at MCS (4 x per year)
- Newly started: testing alkaline phosphatase samples in duplicate with COKZ
- Elaboration of validation data on the alkaline phosphatase method to apply for accreditation in 2008
- Participation in CRL ring trial total aerobic flora
- (F)DIS revised ISO 13366-1 (somatic cell count)
- CRL general meeting (June, Paris)

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CRL general meeting (June, Paris)

- Roll-call of delegates
  - 29 delegates of 27 NRLs of 27 EU Member States and 2 associated countries (NO, CH)
  - EC/DG SANCO "Helath and Consumer Protection" representative
- Minutes of dedicated meeting in 2006 (on TF determination by alternative (instrumental) methods
- Latest legislation matters concerning the topic
- Update on IDF/ISO works
- 2006 PT trial on alkaline phosphatase
- Planning of work in the coming years
- Dedicated workshop on Alkaline phosphatase in 2008

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CRL general meeting (June, Paris)

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