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# Pathology Division

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## Annual Report 2009

### Introduction

Pathology Division comprises three Sections – Histopathology, Clinical Chemistry and Dublin Regional Veterinary Laboratory. The primary role of the Division is to provide diagnostic pathology expertise and resources in support of the Department's responsibilities in relation to animal health. This includes providing laboratory support for national disease control and eradication programmes, as well as specialist pathology support for the Department's Regional Veterinary Laboratories (RVLs).

The Division also provides Project Management for the Veterinary Laboratory Service Laboratory Information Management System (LIMS). The LIMS – which is networked throughout the Department's Veterinary Laboratory Service – is used to manage laboratory data throughout its entire cycle from sample reception, through testing and reporting, to data retrieval, reporting and analysis.

Three key posts in Pathology Division remained unfilled during 2009 – Deputy Chief Analyst (last occupant retired February 2009), Senior Research Officer in charge of Clinical Chemistry Section (last occupant retired March 2009) and a fulltime LIMS administrator (post vacant since its sanctioning in 2008).

### Dublin Regional Veterinary Laboratory

Dublin Regional Veterinary Laboratory (DRVL) provides a diagnostic pathology service to the agriculture industry in the North Leinster area and in counties Monaghan and Cavan. It is part of the Department's network of Regional Veterinary Laboratories - which provide specialist diagnostic pathology facilities at strategic locations throughout the country. In 2009, Dublin RVL completed its second full year of provision of service from its new location at Backweston. Submission numbers

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continued to increase in almost every category during 2009 - reflecting wider client familiarity with the services available, as well as the location of the new facility at Backweston.

### **Diagnostic Submissions and Investigations (Statutory and Surveillance)**

Total numbers of carcass and clinical pathology submissions from live animals to Dublin RVL are given in Table 1. As investigations by may include a wide range of supporting laboratory tests and analyses such as microbiology, parasitology, biochemistry, toxicology, and haematology, the figures below only give an indication of the spread of work encountered across the various species of farm and wild animals.

• Table 1: Submissions to Dublin RVL in 2009.

<b>Species</b>	<b>Carcass<sup>1</sup></b>	<b>Clinical Pathology<sup>2</sup></b>
Avian	401	119
Bovine	310	1169
Ovine	106	106
Porcine	220	89
Caprine	74	19
Badger	130	2
Fox	56	
Other <sup>3</sup>	11	25
Totals	1,308	1,529

1 Includes carcasses, part-carcasses and aborted fetuses.

2 Blood samples, swabs, faeces, etc.

3 Includes equine, cervine, canine, feline, exotic submissions.

Dublin RVL provided continued support for the Department's control and eradication schemes for TSEs (BSE and scrapie), tuberculosis, and brucellosis in 2009. This included a collaboration with Dublin-Wicklow DVO in the investigation of tuberculosis in East Wicklow and South County Dublin.

Notifiable diseases diagnosed in submissions to Dublin RVL during 2009 included, tuberculosis (*Mycobacterium bovis* infection) in goats, badgers, and calves, Caseous Lymphadenitis (*Corynebacterium pseudotuberculosis* infection) in sheep, *Mycoplasma gallisepticum* in turkeys, *Salmonella typhimurium* in pigs and cattle, and *Trichinella spiralis* in foxes.

Dublin RVL also undertook field investigations of serious disease outbreaks. In 2009, these included an investigation of multifocal haemorrhage in a four-month-old calf that was associated with BVD virus. One of the factors that had to be considered in the investigation of this case was the emergence in neighbouring countries of a new disease of 'Haemorrhagic Diathesis in calves'. Although this incident was confirmed as related to BVD virus infection, the seriousness with which surveillance for emerging diseases is taken by the Department is highlighted by its standing offer to waive post mortem examination charges for any submissions that meet the clinical description of multiple haemorrhages in a calf of less than one month of age.

Dublin RVL also continued its involvement in the diagnostic pathology investigations of outbreaks of tuberculosis in lactating goat herds. This included pathological confirmation of tuberculosis in affected goats in a field application of the intradermal tuberculin test and gamma interferon assay. A paper arising from this investigation has been accepted for publication in the peer-reviewed

journal Veterinary Record - *A Concurrent Outbreak of Tuberculosis and Caseous Lymphadenitis in an Irish Goat Herd*. A.E. Sharpe, C.P. Brady, A. Johnson, W. Byrne, K. Kenny and E. Costello.

Dublin RVL was also involved in the investigation of tuberculosis in calves from a farm in Co. Wicklow. It also provided pathology support to the CVRL TB Section in its investigations of a farm in Munster, as well as studies in the TB isolation unit on the CVRL Abbotstown Farm.

### **Projects and Special Investigations**

Participation by a Dublin RVL pathologist in a multi-agency research project on *Toxoplasma gondii* abortion in sheep continued in 2009. The focus during the year was on screening sera harvested from *Toxoplasma gondii* challenged dams and their fetuses for specific IgG and IgM responses employing ELISA techniques. Other immunology and pathology data has already been collected from the same challenge experiment. The purpose of the work undertaken in 2009 was to combine the serology findings with the immunology and pathology data with a view to generating a publication provisionally entitled “*Sequential study of maternal and foetal immune responses of pregnant sheep challenged with Toxoplasma gondii*.”

Other projects where Dublin RVL provided post mortem or full pathology support included:

- Project led by Bacteriology Division as part of the DAFF response to the dioxin animal feed contamination incident. This involved euthanasia and sampling of over 170 pigs of various ages and about 20 two-year-old beef bulls and heifers through the Dublin RVL Post Mortem facilities.
- Undertaking gross and histopathology examinations in support of DAFF Special Investigation Unit investigations of suspected interference of the TB tuberculin test.
- Pathology examinations for highly pathogenic H5N1 strain of Avian Influenza. Systems were in place for submission, examination and sample collection from wild and domestic birds (or for porcine investigations in consideration of the perceived risks from pigs of the H1NI influenza virus). In addition to examination of wild birds, Dublin RVL also examined and collected samples from domestic poultry with a history of suspicious outbreaks of mortality. All examinations were negative for Avian Influenza.

Dublin RVL, along with the five other RVLs, and in collaboration with Agriculture House and District Veterinary Offices, participated in a survey of *Trichinella spiralis* in foxes. This was carried out as required by EU Directive.

DRVL staff also contributed to the production of the ‘*Regional Veterinary Laboratories Disease Surveillance Report 2009*’. This report, which has been widely distributed to interested parties in the agriculture sector and media, comprises an analysis of the most frequently diagnosed causes of disease and deaths identified in investigations on submissions to the Veterinary Laboratory Service. Its objective is to provide information on the occurrence of disease in farm animals, and to provide a basis for national and farm-level disease control measures. From an international perspective, it also contributes to evidence of national surveillance of the Irish livestock population in support of measures to demonstrate freedom from specific diseases which are of significance in relation to international trade and animal welfare.

Along with the other RVLs, Dublin RVL provides a monthly report of case histories for inclusion in the monthly summary RVL Report in the Irish Veterinary Journal. These monthly reports provide an up-to-date source of information for veterinary practitioners regarding disease conditions that are being investigated by the RVLs. See link below to example RVL Monthly Report on DAFF website:

<http://www.agriculture.gov.ie/animalhealthwelfare/laboratoryservices/regionalveterinarylaboratoryreports/rvmonthlyreports2009/june2009rvmonthlyreport/>

### Proficiency Testing and external QA

Dublin RVL participates in external proficiency ring trials provided by the UK Veterinary Laboratories Agency proficiency testing service VETQAS. Test types and distribution dates are given in Table 2. Monitoring performance in these tests provides a regular comparison with results from other similar laboratories – and is an integral part of the Dublin RVL laboratory quality control system.

• Table 2: DRVL proficiency trials 2009

	Microbiology <sup>1</sup>	Haematology <sup>2</sup>
9/1/2009	√	NA
30/1/2009	√	NA
27/2/2009	√	NA
3/4/2009	√	√
1/5/2009	√	NA
29/5/2009	√	√
3/7/2009	√	√
4/8/2009	√	√
8/9/2009	√	√
6/10/2009	√	√
3/11/2009	√	√
1/12/2009	√	√

1 Microbiology culture & isolation - Farm animals.

2 Ruminant blood.

Dublin RVL pathologists also participate in regular histopathology slide assessment sessions along with UCD veterinary pathologists. The slides for these sessions are distributed to veterinary pathologists in the US and Europe by the AFIP (American Forces Institute of Pathology).

## Histopathology Section

Histopathology Section is responsible for the histological processing of all animal tissue sections within the Veterinary Laboratory Service. This involves mounting, cutting and staining of tissue sections. Tissue submissions originate from post-mortem examinations carried out by pathologists in the six RVLs, from pathology examinations in CVRL Backweston as part of special investigations, from research projects, from samples collected as part of DAFF disease surveillance schemes, e.g. suspect tissues collected in abattoirs as part of the TB eradication scheme, and from external sources such as private veterinary practitioners.

The Section also provides a specialist histopathology referral service for the RVLs and external clients.

As the EC National Reference Laboratory for TSEs, Histopathology Section is also responsible for the confirmatory diagnosis of all suspect TSE cases identified in the State. Additional responsibilities assigned to the Section under the EC TSE legislation (999/2001) include the approval and ongoing monitoring of private laboratories carrying out rapid screening tests for TSEs. The TSE NRL also maintains an archive of TSE-positive tissues. The latter is used to support quality control of TSE screening tests – as well as local and international research projects.

## General Histopathology

### Case Throughput (includes TSE-related tests)

Details of case throughput for Histopathology Section in 2009 are given in Table 3.

- Table 3: Histopathology Section tests and procedures in 2009.

Description	Number <sup>1</sup>
<b>Routine Stains:</b>	
<i>Haematoxylin and Eosin staining (H&amp;E)</i>	18,016
<b>Special Stains:</b>	
<i>Gram</i>	334
<i>Ziehl-Neelsen</i>	339
<i>Grocott stain for fungal hyphae</i>	99
<i>PAS stain for fungal hyphae</i>	122
<i>Perl's Prussian Blue stain for iron</i>	49
<i>Toluidine Blue stain for mast cells</i>	11
<i>Warthin Starry stain for spirochetes</i>	87
<i>Miscellaneous</i>	166
<b>Immuno-histochemistry:</b>	
<i>TSE (BSE and scrapie)</i>	785
<i>Porcine Circovirus</i>	96
<i>Chlamydia (agent of ovine enzootic abortion)</i>	7
<i>Toxoplasma</i>	19
<i>Neospora</i>	92
<i>Mycoplasma bovis</i>	7
<b>Western Blots:</b>	<b>No. Tests<sup>2</sup></b>
<i>PrP Western Blot</i>	39
<i>PrP Western Blot BSE Strain</i>	7
<i>PrP Western Blot Discriminatory</i>	19

1 Counts number of tissue cassettes overnight wax-embedded, thin sectioned, slide-mounted and stained in preparation for histopathology examination. Includes recut requests.

2 Number of tests performed.

## **Developmental Work**

An immuno-histochemical test for bovine viral diarrhoea (BVD) virus which can be performed on formalin-fixed tissues, using commercially available antibodies and published techniques, was added to the inventory of IHC stains available in Histopathology Section during the year. This assay has been used on several clinical cases submitted by pathologists in the RVLs to confirm diagnoses of BVD. It can also be used to differentiate acutely-infected from persistently-infected animals.

A real-time PCR assay for *Mycoplasma bovis* was also developed during the year. This uses published genome sequences to identify the target area. The assay is undergoing optimisation prior to being offered as a diagnostic test.

## **TSE NRL**

### **TSE Confirmatory Diagnosis**

The NRL is responsible for the confirmatory diagnosis of all suspect TSE cases. These comprise clinical suspects which are identified by the Department's passive surveillance systems, as well as rapid-test reactivities identified in the private TSE rapid-test laboratories by the active surveillance program at abattoirs and knackeries. The confirmatory diagnostic procedures carried out by the NRL comprise histopathological and immunohistochemical examination of brain sections, as well immunoblotting to detect disease-specific PrP in CNS target sites.

Brains from TSE clinical suspect cases are removed in a Regional Veterinary Laboratory. They are partially dissected in the RVL, one half being fixed in formal saline and the other half frozen at minus 20°C. The fixed and frozen tissues are submitted to the NRL for confirmatory diagnosis by histopathology, immunohistochemistry and immunoblot.

Hindbrain samples from the RTLs which give a positive result to the rapid test are also submitted to the NRL. These are submitted fresh and are assessed by the pathologist on duty at the NRL when they arrive. If suitable, the tissues are fixed and subjected to histopathology and immunohistochemistry examination. All samples are also tested by immunoblot.

### Bovine Cases

In 2009, confirmatory diagnosis was carried out on samples from a total of 53 bovines. BSE was confirmed in 9 animals. All nine were from RTLs, i.e. active surveillance. The other 44 samples were Clinical Suspect cases (passive surveillance). None of these were confirmed as positive for BSE.

Table 4 shows a breakdown of the histopathological diagnoses reached for these cases.

- Table 4: Histopathological Diagnoses for BSE Clinical Suspects in the TSE NRL 2009.

H&E Result	Number of Cases
Listerial Encephalitis	15
Non-suppurative encephalitis	3
Neoplastic	2
Fibrinosuppurative ventriculitis	1
Progressive Ataxia of Charolais	1
No Specific Findings	22
<b>Total</b>	<b>44</b>

### Ovine Cases

Thirty eight cases of Scrapie were confirmed by the TSE NRL in 2009. Of these, 33 were classified as Classical Scrapie, with the remaining five being classified as Atypical Scrapie (Nor-98).

The 33 confirmed cases of Classical Scrapie were from eight separate flocks. Twenty three had been initially submitted for confirmatory testing to a Rapid Testing Laboratory (active surveillance) – and from there to the NRL for confirmatory testing. The remaining ten were submitted to the NRL as clinical suspect cases *via* the Regional Veterinary Laboratories.

Tissues from four of the five Atypical Scrapie cases were received in the NRL *via* the Rapid Testing Laboratories i.e. they were detected through the Department's active surveillance programme for scrapie. Tissues from the remaining atypical case were submitted to the NRL from a Regional Veterinary Laboratory under the Department's Scrapie Monitor Flock Scheme.

Two clinical suspect cases (passive surveillance), submitted through Regional Veterinary Laboratories, were confirmed as scrapie negative.

Twelve new flocks were identified with scrapie during the year based on the NRL confirmatory diagnosis testing – seven with Classical and five with Atypical scrapie (Nor-98). Co-existence of the classical and atypical forms of scrapie was not detected in any flock during the year.

All ovine samples were also subjected to Discriminatory Western Blot testing in the NRL to differentiate between scrapie and BSE in sheep. All scrapie-positive cases were confirmed by this technique 'Scrapie-like' in 2009 - thus providing no evidence to suggest the presence of BSE in sheep in Ireland.

### **Accreditation of TSE NRL Testing**

A Quality Manager was appointed to the Division in March 2009 with responsibility for the implementation of a quality system in accordance with ISO 17025 - and accreditation of certain procedures to ISO 17025. A Senior Research Officer in the Division was appointed Technical Manager. A defined Quality System was devised, and first implemented in the laboratory in June 2009. A Quality Manual was written and quality and technical SOPs implemented.

A corrective and preventive action system has been implemented in the laboratory. A training programme has been introduced and implemented. The training programme includes initial induction and training of new staff, and ongoing competence of established staff in both technical and quality procedures. An audit program has been implemented. All of the clauses of ISO 17025 are audited, and vertical and witness audits are also performed.

An application for accreditation to ISO 17025 was made to INAB in October 2009 for accreditation of the Discriminatory and Confirmatory Western Blot methods. A pre-assessment inspection by INAB was expected to take place early in 2010.<sup>1</sup>

### **Proficiency Trials in the TSE NRL**

All EU TSE National Reference Laboratories are required to participate in proficiency trials issued by the TSE Community Reference Laboratory. In 2009 the Irish NRL participated in the following rounds:

- BSE Confirmatory Immunoblot test.
- TSE Discriminatory Immunoblot test.
- Scrapie Confirmatory Immunoblot test.
- IHC Technical (Immunohistochemistry).
- BSE and Scrapie Histopathology.

For the immunoblot rounds, five samples of unknown status are tested and results submitted to the CRL through an on-line portal. Reports are issued which include a tabulation of the results for all participants (anonymously), along with the expected results and comments from the Test Consultant.

The IHC Technical round involves the immunohistochemical staining of histological sections from positive and negative BSE or scrapie (classical and atypical) cases. Stained slides are sent to the CRL for assessment of staining intensity and patterns along with the interpretation of findings by the NRL. The CRL Test Consultant issues a report, comparing the staining intensity and case result interpretation by the NRL with that of the CRL.

In 2009, a new web-based portal was introduced for viewing and reporting proficiency round histological sections - stained using either hematoxylin and eosin or immunohistochemical techniques. The pathologist views the slide electronically and enters a result along with any comments they have for each case. When the closing date for the round has passed, each pathologist can view their results comparing them with the intended result, and an analysis of how their interpretation for the case compares with that of other pathologist. Four pathologist from the Irish NRL participated in the 2009 round.

Results of all rounds were deemed to be satisfactory with sufficient competency shown to maintain approval for BSE and Scrapie confirmatory diagnostic techniques as well as differential immunoblotting.

### **Tissue Bank**

The NRL maintains a tissue bank of TSE-positive tissues. Tissues are available from this on request for use in:

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<sup>1</sup> INAB pre-assessment took place on 29 January 2010.

- Preparation of proficiency trials for the RTLs.
- Positive control tissues for the NRL immunoblot.
- Positive control tissues for the rapid tests.
- National and international Research projects.
- For use in EU assessment trials of TSE rapid tests.

In 2009, the tissue bank stock was augmented by addition of BSE and scrapie-positive CNS tissues – as well as TSE-negative bovine and ovine CNS tissue.

Thirteen requests for TSE positive and negative CNS tissues were received from the private RTLs for use as control reagents and as part of their quality assurance programs. All of these requests were approved in accordance with NRL procedures. Following processing, they were packaged and shipped out by trained staff in accordance with national and international transport regulations.

### **Rapid Testing Laboratories (RTLs)**

Under EC 999/2001 the TSE NRL is responsible for monitoring performance of the TSE rapid testing laboratories in Ireland. Table 5 lists the approved TSE rapid testing laboratories in operation in 2009, together the tests they were approved to use.

- Table 5. TSE Rapid testing laboratories.

<b>RTL</b>	<b>Test</b>	<b>Species</b>
Enfer Testing	(Enfer V 2.0 <sup>1</sup> )	(Bovine and Ovine)
	IDEXX Herd Check	Bovine and Ovine
Irish Equine Centre	Prionics Check WB	Bovine
	Prionics Check SR	Ovine and Caprine
	Prionics Priostrip	Bovine
Advanced Micro Services	IDEXX Herd Check	Bovine and Ovine
Identigen	Roboscreen	Bovine
Irish Biotechnology Services (IBS)	BioRad TeSeE	Bovine and Ovine

<sup>1</sup> Approval for use of the Enfer V2 test suspended following approval and introduction of IDEXX test.

#### **LABORATORY APPROVALS**

The TSE NRL is responsible both for the approval of new laboratories to perform TSE rapid testing in Ireland, as well as for the approval of existing laboratories to implement an EC-approved test kits for which they did not previously have approval. Based on guidelines issued by the EU Community Reference Laboratory the National Reference Laboratory must ensure that laboratories approved to perform TSE rapid testing comply with the following:

- Adequacy of the facilities for the type and volume of testing being proposed.
- Experience and training of staff for the proposed test methods and their interpretation.
- Adequate understanding of the regulations and legislation relevant to the proposed work.
- The laboratory has demonstrated its diagnostic competence in proficiency tests organised by the National Reference Laboratory.

- The laboratory participates in regular ring-trial proficiency tests organised by the NRL.
- The laboratory has formally undertaken to notify the NRL of any problems or anomalies encountered in the course of testing.
- The laboratory has external accreditation.
- The laboratory has provided copies of all Standard Operating Procedures relating to its operation as a rapid-testing laboratory.

The NRL approval protocol is designed to ensure compliance with these requirements. The approval process includes meetings with the candidate RTLs, review of procedural and quality system documentation, site visits to assess the suitability of the testing facilities, review of staff qualifications and training, and performance of a series of live test trials involving TSE negative and positive tissues.

Two approvals for change of test were carried out in 2009. The first involved the proposed move by the Enfer laboratory from the Version 2 of the Enfer test to Version 3. However, this approval process was terminated with the change of ownership of the Enfer laboratory. The second approval was for the subsequent adoption of the IDEXX test by Enfer.

#### INSPECTIONS

Unannounced and pre-arranged visits to RTLs are carried out periodically – see Table 6. As the laboratories work overnight, unannounced visits generally commence in the evening – and extend into the night's testing run. The purposes of these visits are to ensure sample traceability throughout the testing process, and to ensure RTL compliance with standard operating procedures and the test manufacturer's instructions.

Each of the RTLs received at least one unannounced inspection visit during 2009. The inspection teams comprised at least one member of staff from the TSE NRL and at least one member of staff from DAFF's TSE and Animal By-Products Division. The main scope of the visits comprised:

- Sample traceability and quality.
- Staff training records and proficiency in relation to sample cutting and grading and test process.
- Quality System documentation to ensure it is consistent with current IFU and NRL sampling and test requirements.
- Implementation of the test process.
- Laboratory waste processing and disposal.
- Result interpretation, reporting, and procedures relating to transmission of samples to the NRL.

• Table 6: Inspection Visits to Rapid Testing Laboratories in 2009

RTL	Date
Enfer Testing	22/6/09
Irish Equine Centre (IEC)	22/9/09
Advanced Micro Services (AMS)	2/11/09
Identigen	2/9/09;
Irish Biotechnology Services (IBS)	28/05/09

Reports were issued to the RTLs following the inspection visits. These outlined the findings of the visits and highlighted areas that needed to be addressed where relevant. Issues for attention were followed up by communications between the inspection teams and the RTLs to ensure compliance.

A secondary inspection was necessitated in one case arising from issues identified in the initial inspection. Meetings with management from the RTL, and staff from the TSE NRL and DAFF TSE and Animal By-Products Division, also took place.

#### ANALYSIS OF RTL RAW DATA

Raw data results from each night's testing are received in the NRL each day by email. Each month, the raw data from at least four night's testing for each RTL is examined in detail in the NRL. The examinations provide a means of randomly checking sample throughput, test results including anomalies and repeat testing, kit references, kit and sample controls and testing durations and sequences. Any issues identified by the raw data analysis are followed up with the RTL concerned.

#### PROFICIENCY RING TRIALS OF RTLs

The NRL arranges and supervises regular proficiency ring trials of all RTLs – see Table 7. This involves the preparation and delivery of coded brain tissue homogenate samples to all RTLs, and subsequent analysis and reporting of the results.

Two RTL proficiency trial rounds were conducted by the TSE NRL during 2009 - in May and November. In May, five bovine and five ovine homogenates (TSE positive and negative) were sent to the RTLs. Each of the RTLs correctly identified all of the homogenates.

• Table 7: Tests used in the 2009 NRL-organised proficiency trials of RTLs.

RTL	Bovine	Ovine
Enfer	IDEXX BSE – Scrapie Antigen Test kit	IDEXX BSE - Scrapie Antigen Test kit
IEC	Prionics WB and Prionics Priostrip	
AMS	IDEXX BSE - Scrapie Antigen Test kit	IDEXX BSE - Scrapie Antigen Test kit
Identigen	Roboscreen EIA	NA
IBS	BioRad TSE Elisa	BioRad TSE Elisa

In November, the design of the proficiency trial was changed. Homogenates were prepared from two BSE-positive bovine brains using the CRL protocol. A single aliquot of each stock homogenate and three aliquots each of 1:10 and 1:20 dilutions were sent to each RTL for testing. Three aliquots of a negative brain homogenate were also sent. The same protocol was also followed for ovine brains.

The criteria for passing the proficiency trial were that positive results should be obtained for undiluted positive homogenates - and negative results should be obtained for negative homogenates. The dilutions were used to compare analytical sensitivity between the laboratories.

The expected results were obtained for each homogenate by each laboratory. The OD values obtained by the two RTLs using the IDEXX test were similar. For future trials it was decided that more titrations will be done on dilutions of homogenates in the NRL to ensure that weaker samples are sent to the RTLs. This should enable more comparisons on analytical sensitivity between RTLs.

#### SAMPLE QUALITY MONITORING

Due to the very localised distribution of disease-specific PrP in the CNS, ensuring correct sample collection (hindbrain) at abattoirs and knackeries has always been a key component of the Department's TSE surveillance programs. While ensuring the quality of sample collection at source is the responsibility of the VPH and TSE ABP Divisions of the Department, the TSE NRL has a role in monitoring the RTL reports on sample quality as delivered - and in ensuring that this information is transmitted to the relevant DAFF Divisions. In 2003, in order to provide a means whereby the RTLs can objectively record sample quality, the NRL introduced a scoring system for RTLs to record sample quality from abattoirs in terms of anatomical suitability (SO and NC grading) and from knackeries in regard to the degree of autolysis. Since 2007, the RTLs have sent monthly sample quality reports to the NRL (abattoir and knackery), and these are used in the NRL to provide quarterly analyses which are forwarded to the VPH and TSE ABP Divisions.

The NRL also uses the RTL monthly sample quality reports to monitor the consistency of sample SO and autolysis grading between RTLs.

#### MONITORING RTL SAMPLE CUTTING PERFORMANCE

In addition to monitoring the quality of samples received by the RTLs, the NRL also has a responsibility to ensure that correct sample cutting procedures are implemented in the RTLs. To this end, the NRL issues a 'Sample Cutting Protocol' to RTLs which is based on the optimal rapid-test target site in the hindbrain as identified in the OIE Manual. This is incorporated into RTL SOPs and training. The TSE NRL also provides advice and support to the RTLs in training staff in sample taking techniques.

The NRL also implements an inspection program to ensure that sub-sampling for rapid testing is maintained to an adequate standard. As well as inspecting cutting technique during regular visits to the RTLs, the NRL oversees a programme of monitoring sampling technique in each RTL – see Table 8.

Prior to June 2009, this inspection of cutting technique involved RTLs submitting cut samples for assessment to the NRL on a quarterly basis. Since June 2009, each RTL has been required to carry out biannual in-house monitoring of sampling technique by each member of staff involved. The RTLs carry out their own internal checks according to a procedure defined by the NRL. At least once per year, the NRL requests that each RTL submit the samples which have been used in an internal assessment. These are subjected to gross and microscopic examination by NRL

pathologists to monitor the quality of the RTL assessments and to ensure sampling is targeted at the optimal sites for rapid testing.

- Table 8: Program of RTL sample cutting checks in 2009.

RTL	Date
Enfer Testing	09/04/09;16/04/09
Irish Equine Centre (IEC)	15/01/09;15/04/09;07/07/09
Advanced Micro Services (AMS)	24/04/09;07/05/09
Identigen	07/01/09;16/04/09
Irish Biotechnology Services (IBS)	24/04/09

The internal assessments are used by the RTLs to identify and address procedural or training needs. Any additional anomalies or deficiencies identified by the NRL external assessments are notified to the RTL concerned to address as required.

### **CRL Issues**

Under EC legislation (999/2001, 882/2004) the TSE NRL is required to collaborate with the TSE Community Reference Laboratory (CRL) - and to ensure dissemination of information supplied by the CRL. During 2009, the NRL was in communication with the CRL on a range of issues – and a member of NRL staff attended the three-day CRL workshop in England in June. The NRL forwards all CRL notifications on test kit issues and batch releases to the RTLs.

### **Scrapie Monitored Flock Scheme**

The Scrapie Monitored Flock Scheme facilitates the trade in breeding sheep and goats. In most cases the brains are screened in one of the private rapid testing laboratories. However, some brains are submitted, *via* Regional Veterinary Laboratories, to the TSE NRL. In 2009 19 sheep brains were examined under this scheme. In one case atypical scrapie was diagnosed.

## **Other**

### **Projects**

#### **MOLECULAR CHARACTERISATION OF TSE STRAINS PRESENT IN CATTLE IN IRELAND**

Until recently BSE was thought to be caused by a unique infectious agent with stable features. However, a number of so-called atypical BSE isolates have been identified in Europe, Asia and the USA. These have been termed H- and L-type due to the molecular characteristics of the abnormal prion protein isolated from them. These cases are have been reported in older animals. A retrospective study of 180 Irish cases of BSE was carried out to determine if there was any evidence of atypical BSE in Ireland. A single case of atypical H-type BSE was identified in an 11-year-old cow. No evidence of L-type BSE was found. No evidence of atypical BSE was found in the animals born after the reinforced feed ban of 1996. The results of this study will be submitted for publication in a peer-reviewed journal.

## LEPTOSPIROSIS IN IRISH FARM ANIMAL SPECIES

A Research Officer in the Division continues to contribute to a collaborative project with colleagues in University College Dublin, aimed at developing improved diagnostic techniques for Leptospirosis in domestic animal species. Recent efforts have focused on the detection of the organism in animal tissues using a quantitative real-time polymerase chain reaction (qPCR).

## Q-FEVER

Q-fever, caused by *Coxiella burnetii*, is a zoonosis as well as a cause of bovine abortions. In 2009, Pathology Division initiated a pilot study to investigate Q-fever seroprevalence in cattle in Ireland by active surveillance. This study examined sera from post-abortion cows and from randomly sampled bovines with a view to estimating individual and herd level prevalence - and to identify factors associated with seropositivity. It is also intended to examine randomly-sampled bulk milk in the future. The results of the study will be disseminated and published when complete, and should be of use from both a veterinary and public health perspective.

## COOPERATIVE PROJECT WITH DAFF NATIONAL DISEASE CONTROL CENTRE ON FOOT AND MOUTH DISEASE TRAINING PROGRAMMES.

A Pathology Division RO spent six months collaborating with colleagues in the National Disease Control Committee. This involved work on the DAFF FMD contingency plan, revising biosecurity protocols, designing information leaflets on FMD and CSF, coordinating bluetongue serosurveillance in sheep and drawing up training programmes and material for VIs. In addition, it involved co-creating and designing a real-time FMD field training programme in Turkey for EU vets for the European Commission for the Control of FMD and acting as a trainer.

## Audits

The TSE NRL was audited by the DAFF Veterinary Service Internal Audit Group (SVSIAG) in February 2009. The objective of the audit was to assess the NRL compliance with Regulation (EC) No 882/2004 regarding its role as a National Reference Laboratory (NRL) for TSEs. The Audit Report concluded that the NRL did carry out its duties as NRL for TSEs as required under Article 33 of Regulation No 882/2004 and Annex X of Regulation 999/2001. It also concluded that the testing methods available at the NRL are in accordance with EU legislation. Additionally, it concluded that the NRL has a comprehensive approval and supervisory system in place for private rapid testing laboratories in accordance with legislation and CRL recommendations. While the Audit Report recorded that the NRL had a system in place to guarantee the quality of the tests results, it noted that none of the confirmatory methods was accredited to ISO 17025, and that at the time of the audit there no Quality Manager.

The NRL Action Plan prepared in response to the audit noted that a Quality Manager had been appointed, and a program was in place for accreditation during 2010. Issues identified in the audit regarding data retrieval were also addressed in the NRL Action Plan – and were fully implemented in 2009.

## Clinical Chemistry

Clinical Chemistry Section (formerly Biochemistry) performs metal (macro, trace and heavy) analyses on animal blood and tissues, and also measures metabolites and enzyme activity in serum to diagnose animal diseases. In addition to direct submissions from private veterinary

practitioners on behalf of their farmer clients, the Section also provides specialist clinical chemistry support to the Regional Veterinary Laboratories.

Because of its expertise in the study of toxicological and production-related diseases, the Section was central to the large inter-Agency investigations in Askeaton and Silvermines in the 1995 to 2002 period. The Section has been involved in several other investigations of animal health problems with an environmental component in counties Kerry, Kildare and Kilkenny. The Section also provided an expert representative on the inter-agency 'Historic Mines Sites' working group. In 2009, the Section provided clinical chemistry support in the investigation of a feed-related selenium toxicity incident in several pig herds. Further details are given below.

### Diagnostic Submissions

The numbers of tests and analyses performed on blood, tissue and other clinical pathology and toxicology samples submissions from farm animals in 2009 are shown in Table 9. These include samples referred from the RVLs, as well as samples submitted directly from veterinary practitioners or external projects (e.g. Teagasc).

- Table 9: Tests and analyses performed in Clinical Chemistry Section in 2009.

Analysis	No.
<b>Metabolites:</b> Albumin, globulin, beta-hydroxybutyrate, calcium (blood and tissue), chloride, creatinine, glucose, non-esterified fatty acid, bilirubin, urea, urinalysis.	1,877
<b>Enzymes:</b> Alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH).	880
<b>Minerals:</b> Arsenic, cobalt, copper, phosphorus, iron, lead, magnesium, molybdenum, selenium, zinc, potassium, sodium.	6,492

### Projects and Special Investigations

In the past, the Section has provided expertise in relation to toxicology, food safety and environmental animal health to DAFF, as well as to other State agencies such as the FSAI and the EPA. It is hoped that the current gap in this level of expertise can be addressed once the Section manager position (SRO) – vacant since March 2009 - has been filled.

In 2009, the Section provided analytical support for the DAFF Feedingstuff Division and Cork RVL investigations of several instances of selenium poisoning in pig herds suspected as having been due to excess inclusion rates in pig feed. This investigation generated around 200 selenium and other mineral and metabolite analyses in the Section on blood and tissue samples from animals on the affected farms. Overall, analytical findings were consistent with selenium toxicity.

## Laboratory Information Management System

Since its introduction in mid-2002, Pathology Division has provided the Project Management function for the Veterinary Laboratory Service Laboratory Information Management System (LIMS). The main functions of this role comprise:

- Monitoring overall performance of the LIMS and liaising with DAFF IT Division and the external IT support contractors (Orbis ) to supply support services as required.
- Coordinating VLS management and user requirements for enhanced or additional LIMS functionality – and liaising with DAFF IT Division and Orbis to implement agreed changes and projects.
- Coordinating local user support of the LIMS and forwarding jobs to the external support contractors.

No progress was made in 2009 on the filling of the vacant full-time LIMS administrator post.

Among the major LIMS issues dealt with in 2009 were:

- Coordination of actions to improve LIMS performance for users – in particular in the RVLs.
- Continued extension of laboratory analytical instrument integration with the LIMS in the CVRL and RVLs.
- Additional work on LIMS workflows and reports to meet ISO 17025 accreditation requirements for CVRL Divisions.
- Implementation of archiving within the Nautilus database. The first run of archiving old data, followed by a database reorganisation, resulted in definite improvement in performance based on user feedback. Unfortunately, what has since been identified as a 'bug' in the Nautilus application lead to problems on the second batch of archiving in August. As a result, the LIMS had to be rolled back two days to its pre-archive status. This lead to a lot of problems for users - as two days-worth of data had to be re-entered on the rolled-back database. This incident was investigated in depth by DAFF IT Division, Orbis and Thermo - the provider of Nautilus. At the request of the LIMS Project Manager, the DAFF Audit Unit also carried out an investigation on the impact of the incident on laboratory work. The Unit has issued a report.
- User training on Infomaker reporting. The external support contractors Orbis provided training to two VLS users to enable them to modify or create database reports using the Infomaker reporting tool.

LIMS Project Management also coordinates internal and external requests for *ad-hoc* and periodic data extracts from the database for use in compilation of Department and EC reports. Besides dealing with many requests for RVL or Section-level data for use in local analysis of data, the following substantial requests were addressed:

- Extract of data for use in compilation of the RVL Disease Surveillance Report. Data is provided under many headings to provide information for the relevant sections of the report.
- Extract and compilation of data for inclusion in the EC Annual Zoonosis Report for Ireland.
- Extract of data for use in compilation of the OIE bi-annual Listed Diseases Report for Ireland.

## Other Division activities

### Health and Safety

In common with Divisions and RVLs across the VLS, Pathology Division staff participated in a detailed hazard and risk assessment of their work areas as part of the updating of the VLS Safety Statement in 2009.

### Other Projects

A pathologist in the Division was involved on a multi-agency project on '*Antimicrobial resistance in commensal and pathogenic bacteria in Irish farm animals*'. This pathologist also provided expert support to the Working Group on the standardisation of Antimicrobial Antibiotic Sensitivity Testing in the RVLs.

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Paul Collery SSRO  
24 March 2010