



NRL Contacts

**Antimicrobial
Resistance
Zoonoses
(salmonella)**
Dr M Gutierrez

**Listeria
Staphylococci
Milk & Milk Products**
Ms B Hickey

Ecoli (VTEC)
Dr L Scott

Parasites
Dr T Murphy

TSE's
Dr P Collery

**Residues/Chemical
Elements**
Mr P Rafter

Pesticide Residues
Dr D O'Sullivan

Campylobacter
Dr J Egan

Animal Proteins
Mr G Roe

Activities of National Reference Laboratories (NRL's)

Introduction

In 2006 following the designation of a number of additional Community Reference Laboratories (CRL's) by EU, Member States were required under Article 33 of Regulation 882 / 2004 to designate one or more National Reference Laboratory (NRL) for each CRL. The Departments of Health and Children and Agriculture and Food, as the Irish Competent Authorities, assigned these NRL functions to a number of laboratories including those within the Backweston Laboratory Campus. See list of NRL's outlined in Appendix.

In this issue:

- (a) CVRL is awarded VetNet certification in Pulsed Field Gel Electrophoresis (PFGE) for Salmonella.
- (b) Twelfth Workshop of the Community Reference Laboratory on Milk and Milk Products, AFSSA, Paris, 28th - 29th May 2009.
- (c) Third Workshop of the Community Reference Laboratory on Coagulase - positive staphylococci AFSSA, Paris, 15th and 16th June 2009.
- (d) Third Workshop of the Community Reference Laboratory on Listeria monocytogenes, AFSSA, Paris, 14th - 15th May 2009.
- (e) Short Report on the 14th International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Madrid, 17 - 20 June 2009.

Report

CVRL is awarded VetNet certification in Pulsed Field Gel Electrophoresis (PFGE) for *Salmonella*

A multi task project entitled "Genomics of Gram Negative Food Poisoning Bacteria of Animal Origin", funded by DAFF through the Food Institutional Research Measure (FIRM project network no.: 06/TNII/UCD/10), has been on going since 2008. This multi centre project, led by Professor Séamus Fanning of UCD, has scientists and food safety experts participating from a number of centres including the DAFF laboratories in Backweston, UCD, AFRC, MFRC and FSAI. One of the specific tasks of the project is the transfer of molecular techniques for application at the NRL's in Backweston and the establishment of a VetNet database compatible with those hosted by the United States Department of Agriculture (USDA).

The CVRL which undertakes a number of National Reference Laboratory functions for Food, Feed and Animal Health has recently been VetNet certified in Pulsed Field Gel Electrophoresis (PFGE) for *Salmonella*. USDA VetNet was established in 2003 and was modelled after PulseNet USA, the national molecular subtyping network for food borne disease surveillance. The objectives of VetNet are to use PFGE to subtype zoonotic pathogens, compare VetNet and PulseNet PFGE patterns, and to use the comparative data for surveillance and investigation of food borne disease outbreaks. The certification process involved two weeks PFGE training for CVRL and UCD participants at the USDA, Richard B Russell Agricultural Research Centre, in PulseNet standardised PFGE protocols and VetNet BioNumerics software in concordance with the CDC-PulseNet program. Following the training procedures the CVRL laboratory was set up to USDA VetNet standard and PFGE was successfully performed on a certification set of isolates for both TIFF images and bundle files (created through the BioNumerics database).

PFGE is an invaluable tool that can be used to determine the epidemiological link between isolates from human and environmental sources. It will allow the CVRL to better assist in outbreak investigations. The establishment of a VetNet database compatible with those hosted by the USDA will further enhance food safety surveillance in Ireland. VetNet certification for *E. coli* O157:H7 PFGE is currently underway.

Report

12th Workshop of the Community Reference Laboratory on Milk and Milk Products, AFSSA, Paris, 28th - 29th May 2009.

NRL Representative: Bernadette Hickey, DSL

The following items were discussed:

1. Task and duties of NRL's and Accreditation for Official controls

The duties outlined in Article 33 of Regulation 882/04 were outlined. Test procedures used for official controls must be accredited (Regulation 882/04 Articles 12 and 33). Regulation 2076/2005 gave derogation until 31st December 2009. Difficulties in accrediting the Somatic cell count method were highlighted, as it is not used routinely. There is no Proficiency Scheme available for counting SCC by Reference method and there are no reference materials. CRL are unable to carry out an Interlaboratory trial in 2010. CRL are to discuss the matter with DG Sanco.

A discussion took place on the current mandate for the CRL on Milk. It is currently confined to hygiene parameters. The scope of activity of the CRL was larger under the Directive 92/46. Currently there are no CRL's to deal with parameters affecting milk and milk products for the following – Enterobacteriaceae, *Enterobacter sakazakii* and *B.cereus*.

2. Total Bacterial Count - Reference Methods

2.1 The results of the Interlaboratory trial organised in 2007 on Total Bacterial Count were presented and the performance of the NRL network was satisfactory.

2.2 Use of Preservatives in Interlaboratory trials

The CRL is carrying out studies on the use of 3 preservatives, Sodium Azide, Boric acid and Boric acid mixture (Boric acid, glycerol and potassium sorbate) and testing the stability of the prepared samples over time. The purpose of the study is to allow CRL to prepare their own samples for the Interlaboratory trials.

2.3 Analysis of Colostrum

DG Sanco intends to add micro criteria for colostrum to Regulation 853 /2004.

CRL have carried out some analysis on testing but have difficulty getting colostrum for testing due to short calving period and colostrum is not preserved.

3. Milk Hygiene Total Bacterial Count - Alternative Methods

3.1 Study of the conversion relationship between the reference method and the Bentley Bactocount method.

The CRL have a Bactocount machine manufactured by Bentley.

Commission Regulation 1664/06 permits the use of alternative methods if compared to reference method in accordance with ISO 16140. The conversion relationship is defined in EN ISO 21187.

The purpose of the study is to investigate the relationship between Bactocount and the Reference method ISO 4833 and to evaluate factors that affect it. The Institute in Kiel have carried out similar research on the Bactoscan and have assisted the CRL.

The study will continue for 2009 and 2010.

3.2 Check list for laboratory visits on conversion characteristics by NRL's

The CRL, with the help of some NRL's, compiled a document detailing check list questions that an NRL should use when checking the establishment of the conversion factor in their country.

3. 2008 Questionnaire on Raw Milk

The CRL circulated a questionnaire in 2008 on milk production, collection, and testing in Member states.

- The number of Dairy Farms decreasing.
- Milk from cow's account for 99% of farms with an average herd size of 49 cows.
- Goat's and sheep account for 4% of farms with an average herd size of 114 for goat's and 99 for sheep.
- Buffalo milk is produced in CH and NL
- Horse Milk is produced in DE and NL.
- Majority of milk is delivered every day / every second day to the dairies.
- 95% of samples are analysed for both quality and payment purposes.
- The methods used are mainly Bactoscan and EN ISO 4833.

3. Update on ISO / IDF works

ISO / IDF 161 Quantitative determination of bacteriological quality- Protocol for the evaluation of routine or alternative methods. It was originally thought that this standard could be incorporated as an Annex into the revision of EN ISO 16140 but this proved too complicated. The ISO/ IDF 161 standard will now be updated. A draft has been circulated for comment and it will be included in the IDF Work Programme.

4. Phosphatase

4.1 Cheese

CRL are working to establish suitable limits for cheese by type. When cheese is pasteurised there is little difference in the results obtained using milk for the extraction step or cheese extraction buffer. When milk is unpasteurised there is a large difference in the results between milk as the extraction buffer and the cheese extraction buffer.

Report

3rd Workshop of the Community Reference Laboratory on Coagulase - positive staphylococci AFSSA, Paris, 15th and 16th June 2009.

NRL Representative: Bernadette Hickey, DSL

The following items were discussed:

1. Accreditation for Official Controls (Adrien Assere CRL AFSSA)

Test procedures used for official controls, including Enumeration of Coagulase positive staphylococci (CPS) and Detection of Staphylococcal Enterotoxins (SE), must be accredited by 31st December 2009. As there is no Proficiency Scheme available for detection of SE (CRL only provider of an Interlaboratory trial in 2008) and there are no reference materials it will not be possible to achieve accreditation for the methods in the timeframe required. DG Sanco will contact IRMM in Geel, Belgium to discuss the preparation of a reference material.

1.2 Requirements for the validation of alternative methods (B.Lombard CRL AFSSA)

Article 5, Regulation 2073/2005

The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in EN/ISO standard 16140 or other internationally accepted similar protocols, is used.

If the food business operator wishes to use analytical methods other than those validated and certified as described in paragraph 3 the methods shall be validated according to internationally accepted protocols and their use authorised by the competent authority.

The paragraph in Article 5 is not clear.

The CRL and EU representative confirmed that validations carried out by Microval and AFNOR meet EN 16140 and are validated against a reference method. AOAC is not acceptable currently as their validation protocol differs from EN 16140 and they validate against BAM/ FDA methods.

1. Update on EFSA activities and CPS reporting in animals and food. (Pierre-Alexandre BELOEIL, EFSA, Parma)

The Zoonoses Directive places an obligation on EU member states to report annually on investigated food borne outbreaks. A new reporting system was introduced in 2007. Outbreaks can now be categorized as (a) possible outbreaks, (b) Verified outbreaks.

Staphylococcal enterotoxins were the causative agents in 4.6% of outbreaks reported in 2007. 11 countries reported verified outbreaks with the highest numbers reported in France. 3 deaths were attributed to staphylococcal enterotoxins in 2007. The implicated food sources were Cheese 8%, Poultry products 6%, Minced meats 6%, Bovine meat products 5%, Fish products 4%, Cereals 4%, Eggs and egg products 4%, pig meat products 4%, Other foods 23%, Unknown 29%. There was a higher % of verified outbreaks for meat products than for dairy and fish. Discussion took place on the absence of microcriteria for products other than dairy and fish. DG Sanco will ask EFSA to look at this issue.

France have a high degree of organization and communication between agencies and it is mandatory to report outbreaks of food related and this has led to a higher incidence of verified outbreaks. Protocols differ from country to country and reporting of food related illness is not mandatory.

2. CPS detection and enumeration

2.1 Optimisation of reference method (confirmation step) EN ISO 6888-1

The confirmation step in EN ISO 6888-1 is the tube coagulase test. The CRL investigated the use of an alternative confirmation step, stabbing of Baird Parker (BP) + RPF. The typical reaction for coagulase positive colonies is black coloration and a positive coagulase reaction on the BP + RPF. The CRL examined the performance criteria for inclusivity and exclusivity using 31 pure CPS strains and 23 non CPS strains. There was no statistical difference between the tube coagulase confirmation method in ISO 6888-1 and the alternative method (BP + RPF). The advantage of BP+ RPF is that it is easier to perform, quicker and easier to interpret results.

The CRL is to recommend to ISO /TC34/SC9 in charge of Standard EN ISO 6888-1 that the stabbing of BP + RPF be included in the standard as an alternative confirmation step.

2.2 Homogeneity and stability study of samples used for Interlaboratory trials

CRL have carried out studies to check the performance of bacteriostatic agents on cheese samples used for the preparation of samples for Interlaboratory trials.

The examined boric acid and boric acid mixture (boric acid, glycerol and potassium sorbate) over different inoculation levels of flora and temperatures of 8°C and 12°C. Boric acid mixture is the most suitable.

3. Staphylococcal Enterotoxins

3.1 Inter laboratory trial 2008

The results of the Interlaboratory trial on the detection of Staphylococcal Enterotoxins (SEA - SEE) in milk products in 2008 was presented. 6 cheese samples containing, blank (0ng/g), low (0.05ng/g) and high(0.15ng/g) levels were dispatched.

27 laboratories participated and used the European Screening method of CRL 2nd version April 2008. 20 laboratories used the VIDAS SET 2 Kit, 13 labs used Transia Plate SE kit and 6 laboratories used both kits. The test is qualitative and 24 /26 laboratories had satisfactory performance for qualitative results.

The CRL examined the influence of the batches of test kits used. Vidas SET 2 : 7 batches used by NRLs's. No statistical difference between the batches.

Transia Plate SE kit: 6 batches used by NRLs'. Statistical difference between batches.

The CRL will look at validating Vidas for Part B, detection of SE's in food products other than milk products.

3.2 Evaluation of Trichloroacetic acid (TCA) for the precipitation and extraction step in the detection of SE types, SEA - SEE

CRL investigated the use of TCA in the detection of SE's. TCA is less expensive and faster than dialysis concentration. However TCA is toxic, damaged the toxins and there were technical difficulties with protein pellet dissolution. The toxin recovery was less than that achieved using dialysis concentration. TCA is also unsuitable as there is a risk of obtaining false negatives.

3.3 Evaluation of commercially available antibodies against SEA to SED and SEH.

The CRL uses an Elisa double sandwich type in house antibodies to quantify toxin types SEA to SED. They want to design a quantitative Elisa with commercially available antibodies and to transfer the step to NRLs. They are evaluating commercially available antibodies with single and double sandwich Elisa

design.

3.4 Development of immuno qPCR test to detect SE's in food samples

CRL are working on this method. The benefits are sensitive quantification, improvement of LOD, ability to analyse a lot of samples at same time, a small sample volume required and compatible with complex biological matrices. The disadvantages are, expense of antibody/ DNA conjugate and synthesis of tailored reagents requires knowledge of protein chemistry.

3.5 CEN Standardisation of Staphylococcal Enterotoxins and detection.

A proposal has been submitted to WG 6 of CEN TC275 in March 2009. The method as written must be changed as the CRL method specifies Transia and Vidas as the detection kits. These cannot be specified and the method must be written defining specific and performance characteristics. A CEN mandate is required to get funding to organize Interlaboratory trial that is required for standardization.

3.6 Use of quantitative MS to improve the investigation of Staphylococcal enterotoxin food poisoning.

CRL working on Mass spectrometry to help identify Staphylococcal Enterotoxins in food poisoning investigations.

4. Strain Typing / Epidemiology.

4.1 Coagulase positive Staphylococci Lyon FR.

Mona DUMITRESCU gave an overview of methods in use for clinical samples and investigations. The tools used depend on the information required and purpose. For local comparisons of strains both MSSA and PFGE are useful except in the case of MRSA where clonal spreading makes PFGE less useful. For the global epidemiological purposes MLST is most useful. In future more use will be made of DNA chips.

4.2 Update on National Epidemiology data. (Marie-Laure DE BUYSER -CRL, AFSSA)

Staphylococcal food poisoning outbreaks (SFPO) rank second for number of food poisoning outbreaks and 3rd for the number of cases in France in 2006 and 2007. *S.aureus* is the first agent responsible or suspected in cases associated with milk and milk products. There are more outbreaks suspecting *S.aureus* and the etiological agent than confirmed cases.

4.3 Outcome of EFSA enquiry on molecular typing. (Pierre-Alexandre BELOEIL, EFSA, Parma).

EFSA circulated a questionnaire on molecular typing in MS in autumn 2008. 26 MS replied. Some MS not performing typing

and other purchase the service from other countries. Only a few countries type isolates on a routine basis and this is on food isolates.

4.4 Development of SE genes by PCR

The CRL have identified 21 SE genes by PCR. SE types A, B, C, D, E are classical Enterotoxins and are emetic. Only these 5 types can be detected by commercial kits. Types G, H, I, R, S, T are new Enterotoxins and are known to be emetic. SEI and SET show reduced emetic activity.

The CRL worked on the development of SE genes detection by PCR using simplex / multiplex PCR. A good concordance was observed between the 2 methods but the PCR multiplex was better as the genes in some isolates were detected by multiplex only. The multiplex can detect 2 additional genes ser and sep. The CRL will circulate the new procedure.

4.5 Optimisation of molecular typing by PFGE

CRL examined critical points to optimise PFGE for *S.aureus*. These include:

- Bacterial cell harvesting: Agrose plate vs liquid broth . The liquid broth enabled the washing of cells and is a very important step to improve extraction efficiency.
- Proteinase K digestion; Overnight vs 2hr with better results from overnight digestion.
- Plug shape: Square plug vs agarose disk. Better results with square plug.
- Extraction Buffer: TE vs PIV salty buffer. PIV agarose solution gets solid at 52°C and does not support microwave treatment.
- Lysostaphin conditioning: frozen vs freshly prepared. Freshly prepared lysostaphin is time consuming to prepare. Results acceptable with frozen lysostaphin.
- Improvements in low bands separation and sharpness. Work in progress.

Report

3rd Workshop of the Community Reference Laboratory on *Listeria monocytogenes*, AFSSA, Paris, 14th - 15th May 2009

NRL Representative: Bernadette Hickey, DSL

1.0 EFSA activities on data collection for *L. monocytogenes*

Pia MÄKEKÄ presented the EFSA activities on collection of

zoonotic data relating to *Listeria* in the 2007 EFSA report: Stable number of listeriosis cases after a significant increase in the previous years: 1554 cases (1583 in 2006) in total from 25MSs; Ready-to-eat foods (RTE) are the main source of food-borne listeriosis infections. It was noted that there were more cases of listeriosis reported with pasteurised soft/semi soft cheeses than with raw milk's cheeses.

1.1 Proposal for a EU wide survey on *L. monocytogenes* in RTE food - Baseline survey

P. MÄKELÄ presented an ongoing EFSA project, i.e., the setting-up of a technical protocol for a baseline survey on *L. monocytogenes* in the European Union for RTE foods, focused on 3 food categories: packaged, smoked fish, semi-soft cheese, and heat-treated meat products. This survey will be a community specific survey and take place in 2010.

2. Detection/enumeration of *L. monocytogenes*

2.1 Interlaboratory trial

The results of the Interlaboratory trial on the detection of *L. monocytogenes* in salmon were presented. 30 NRLs participated in the PT trial and provided results, which could be taken into account for the statistical analysis. The statistical analysis showed that the performance of the network was good with an accuracy rate of 97.3%.

2.2 Study on the detection method

N. GNANOU-BESSE presented the progress of the CRL Lm study on overgrowth in *Listeria* populations undergoing enrichment culturing. No significant difference between 24 h and 48 h second enrichment in full Fraser was observed. The 2nd enrichment could not be avoided because stressed *Listeria* bacteria at low levels could be hidden by high level of competitive flora. It may be possible to reduce the duration of the 2nd enrichment

to 24 h but there was a need of a further study in natural contamination. The EC baseline survey may be a source of samples.

2.3 *L. monocytogenes* enumeration at low contamination levels in food using a membrane filtration method

L. BARRE presented the progress of the evaluation study of the enumeration method using membrane filtration at low levels. The method has been validated for cold smoked salmon. CRL will continue to test suitability of method for other food matrices.

2.4. Measurement uncertainty

M. CORNU presented the estimation of measurement uncertainty (MU) which should be performed according to ISO/TS 19036 and the Guide on measurement uncertainty for the enumeration of *L. monocytogenes* (CRL Guide MU, see CL 2009/02 - dated 30 March 2009). ISO/TS 19036 states that MU should be calculated on naturally contaminated product. This is impossible due to unavailability of naturally contaminated product.

The Guide and ISO/TS 19036 refers to defining MU for "consistent group" of bacteria, in terms of MU value. In the CRL Guide, the MU for Lm was estimated based on the MU value for total plate count. Several participants did not agree with this approach. The CRL are to prepare a second version of the CRL Guide.

2.5 Update on ISO standardization

N. GNANOU-BESSE and Enne DE BOER (NL-NRL) presented an update on the revision of EN ISO 11290-parts 1 & 2, undertaken by an Ad'hoc group *Listeria* of CEN/TC 275/WG 6.

The group were set up to update EN ISO 11290-1 and 2

Aim	Proposal
Simplification of the confirmation step.	Detection: 1 typical colony to be selected and if negative 4 more colonies to be selected. Enumeration: 5 typical colonies for confirmation. Considering removing CAMP test as not robust.
Extension of the scope to include other <i>Listeria</i> species.	Title to change and other <i>Listeria</i> species to be added to title.
Replacement of phosphatidyl inositol by soya lecithin.	PI to remain main formulation but footnote to allow PI replacement by soya lecithin.
Plating out agar for Detection.	ALOA and Second selective media such as Oxford, Palcam or other chromogenic media.

Aim	Proposal
Preparation of homogenate and resuscitation in enumeration method.	A common preparation for Detection and Enumeration will be included. Resuscitation step will be removed from enumeration method.
Extension of scope to include environmental samples.	Further information required.
Correct errors and inconsistencies between I 1290-1 and 2.	To carry out. Incubation 37°C, reference to 35°C removed.
Characterisation tests.	Agar for selection of colonies for confirmation -non selective agar such as blood agar, nutrient agar, tryptone soya agar. Catalase reaction (optional). Haemolysis: Stab and streak colony onto surface of agar. New table of characterization tests for presumptive L.monocytogenes and other presumptive Listeria species. Carbohydrate utilization: Voges- Proskauer VP reaction.

3. Microbial shelf-life studies and predictive microbiology

3.1. CRL Lm Technical Guidance Document on shelf-life studies

Marie CORNU, MQR Unit (CRL Lm), presented the Technical Guidance Document on shelf-life studies for L. monocytogenes in ready-to-eat foods, Version 2 of 14/11/2008 (TGDv2), on how to conduct challenge tests and durability studies. This guide had been prepared by the CRL, in collaboration with a WG of NRLs.

The document was finalised in a short period of time as it was required by DG Sanco as supporting documentation for a meeting of Codex Committee on Food Hygiene that took place in December. CRL will work on a new version of the guide.

3.2 Implementation of the CRL Lm Technical Guidance Document

Hélène BERGIS, MQR Unit (CRL Lm), presented a survey related to the implementation of the Technical Guidance Document. NRLs (85%), were aware of the document, a third (30%) apply it and 45% dispatched it to laboratories undertaking this area of work.

4. Strain typing / epidemiology

4.1. Outcome of EFSA questionnaire on Lm molecular typing

P. MÄKELÄ presented the results of an EFSA survey circulated to MS in autumn 2008 on the use of molecular typing methods. Some MS not performing typing and other purchase the service from other countries. Typing is performed mainly for pathogens

(Salmonella and Listeria) Only a few countries type isolates on a routine basis and this is on food isolates.

4.2 PT trial on agglutination/molecular serotyping and on PFGE typing

Benjamin FELIX presented the PT trial organization and results. The report is scheduled to be dispatched by the end of June. The outcome was really good, especially for a first trial. The drafting of a harmonized guide on agglutination serotyping was suggested, it would be reconsidered at the next workshop based on the final report of the trial. The next PT trial on PFGE would include gel interpretation with BioNumerics software.

Report

Short Report on the 14th International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Madrid, 17 - 20 June 2009

Attended by: Dr Paul Collery, CVRL

The functions and mission of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) can be summarised as:

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- Disseminate information relating to the diagnosis of animal diseases;
- Coordinate diagnostic activities of regulatory, research and service laboratories;
- Establish uniform diagnostic techniques;
- Improve existing diagnostic techniques;
- Develop new diagnostic techniques;
- Establish accepted guidelines for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities;
- Facilitate the organization of associations of veterinary laboratory diagnosticians in all countries of the world;
- Provide consulting assistance to countries wishing to build and operate veterinary diagnostic laboratories.

The WAVLD conference comprised:

- A half-day pre-meeting symposium on 'The Development and Implementation of Veterinary Diagnostic Laboratory Networks'. This was presented by staff from the US National Animal Health Laboratory Network (NAHLN).
- The two-day WAVLD Conference proper which comprised presentations on a range of topics related to veterinary laboratory diagnostics.
- A full-day OIE seminar on 'Veterinary Laboratory Networks and Networking' - with presentations from OIE staff.

If there was a single key message to be taken from this conference - both from the presentations and from discussions with delegates - then it was that diagnostic laboratory expertise must be well dispersed nationally. While there are benefits to be gained from locating specific skills in individual laboratories, effective surveillance requires adequate national coverage, and a secure system of communication. The OIE refers to national laboratory networks as 'global public goods' - an investment by one country benefits all. A secondary message from the conference was the importance of having common procedures and standards within national laboratory networks. Two of the presentations which covered these themes are summarised below.

Barbara Martin gave a presentation on The US National Animal Health Laboratory Network. The United States National Animal Health Laboratory Network (NAHLN) was established in 2002 to enhance the detection and response to animal health emergencies - including bioterrorist events, newly emerging diseases, and exotic diseases. The NAHLN is a collaborative effort between the US Dept of Agriculture and the American Association of Veterinary Laboratory Diagnosticians (AAVLD). From an initial group of 12 laboratories the NAHLN has now expanded to 54 laboratories in 45 US states.

The founding principles of the NAHLN comprised:

- Standardized, rapid diagnostic techniques;
- Secure communications, alerting, and reporting systems;
- Modern equipment and trained personnel;
- Training, proficiency testing, and quality assurance programs;
- Facilities that meet biocontainment and security requirements;
- Scenario testing in support of regional and national training exercises;
- Quality management system compliant with international standards.

After seven years of operation, the NAHLN has become the laboratory backbone of the United States emergency response and recovery program, and has enabled implementation of national, standardized surveillance for high priority diseases. NAHLN laboratories are currently participating in surveillance for avian influenza (AI), exotic Newcastle disease, classical swine fever, BSE, chronic wasting disease of deer, and scrapie.

The presentation also outlined the NAHLN involvement in recent surveillance for swine flu to illustrate its activities. The speaker stressed the importance of training, proficiency testing, and

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quality assurance programs in maintaining a network of laboratories with comparable diagnostic standards. Because of the number of laboratories involved, the NAHLN uses programs of 'training the trainers' wherever possible. This also compensates for staff movements - provided a trainer remains available in-situ.

Terry McElwain of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) Accreditation Committee gave a presentation on 'Quality Assurance and Accreditation'.

The presentation covered the recognised standards in the US - and their application to laboratory networks. These comprise the AAVLD, ISO and OIE standards. It was stressed that accreditation to a standard is an evaluation of competency. It is not a product guarantee or certification.

The speaker referred specifically to the ISO standard 15189 for medical laboratories. He was not aware of any veterinary laboratory accredited to this standard. While the OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008 are also comparable to ISO 17025, the OIE is not an accreditation body - so the OIE standard must first be adopted by a national accreditation body before it can be applied in practice. The AAVLD has adopted the OIE standard - and applies it in its accreditation program for publicly funded veterinary laboratories in the US.

According to the speaker, the OIE standard is very well suited for veterinary laboratories. It has eliminated many of the calibration components of ISO 17025.

Accountability for accreditation of the NAHLN laboratories goes to a high level in each State in the US. The accreditation certification for each laboratory is signed off annually by the Director of that laboratory, the Biosafety Officer, and the State Veterinarian.

The rest of the presentation was a detailed coverage of the essential components of accredited quality systems - and their application and significance in laboratories accredited by the AAVLD. Quality Assurance is an ongoing process to ensure the quality of a service. It creates a standard and accountability for work performed, and provides a system to ensure that the conduct of procedures meet the expectations of the client. The importance of staff training, client confidentiality - as well as method transparency vis-à-vis clients - were stressed. Also the need for good internal communications, periodic management reviews, and a time-frame for internal auditing.

Matrix/ Parameter	CRL	Head of NRL	Contact person (if different to Head NRL)
Milk and milk products	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort, France	Bernadette Hickey Tel: +353 6157452 Fax: +353 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
Zoonoses (salmonella)	Rijksinstituut voor Volksgezondheid en Milieu (RIVM) 3720 BA Bilthoven The Netherlands	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Dr Montserrat Gutierrez Tel: +353 6157222 Fax: +353 6157116 Email: mm.gutierrez@agriculture.gov.ie
Listeria monocytogenes	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort France	Bernadette Hickey Tel: +353 6157452 Fax: +353 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
Coagulase positive <i>Staphylococci</i> , including <i>Staphylococcus aureus</i>	AFSSA - Laboratoire d'études et de recherches sur la qualité des aliments et sur les procédés agroalimentaires (LERQAP) F-94700 Maisons-Alfort France	Bernadette Hickey Tel: +353 6157452 Fax: +353 6157454 Email: Bernadette.hickey@agriculture.gov.ie	
<i>Escherichia coli</i> , including Verotoxigenic E. Coli (VTEC)	Istituto Superiore di Sanità (ISS) I-00161 Roma Italy	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Dr Lourda Scott Tel: +353 6157352 Fax: +353 6157353 Email: lourda.scott@agriculture.gov.ie
Campylobacter	Statens Veterinärmedicinska Anstalt (SVA) S-751 89 Uppsala Sweden	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Dr John Egan Tel: +353 6157138 Fax: +353 6157116 Email: john.egan@agriculture.gov.ie
Parasites (in particular <i>Trichinella</i> , <i>Echinococcus</i> and <i>Anisakis</i>)	Istituto Superiore di Sanità (ISS) I-00161 Roma Italy	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Paul Rafter/Dr Tom Murphy Tel: +353 6157350 Fax: +353 6157361 Email: paul.rafter@agriculture.gov.ie
Antimicrobial resistance	Danmarks Fødevareinstituttet DK-1790 København V Denmark	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Dr Montserrat Gutierrez Tel: +353 6157222 Fax: +353 6157116 Email: mm.gutierrez@agriculture.gov.ie
Animal proteins in feedingstuffs	Centre Wallon de recherches agronomiques (CRA-W) B-5030 Gembloux, Belgium	Gabriel Roe Tel: +353 6302902 Fax: +353 6280634 Email: Gabriel.roe@agriculture.gov.ie	
Transmissible spongiform encephalopathies (TSEs)	The Veterinary Laboratories Agency Surrey KT15 3NB United Kingdom	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Dr Paul Collery Tel: +353 6157203 Fax: +353 6157199 Email: paul.collery@agriculture.gov.ie
Chemical elements in food of animal origin	Istituto Superiore di Sanità Viale Regina Elena 299 00161 Rome, Italy.	Dr John Ferris Tel: +353 6157106 Fax: +353 6157197 Email: john.ferris@agriculture.gov.ie	Paul Rafter Tel: +353 6757350 Fax: +353 6157361 Email: paul.rafter@agriculture.gov.ie