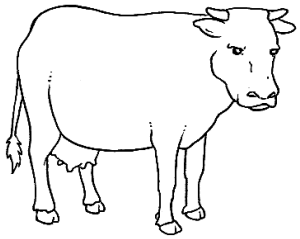
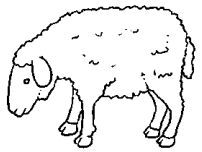
2019 round of TSE EURL EQAs: Results feed-back

Torino/Rome, Italy

Discriminatory Western Blot in Bovine & Small Ruminants EQA

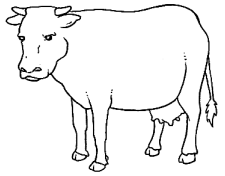




Gabriele Vaccari

TSE EURL
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



- Proficiency Test Samples has been prepared by homogenisation of brain tissue using a 50/50 mix of tissue and distilled water. Aliquots have been tested with discriminatory Western blot

- Each sample was identified with a unique alphanumerical code common to all laboratories

- Each laboratory received its own individual “laboratory code” for each PT

- Sample have been dispatched in dry ice after the receipt of the import permit

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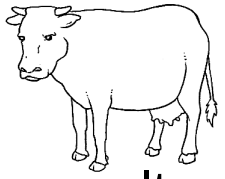
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



- Samples has been dispatch the 12/Nov/2019 and dead line for results submission was the 31/Jan/2020

- Results (excel file and raw data) to be returned to the email addresses EURL.TSE.PTDS@ISS.IT and EURL.TSE@izsto.it

- For bovine there is not a positive list of methods. Laboratories can participate with in house method or with “The APHA Bio-Rad TeSeE-based Hybrid Western blotting Method” reported “TSE Strain Characterisation in small ruminants – A Technical handbook for NRL in the EU”

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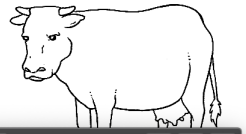
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



Guidance “Approach for the provisional Classification of bovine TSE isolates” is available on the internet at http://www.izsto.it/images/stories/EURL_guidance.zip

TSE EURL

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta

Istituto Superiore di Sanità

APPROACH FOR THE PROVISIONAL CLASSIFICATION OF BOVINE TSE ISOLATES

Version 1.0 June 2020

The examination by histopathology, immunohistochemistry, Western blotting and bioassay of bovine isolates from individuals with clinical signs throughout the BSE epidemic has supported the hypothesis that the epidemic has been sustained by a single type, or strain, of BSE. However, the development of sensitive PrP^{res} immuno-detection diagnostic techniques and their application through active surveillance in non-suspect populations have led to the detection of a small number of geographically widespread sporadic cases of deviant types, predominantly in older animals. These isolates have now been confirmed in mice as distinct strains, and have been operationally defined as H- (high) or L- (low) type based on the molecular mass of the unglycosylated fragment of PK resistant PrP in Western blot, as opposed to the classical form of BSE (C-type BSE).

L-type behaves in Western blots like the cases initially identified in Italy (initially described as BASE; bovine amyloidotic spongiform encephalopathy). For the time being L-type and BASE are considered to be the same.

It is now a regulatory requirement for all positive bovine isolates to be classified by discriminatory methods in L-type, H-type or C-type based on distinctive molecular characteristics of PK resistant PrP. This discriminatory test shall be performed by a laboratory, appointed by the competent authority, which has participated successfully in the latest proficiency testing organised by the EU reference laboratory for discriminatory testing of confirmed BSE cases.

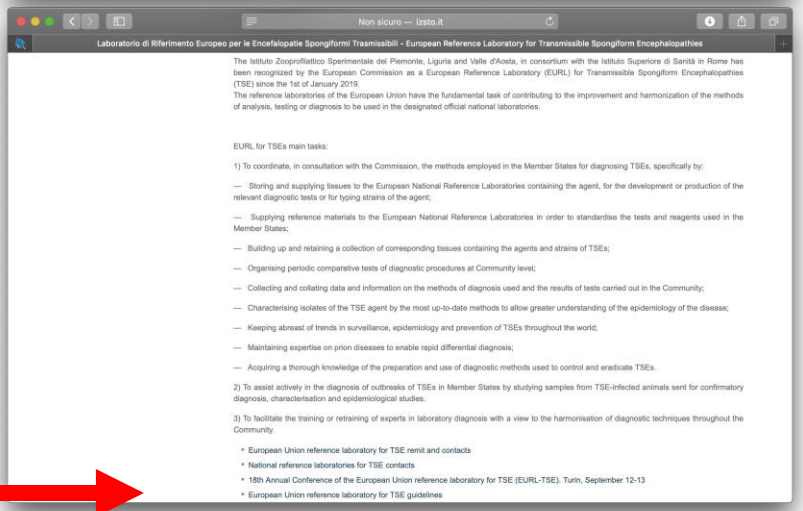
The following blotting guide has been prepared on behalf of the European Union Reference Laboratory (EURL) for the TSE Strain Typing Expert Group by Prof. Jan Langeveld, Lelystad, based on the 2007 publication by Jacobs et al.

In order to have confidence in the results of such a test, it is vital that the appropriate controls should be run on the same gel as the suspect sample. Appropriate controls would include samples previously confirmed as C, H and L type BSE, either in the laboratory of origin, or through referral of the sample to a laboratory with the correct control materials available.

Please note: A protocol for the discriminatory testing of positive BSE samples (The APHA Bio-Rad TeSeE-based Hybrid Western blotting Method) is made available to NRLs in the EU reference laboratory guidelines on discriminatory testing and classification — 'TSE strain characterization in small ruminants: A technical handbook for National Reference Laboratories in the EU'.

Bovine discriminatory guidance
Document v1 June 2020

Page 1 of 10



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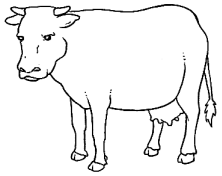
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



Samples dispatched

Sample ID	Expected result
DB1901	L-type BSE
DB1902	C-type BSE
DB1903	H-type BSE
DB1904	C-type BSE

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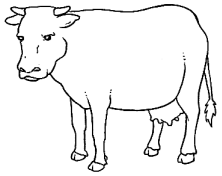
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



Number of Participating Laboratories

	Total
Laboratories participating with one test method	11
Laboratories participating with two test methods	2
Total participating Laboratories	13

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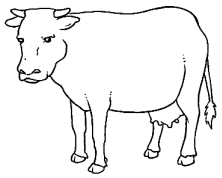
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



- All Laboratories returned the result in the due time
- Results have been returned by e-mail, the excel file and raw data have been received by all participants
- The loading order in the blots, of some raw data, was not always clear as well as the association of antibody used in each membrane

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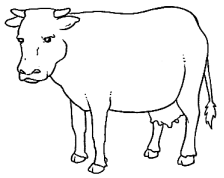
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



- All laboratories, with the exception of two, reported the expected results

Sample ID	Expected result	441	766
DB1901	L-type BSE	BSE	Classical BSE
DB1902	C-type BSE	Scrapie	Classical BSE Atyp.BSE L-type ?
DB1903	H-type BSE	Atypical Scrapie	Atypical BSE H-type
DB1904	C-type BSE	BSE	Classical BSE

- It has been requested to the two laboratories to identify the root cause of the deviation and to adopt appropriate measure
- One laboratory has been supported in the identification and resolution of the critical point

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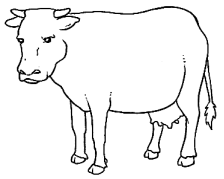
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



- The laboratory has identified the critical point and succeed in the correct identification of sample provided with a second round of the PT

Sample ID	Expected result	766
DB1905	C-type BSE	Classical BSE
DB1906	H-type BSE	Atypical BSE H-Type
DB1907	C-type BSE	Classical BSE
DB1908	L-type BSE	Atypical BSE L-Type

- The identification of the root causes of the deviation from the second laboratory is still missing

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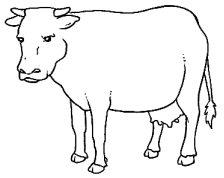
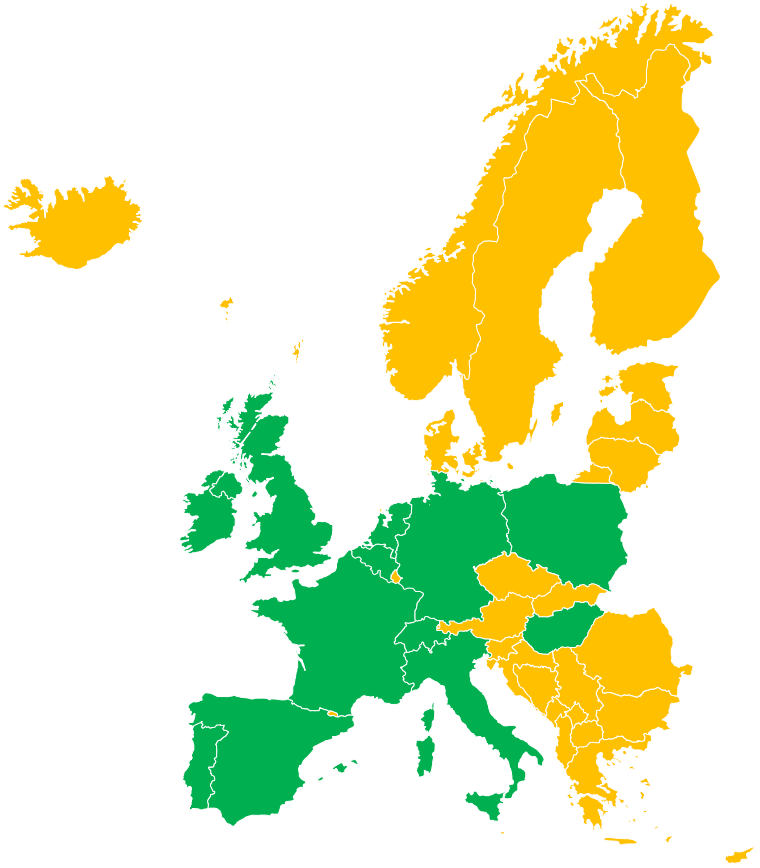
Proficiency testing for Discriminatory Western Blot in Bovine (PTDB19)



12 Participants passed the PT

Belgium
France
Germany
Hungary
Ireland
Italy
Netherlands
Poland
Portugal
Spain
Switzerland
UK



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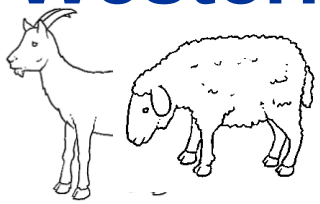
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



- Proficiency Test Samples has been prepared by homogenisation of brain tissue using a 50/50 mix of tissue and distilled water. Aliquots have been tested with rapid test and Discriminatory Western blot

- Each sample was identified with a unique alphanumerical code common to all laboratories

- Each laboratory received its own individual “laboratory code” for each PT

- Sample have been dispatched in dry ice after the receipt of the Import permit

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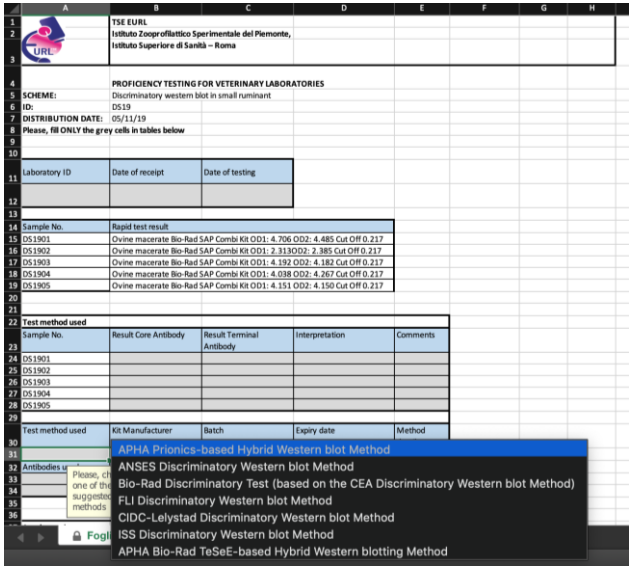
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)

- Samples has been dispatch the 5/Nov/2019 and dead line for results submission was the 10/Jan/2020

- Results (excel file and raw data) to be returned to the email addresses EURL.TSE.PTDS@ISS.IT and EURL.TSE@izsto.it

- The excel file contained a drop-down menu to select the method used

- Methods were those included in “TSE Strain Characterisation in small ruminants – A Technical handbook for NRL in the EU”



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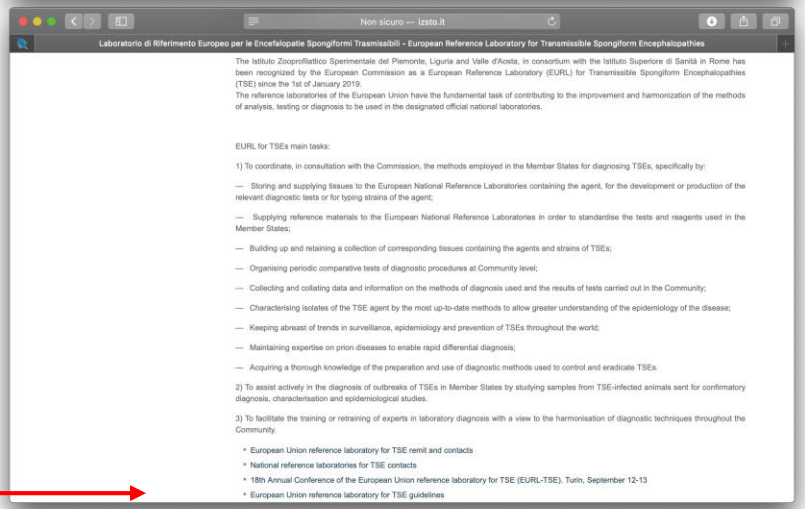
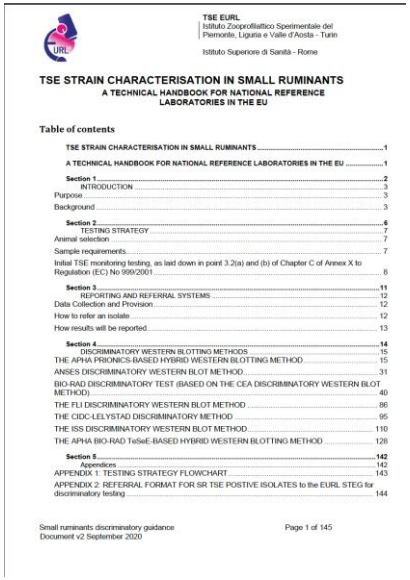
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)

"TSE Strain Characterisation in small ruminants – A Technical handbook for NRL in the EU" re-edited available on the internet at http://www.izsto.it/images/stories/EURL_guidance.zip





TSE STRAIN CHARACTERISATION IN SMALL RUMINANTS
A TECHNICAL HANDBOOK FOR NATIONAL REFERENCE LABORATORIES IN THE EU

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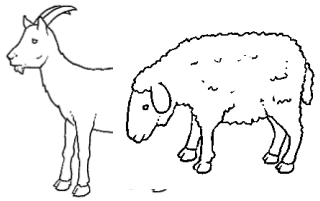
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



Samples dispatched

Sample Name	Expected result	Ovine macerate Bio-Rad SAP Combi Kit - Cut Off 0.217
DS1901	Classical scrapie	OD1: 4.706 OD2: 4.485
DS1902	BSE-like	OD1: 2.313 OD2: 2.385
DS1903	Classical scrapie	OD1: 4.192 OD2: 4.182
DS1904	Classical scrapie	OD1: 4.038 OD2: 4.267
DS1905	Classical scrapie	OD1: 4.151 OD2: 4.150

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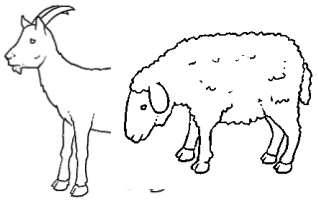
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



- All Laboratories returned the result in the due time
- Results have been returned by e-mail with the excel file compiled with some unexpected modification of the name of the test method used
- Raw data have been received from all participants. The loading order in the blots, of some raw data, was not always clear as well as the association of antibody used in each membrane
- Reagents reported, in one case, was not corresponding to the method reported and not corresponding to the reagents indicated in the Technical handbook for NRL in the EU

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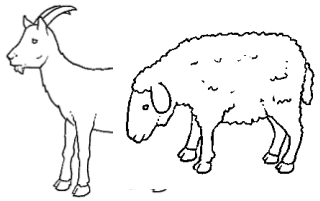
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



Number of Participating Laboratories

	Total
Laboratories participating with one test method	16
Laboratories participating with two test methods	2
Laboratories participating with three test methods	1
Total participating Laboratories	19

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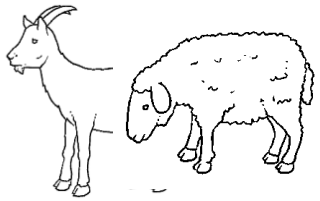
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



Test Method Used on the PT

Test method used	Number of laboratories
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	9
APHA Prionics-based Hybrid Western blot Method	6
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)	4
FLI Discriminatory Western blot Method	2
ANSES Discriminatory Western blot Method	1
ISS Discriminatory Western blot Method	1

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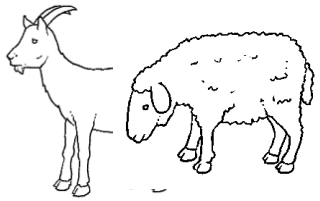
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



- All laboratories, with the exception of two, reported the expected results

Sample ID	Expected result	118	287
DS1901	Classical scrapie	Scrapie	Scrapie
DS1902	BSE-like	Scrapie	BSE
DS1903	Classical scrapie	BSE-in-sheep suspect, requires further testing	BSE
DS1904	Classical scrapie	Scrapie	BSE
DS1905	Classical scrapie	Scrapie	Scrapie

- It has been requested to the two laboratories to identify the root cause of the deviation and to adopt appropriate measure

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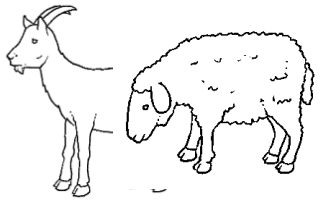
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



- One laboratory has immediately identified the critical point and succeed in the second round of the PT

Sample ID	Expected result	118
DS1906	Classical scrapie	Scrapie
DS1907	Classical scrapie	Scrapie
DS1908	BSE like	BSE-in-sheep suspect, needs further testing
DS1909	Classical scrapie	Scrapie
DS1910	Classical scrapie	Scrapie

- The identification of the root causes from the second laboratory is still missing

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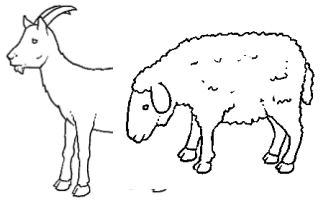
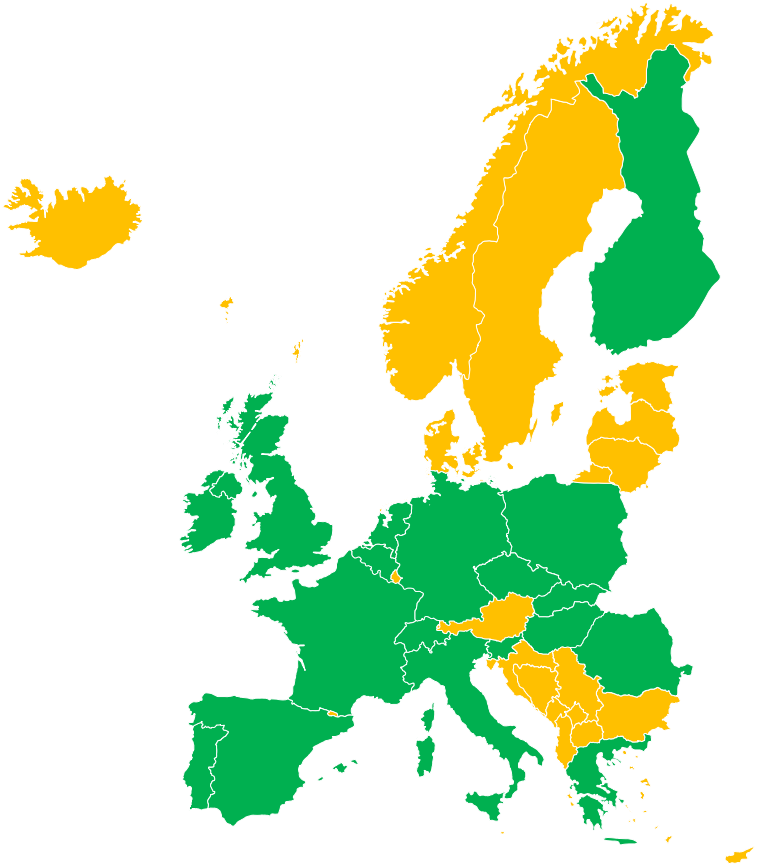
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS19)



18 Participants passed the PT

Belgium
Czech Republic
Finland
France
Germany
Greece
Hungary
Italy
Ireland
Netherlands
Poland
Portugal
Romania
Slovakia
Slovenia
Spain
Switzerland
UK



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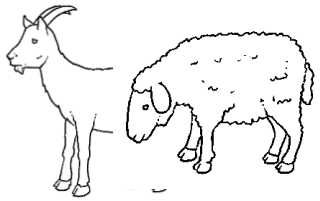
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS20)



Minor Tips

- Discriminatory test used must be one of those reported in the “TSE Strain Characterisation in small ruminants – A Technical handbook for NRL in the EU” and the protocol reported should be followed

- Raw data are expected with clear information

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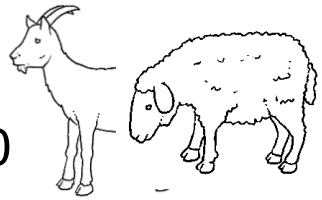
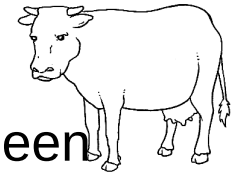
Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS20)



- Samples will be prepared and dispatched by November 2020
- The submission of the results in the next PT (excel file and raw data) will be by a web repository folders

Proficiency testing for Discriminatory Western Blot in Bovine (PTDB)



- Due to the shortage of reference material for atypical BSE it has been agreed with the European Commission to postpone the PT planned for 2020 in 2021

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EQA rounds

EQA for BSE / scrapie discriminatory western blotting of small ruminants
EQA for H, L and C-BSE classification

Contact details
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+39 06 49902139
Gabriele.vaccari@iss.it

Technical aspect of Discriminatory Western Blot blotting of small ruminants and BSE Classification

Contact details
Laura Pirisinu
+39 06 49903626
Laura.pirisinu@iss.it

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Istituto Superiore di Sanità - Rome

ISS
Michele Angelo Di Bari
Laura Pirisinu
Ilaria Vanni
Romolo Nonno
Geraldina Riccardi
Elena Esposito
Stefano Marcon

IZPLVdA
Giuseppe Ru
Daniela Meloni
Elena Bozzetta

FLI
Christine Fast

DAFM
Ann Sharpe
Darren Hand

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Istituto Superiore di Sanità - Rome


Thank to all participants