
Pathology Division

Annual Report 2010

Introduction

Pathology Division comprises three Sections – Histopathology Section, Clinical Chemistry Section 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).

A project to enhance the provision of pathology services to the pig production industry was initiated in 2010. The purpose of this is to improve links with the pig sector, to develop closer communication links with specialist veterinary practitioners, and to develop a deeper knowledge and awareness of pig industry issues which could affect the broader economy in terms of public health and food safety.

Divisional staff, in conjunction with DAFF IT Division, were involved with setting up an on-line video microscopy facility. The facility allows for inter-laboratory discussion of histopathology cases. Microscope slides can be viewed simultaneously by participants in the Central Veterinary Laboratory and one or more Regional Veterinary Laboratories. It is hoped that the system will facilitate discussion of unusual cases between pathologists in different locations as well as being a useful training resource.

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.

Division Quality Management Systems

The Pathology Division has implemented a Quality Management System based on the ISO17025:2005 standard that meets the requirements of the National Accreditation Board (INAB) and other relevant regulatory requirements. An application was made to the Irish National Accreditation Board in late 2009. Following a procedure of assessment and auditing by INAB, the laboratory was awarded accreditation in September 2010 for a defined scope of testing. The scope currently covers the detection of prion protein by the discriminatory and confirmatory western blot tests. It is envisaged that this scope will be expanded to cover other testing activities within the TSE NRL and other areas of the Division.

In line with the ISO17025:2005 standard, the laboratory has implemented a comprehensive audit schedule. It participates in internationally recognised proficiency testing schemes, and maintains extensive and controlled records of all aspects of its activities. Significant resources from within the laboratory are deployed in the maintenance of this system. Successful operation of this system ensures all statutory standards for laboratories involved in official testing are met, and clients are assured of the quality of the laboratory's work.

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 2010, Dublin RVL completed its third full year of provision of service since the move from Abbotstown to Backweston. Submission numbers continued to increase in almost every category during 2010 - reflecting wider client familiarity with the services available.

Dublin RVL Research Officer staff also took on some duties in support of CVRL TB Section in 2010. These included overall responsibility for the tuberculin Potency Assays and associated animal management functions at Longtown, as well as involvement in the Department program for training veterinarians in field tuberculin testing.

Diagnostic Submissions and Investigations (Statutory and Surveillance)

Total numbers of carcass and clinical pathology submissions to Dublin RVL are given in Table 1. As investigations 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 2010.

Species	Carcass ¹	Clinical Pathology ²
Avian	375	77
Bovine	364	782
Ovine	117	31
Porcine	53	82
Caprine	13	26
Badger	40	-
Fox	66	-
Canine	-	15
Equine	-	9
Other ³	1	2
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 2010. 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 2010 included, tuberculosis (*Mycobacterium bovis* infection) in badgers, and a seven-year-old male pet Llama, Enzootic abortion (*Chlamydophila abortus* infection) in sheep, Salmonella typhimurium in pigs and cattle (including in dairy cows in one herd in which an incidence of zoonotic infection occurred), and *Trichinella spiralis* in foxes.

Dublin RVL also undertook investigations of suspected exotic OIE listed disease. In 2010, these included an investigation of a case of suspect foot and mouth lesions in a heifer that had died and was presented for post mortem examination at Dublin RVL.

As for other RVLs and in collaboration with the histopathology section, Dublin RVL was involved in the diagnosis of the emerging disease Bovine neonatal pancytopenia BNP in neonatal calves. 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.

A paper arising from Dublin RVL's involvement in the diagnostic pathology investigations of outbreaks of tuberculosis in lactating goat herds 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.

Projects and Special Investigations

Participation by a Dublin RVL pathologist in a multi-agency research project on *Toxoplasma gondii* abortion in sheep continued in 2010. The focus during the year was on screening sera harvested

from *T. gondii*-challenged dams and their foetuses for specific IgG and IgM responses employing ELISA techniques. Screening for IgG was carried out successfully - though attempts to screen for IgM did not prove successful. However, other immunology and pathology data has already been collected from the same challenge experiment. The findings are being used to generate a publication provisionally entitled "*Sequential study of maternal and foetal immune responses of pregnant sheep challenged with Toxoplasma gondii*", which it is hoped to submit for publication during 2011.

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

- 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 H1N1 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*'. 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 the 2010 RVL Monthly Reports on the DAFF website:

<http://www.agriculture.gov.ie/animalhealthwelfare/laboratoryservices/regionalveterinarylaboratoryreports/rvlmonthlyreports2010/>

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 2010

	Microbiology ¹	Haematology ²
5/1/2010	√	√
2/2/2010	√	√
2/3/2010	√	√
6/4/2010	√	√
4/5/2010	√	√
8/6/2010	√	√
6/7/2010	√	√
3/8/2010	√	√
7/9/2010	√	√
5/10/2010	√	√
2/11/2010	√	√
30/11/2010	√	√

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 2010 are given in Table 3.

- Table 3: Histopathology Section tests and procedures in 2010.

Description	No. Slides ¹
Routine Stains:	
<i>Haematoxylin and Eosin staining (H&E)</i>	23,459
Special Stains:	
<i>Gram</i>	503
<i>Ziehl-Neelsen</i>	263
<i>Grocott stain for fungal hyphae</i>	52
<i>PAS stain for fungal hyphae</i>	218
<i>Perl's Prussian Blue stain for iron</i>	39
<i>Toluidine Blue stain for mast cells</i>	7
<i>Warthin Starry stain for spirochetes</i>	41
<i>Miscellaneous</i>	138
Immuno-histochemistry:	
<i>TSE (BSE and scrapie)</i>	530
<i>Porcine Circovirus</i>	100
<i>Chlamydia (agent of ovine enzootic abortion)</i>	6
<i>Toxoplasma</i>	15
<i>Neospora</i>	7
<i>Mycoplasma bovis</i>	-
Western Blots:	No. Tests²
<i>PrP Western Blot</i>	108
<i>PrP Western Blot BSE Strain</i>	-
<i>PrP Western Blot Discriminatory</i>	29

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

2 - Number of western blot tests performed.

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 reactives 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 (western blot) to detect disease-specific PrP in CNS target sites. The TSE NRL has INAB accreditation to ISO 17025 for PrP immunoblotting.

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 Rapid Testing Laboratories (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 2010, confirmatory diagnosis was carried out on samples from a total of 37 bovine animals. BSE was confirmed in two animals. Both samples from positive animals were detected in the Rapid Testing Laboratories (active surveillance) and forwarded to the NRL for confirmatory diagnosis.

Samples from 35 Clinical Suspect cases (passive surveillance) were submitted to the NRL for confirmatory diagnosis. 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 in 2010.

H&E Result	Number of Cases
Listerial Encephalitis	5
Hepatic Encephalopathy	3
Neoplastic	2
Viral Encephalitis	1
Cerebrocortical Necrosis	1
No Specific Findings	23
Total	35

Ovine Cases

Twenty four cases of Scrapie were confirmed by the TSE NRL in 2010. Of these, 22 were classified as Classical Scrapie, with the remaining two being classified as Atypical Scrapie.

The 22 confirmed cases of Classical Scrapie were from nine separate flocks. Seventeen had been initially submitted for confirmatory testing to a Rapid Testing Laboratory (active surveillance – i.e. mostly part of depopulation programmes in scrapie-positive flocks) – and from there to the NRL for confirmatory testing. The remaining five were submitted to the NRL as clinical suspect cases *via* the Regional Veterinary Laboratories.

Tissues from both of the 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.

Seven new flocks were identified with scrapie during the year based on the NRL confirmatory diagnosis testing – five with Classical and two with Atypical scrapie. 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 as 'Scrapie-like' in 2010 - thus providing no evidence to suggest the presence of BSE in sheep in Ireland.

External 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 2010 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.

A web-based portal is used 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 pathologists. Four pathologists from the Irish NRL participated in the 2010 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:

- 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 2010, 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.

The Tissue Bank provided tissues and tissue homogenates during the year in response to requests under the following headings:

- Provision of stock and multiple dilutions of TSE positive and negative tissue homogenates for NRL-run proficiency trials of the private RTLs.
- Provision of stock TSE positive and negative tissue homogenates for use in NRL internal proficiency trials.
- Provision of TSE-positive tissue to private RTLs for use as test controls.
- Provision of TSE-positive tissue to private RTLs using the IDEXX test to be used as controls in connection with their use of the heat treatment re-test protocol.
- Provision of TSE-positive and negative tissues to private RTLs for use in a) a new laboratory approval, and b) a change of test approval.

All of these requests were responded to in accordance with NRL procedures. Further details are included in the section of this report on Proficiency Trials. 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 2010, together the tests they were approved to use during the year.

• Table 5. Approved TSE Rapid testing laboratories in 2010.

RTL	Location	Test	Species
Advanced Micro Services (AMS)	Cork	IDEXX Herd Check	Bovine and Ovine
AMS (TSE Testing)	Newbridge, Co. Kildare	IDEXX Herd Check	Bovine and Ovine
Irish Equine Centre	Kill, Co. Kildare	Prionics Check WB	Bovine
		Prionics Priostrip	Bovine
		Prionics Check WB SR ¹	Ovine and Caprine
Identigen	Pearse St. Dublin	Roboscreen	Bovine
Irish Biotechnology Services (IBS)	Longford	BioRad TeSeE	Bovine and Ovine
		IDEXX Herd Check ³	Bovine and Ovine
Enfer Laboratories ²	Naas	Enfer V3	Bovine

1 - Prionics Check WB Small Ruminant test no longer approved for scrapie testing in EC from 1 January 2011.

2 – Approval to operate as TSE RTL issued in December 2010. Testing commenced January 2011.

3 – approved for use in December 2010.

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 were carried out by the NRL in 2010. One involved the approval of a new TSE rapid testing laboratory – Enfer Laboratories Naas. Approval was issued in December 2010 and the

laboratory commenced testing in January 2011. The second approval was a change of test by an existing RTL. The process was completed, and approval issued, in December 2010.

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 one unannounced inspection visit during 2010. 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 2010

RTL	Date
Advanced Micro Services (AMS)	25/08/2010
Advanced Micro Services (TSE Testing)	02/06/2010
Irish Equine Centre (IEC)	02/06/2010
Identigen	23/08/2010
Irish Biotechnology Services (IBS)	30/06/2010

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.

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 2010 - in June and November.

- Table 7: Tests used in the 2010 NRL-organised proficiency trials of RTLs.

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

Homogenates for the proficiency trials are prepared by the NRL from TSE-positive brains (bovine and ovine) using the CRL protocol. A single aliquot of each stock homogenate (dilution 1:2), and three aliquots each of 1:20, 1:80 and 1:160 dilutions were sent to each RTL for testing. Three aliquots of a negative brain homogenate were also sent.

The criteria for passing the proficiency trial are that positive results should be obtained for the positive stock homogenates - and negative results should be obtained for negative homogenates.

Each of the RTLs got the expected results for the positive bovine and ovine stock homogenates and for the negative bovine homogenates. Three RTLs got the expected results for all three negative ovine homogenates. One RTL reported one of the negative ovine homogenates as positive. A full investigation by the NRL and by the RTL concluded that the incorrect result was due to an inaccurate interpretation of the result of a single assay rather than a failure of the test. It was decided that there was no requirement for the RTL to participate in an additional proficiency trial at that time.

The dilution series of homogenates were used to monitor analytical sensitivity – both between the RTLs in the trial and with the results of previous trials incorporating dilutions series.

The design of the November proficiency trial was the same as that of the June trial. Each of the RTLs got the expected results for the positive bovine and ovine stock homogenates and for the negative bovine and ovine homogenates. The analytical sensitivities for all laboratories were broadly similar to the June results. Minor differences may have related to the use of different batches of test kits and/or biological variations in the homogenates.

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. Scoring systems are in place in the RTLs to record a) the quality of bovine samples from abattoirs in terms of anatomical suitability (SO and NC grading) and b) the degree of autolysis of samples received from knackeries. The RTLs send monthly bovine sample quality reports to the NRL (abattoir and knacker), and these are used to provide quarterly analyses which are forwarded to DAFF Veterinary Public Health and TSE & ABP Divisions.

The NRL also uses the RTL monthly bovine sample quality reports to monitor the consistency of sample SO and autolysis grading between RTLs. Significant deviations and trends are followed up with the RTLs concerned.

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.

Each RTL is required to carry out quarterly in-house monitoring of sampling technique to ensure that each member of staff involved is assessed at least twice a year. 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 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 inspected by the NRL in 2010.

RTL	Date
Irish Equine Centre (IEC)	13th January 2011*
AMS Cork	5th May 2010
AMS (TSE Testing) Newbridge	13th January 2010
Identigen	15th January and 13th October 2010
Irish Biotechnology Services (IBS)	30th April and 28th October 2010

*The 2010 assessment for the IEC was conducted in early January 2011

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 2010, the NRL was in communication with the CRL on a range of issues – and a member of NRL staff attended the 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 to one of the private rapid testing laboratories. However, some brains are submitted *via* Regional Veterinary Laboratories to the TSE NRL. In 2010, eight sheep brains were examined under this scheme. All tested scrapie-negative in the NRL.

Other Areas (Histopathology Section)

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.

Q-FEVER

A Research Officer and Laboratory Analyst in the Section conducted a project investigating the prevalence of antibodies to *Coxiella burnetii* (the Q-fever agent) in Irish cattle, sheep and goats, and the associated risk factors. This work was carried out in collaboration with Teagasc Moorepark and the Centre for Veterinary Epidemiology and Risk Analysis (CVERA) in UCD. The bovine portion was published in the journal *Epidemiology and Infection* (Ryan *et al.*, 2010), and the sheep and goat section has been submitted for publication to a peer-reviewed journal.

DEVELOPMENT OF CLASS A FRAMEWORK ON ANIMAL HEALTH COMPUTER SYSTEM

Pathology Division staff were involved in advising on the development of a class A framework on the DAFF AHCS system.

FOOT AND MOUTH DISEASE

A Research Officer from the Section acted as a training consultant for the European Commission for the Control of FMD (a section of the FAO) on a field session held in Turkey. This was part of the continuing program of training offered to European veterinary services with which this officer had

been involved in developing during 2009. The officer was subsequently co-opted onto the research group committee of the EuFMD, attending policy and research meetings in this capacity.

BSE EPIDEMIOLOGY

A Histopathology Section Research Officer conducted a project investigating the epidemiology of BSE cases born after the reinforced feed ban (so-called 'BARB' BSE cases), as part of an MSc. This work was conducted in collaboration with CVERA and with the Royal Veterinary College, London. The results will shortly be submitted for publication in a peer-reviewed epidemiology journal.

RESEARCH PROJECT ON BOVINE NEONATAL PANCYTOPENIA (BNP)

Histopathology Section staff were involved in designing and implementing a large-scale animal experiment investigating the immunopathogenesis of bovine neonatal pancytopenia. This work is being conducted in collaboration with CVRL Virology Division and the Moredun Research Institute, UK. The project involves animal work at Longtown Research Farm, investigating the relationship between hypervaccination of cows, haematological and immunological markers in cow and calf serum, colostrum, and the potential development of clinical or subclinical disease. This work is ongoing.

ANIMAL WELFARE BODY

A Research Officer from the Section is a member of the new CVRL animal welfare body, tasked with overseeing the welfare of animals on Longtown Research Farm and ensuring all appropriate legislation and guidelines are complied with. This involves regular meetings and inspections in Longtown Farm.

Clinical Chemistry Section

Clinical Chemistry Section 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, to animal research projects throughout the VLS, and to outside agencies such as Teagasc and the universities.

Diagnostic Submissions

The numbers of tests and analyses performed on blood, tissue and other clinical pathology and toxicology samples submissions from farm animals in 2010 are shown in Table 9. These include samples referred from the RVLs, as well as samples submitted directly from veterinary practitioners or special projects.

• Table 9: Tests and analyses performed in Clinical Chemistry Section in 2010.

Analysis	No.
Metabolites: Albumin, globulin, beta-hydroxybutyrate, calcium (blood and tissue), chloride, creatinine, glucose, non-esterified fatty acid, bilirubin, urea, urinalysis.	852
Enzymes: Alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH).	748
Minerals: Arsenic, cobalt, copper, phosphorus, iron, lead, magnesium, molybdenum, selenium, zinc, potassium, sodium.	5,034

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.

The Section organised a day-long Workshop on Bovine Clinical Chemistry and Nutrition in October 2010. The invited speakers were experts in the fields of clinical chemistry and cattle nutrition.

VLS 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 substantive progress was made in 2010 on the filling of the vacant full-time LIMS administrator post.

Among the major LIMS issues dealt with in 2010 were:

- Coordination of actions to improve LIMS performance for users in the Central and Regional Veterinary Laboratories.
- Continued extension of laboratory analytical instrument integration with the LIMS in the CVRL and RVLs.

- Coordination of user familiarisation on instrument connectivity to LIMS.
- Additional work on LIMS workflows and reports to meet ISO 17025 accreditation requirements for CVRL Divisions.
- Project management of the laboratory side of an IT Division program to upgrade the LIMS servers and database.
- Advisory role in an ongoing project to provide full electronic data communication facilities between the Central Veterinary laboratory and DAFF HQ and DVOs for CLASSA disease surveillance and control - *via* LIMS and AHCS.

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.
- Extract of data for use in compilation of the OIE Wildlife Diseases Report for Ireland.

Other Pathology Division activities

Health and Safety

Division hazard and risk assessments were reviewed and updated as required during 2010.

Other Projects

The Division provided expert input for the Working Group on the standardisation of Antimicrobial Antibiotic Sensitivity Testing in the RVLs.

Pathology Division Publications

Peer Reviewed

Mammary carcinoma in a ewe. McElroy, MC and Bassett, HF. **J. Vet Diagn Invest.** 2010 Nov: 22 (6) 1006-1007.

Neuropathologic features of Aleutian disease in farmed mink in Ireland and molecular characterization of Aleutian mink disease virus detected in brain tissues. Jahns H, Daly P, McElroy MC, Sammin DJ, Bassett HF, Callanan JJ **J. Vet Diagn Invest.** 2010 Jan;22 (1):101-5.

Detection and quantification of *Toxoplasma gondii* in ovine maternal and foetal tissues from experimentally infected pregnant ewes using real-time PCR. J. Gutierrez, J. O'Donovan, E. Williams, A. Proctor, C. Brady, P.X. Marques, S. Worrall, J.E. Nally, M. McElroy, H. Bassett, D. Sammin, D. Buxton, S. Maley and B.K. Markey. **Veterinary Parasitology.** 172 (2010) 8–15.

Identification of immunologically relevant proteins of *Chlamydia abortus* using sera from experimentally infected pregnant ewes. Marques PX, Souda P, O'Donovan J, Gutierrez J, Williams EJ, Worrall S, McElroy M, Proctor A, Brady C, Sammin D, Basset HF, Whitelegge JP, Markey BE, Nally JE. **Clin. Vaccine Immunol.** 2010 Jun 16. [Epub ahead of print].

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. **Veterinary Record.** 2010 May 8;166(19):591-2.

Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples. Ryan E, Kirby M, Collins D, Sayers R, Mee J, Clegg T. **Epidemiology and Infection.** 2010. doi doi:10.1017/S0950268810002530.

Short-lived human nasal carriage of foot-and-mouth disease virus after exposure to infected animals. Wright C, Gloster J, Mazelet L, Paton DJ, Ryan ED. **Veterinary Record,** 2010. 167(24): 928-31.

Options for decentralized testing of suspected secondary outbreaks of foot-and-mouth disease. Sammin D, Ryan E, Ferris NP, King DP, Zientara S, Haas B, Yadin H, Alexandersen S, Sumption K, Paton DJ. **Transboundary and Emerging Diseases.** 2010. 57(4): 237-43.

Not Peer Reviewed

Bovine neonatal pancytopenia in calves in Ireland. C. Sánchez-Miguel, M. McElroy and E. Walsh. **Veterinary Record.** 2010 May 22;166(21):664.

Presentations

An atypical BSE case in the Republic of Ireland. Fox, M., Jahns, H. and McElroy, M. AVTRW 44th Scientific Session, Newry, 1 October 2010.

Nutritional bone disease in calves: a case report. McElroy, MC, Sammin, DS and McAllister, H. CVRL Scientific Seminar 25-26 Nov 2010.

Q fever in Ireland: prevalence and risk factors. Ryan E, CVRL Scientific Seminar 25-26th November 2010.

Paul Collery SSRO
7 June 2011