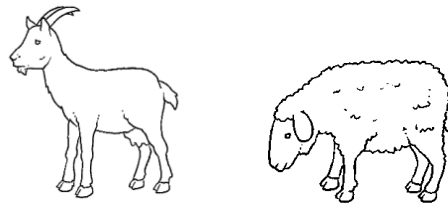


2020 round of TSE EURL EQAs: Results feed-back

Discriminatory Western Blot in Small Ruminants EQA (DS20)



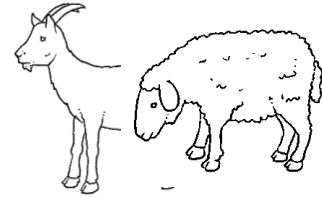
Laura Pirisinu



TSE EURL

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Istituto Superiore di Sanità - Rome

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



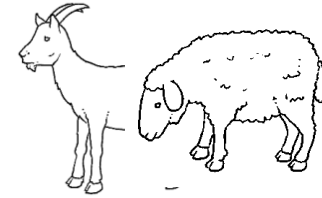
- All NRLs received an email (4th December 2020) informing the imminent dispatch of samples for the 7th December 2020
- Forms for returning the results were attached (and also supplied in the box in paper format)
 1. Cover Letter
 2. Electronic form for submitting the results



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



1. Cover Letter explaining:

a) How the samples were prepared:

- » by homogenisation of brain tissue using a 50/50 mix of tissue and distilled water. Aliquots tested by Discriminatory WB

b) Technical information for preparing the sample test

- » storage temperature (-70°C)
- » treatment of samples as tissue (not considering the water content)
- » How to work the samples (shake/vortex, collect the tissue,..)

c) Safety information:

- » “MATERIAL IS FOR “IN VITRO” USE ONLY AND MUST BE TREATED AS HAZARD GROUP 3** SUBMISSION AND BE HANDLED ACCORDINGLY IN A DEROGATED CONTAINMENT LEVEL 3 LABORATORY”.

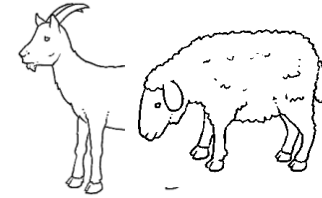


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Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS20)

1. Cover Letter explaining:



d) How submit the results

- » The link to upload the results (<https://forms.gle/NA5RbLdsx8znSbin6>)
- » The excel file Submission PT DS20.xlsx
- » Raw data (blot images)
- » The format (*.zip, *.rar)

e) Deadline

- » 5th February 2021

f) Contacts

- » Gabriele Vaccari

e-mail: gabriele.vaccari@iss.it

telephone number: +39 339 6846007




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

2. Submission file (excel format):

- Identification of the PT scheme

	A	B	C	D
1		TSE EURL		
2		Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino		
3		Istituto Superiore di Sanità – Roma		
4	PROFICIENCY TESTING FOR VETERINARY LABORATORIES			
5	SCHEME:		Discriminatory western blot in small ruminant	
6	ID:		DS20	
7	DISTRIBUTION DATE:		07/12/2020	
8	Please, fill ONLY the grey cells in tables below			
9				
10				
11	Laboratory ID	Date of receipt		
12				
13				
14	Test method used			
15	Sample No.	Result	Comments	
16	DS2001			
17	DS2002			
18	DS2003			
19	DS2004			
20	DS2005			
21				
22	Test method used	Kit Manufacturer	Batch	Expiry date
23				
24	Antibodies used	Batch	Expiry date	Date of testing
25				
26				
27				
28				
29	1st alternative test			
30	Sample No.	Result	Comments	
31	DS2001			
32	DS2002			
33	DS2003			
34	DS2004			
35	DS2005			
36				
37	1st alternative test method used	Kit Manufacturer	Batch	Expiry date
38				



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

2. Submission file (excel format):

- Identification of the PT scheme
- Laboratory ID

The “Laboratory identification number” is the identification code of each lab, received via email in 2019.

→ Note that a different code has been assigned to each different EQA (the same laboratory may have more ID numbers!)

	A	B	C	D
1		TSE EURL		
2		Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino		
3		Istituto Superiore di Sanità – Roma		
4	PROFICIENCY TESTING FOR VETERINARY LABORATORIES			
5	SCHEME:	Discriminatory western blot in small ruminant		
6	ID:	DS20		
7	DISTRIBUTION DATE:	07/12/2020		
8	Please, fill ONLY the grey cells in tables below			
9				
10				
11		Laboratory ID	Date of receipt	
12				
13				
14	Test method used			
15	Sample No.	Result	Comments	
16	DS2001			
17	DS2002			
18	DS2003			
19	DS2004			
20	DS2005			
21				
22	Test method used	Kit Manufacturer	Batch	Expiry date
23				
24	Antibodies used	Batch	Expiry date	Date of testing
25				
26				
27				
28				
29	1st alternative test			
30	Sample No.	Result	Comments	
31	DS2001			
32	DS2002			
33	DS2003			
34	DS2004			
35	DS2005			
36				
37	1st alternative test method used	Kit Manufacturer	Batch	Expiry date
38				




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

2. Submission file (excel format):

- Identification of the PT scheme
- Laboratory ID
- Test method info:
 - Results and comments for each sample (unique alphanumerical code common to all laboratories)

	A	B	C	D
1		TSE EURL		
2		Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino		
3		Istituto Superiore di Sanità – Roma		
4		PROFICIENCY TESTING FOR VETERINARY LABORATORIES		
5	SCHEME:	Discriminatory western blot in small ruminant		
6	ID:	DS20		
7	DISTRIBUTION DATE:	07/12/2020		
8	Please, fill ONLY the grey cells in tables below			
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24	Antibodies used	Batch	Expiry date	Date of testing
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31	DS2001			
32	DS2002			
33	DS2003			
34	DS2004			
35	DS2005			
36				
37	1st alternative test method used	Kit Manufacturer	Batch	Expiry date
38				



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

2. Submission file (excel format):

- Identification of the PT scheme
- Laboratory ID
- Test method info:
 - Results and comments for each sample
 - Test method used (drop-down menu to select the method used)

22	Test method used	Kit Manufacturer
23		
24	Antibodies used	
25		
26		
27		
28		

Please, choose one of the suggested methods

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

- Batch and expiry date of kit (if present)



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	A	B	C	D
1		TSE EURL		
2		Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino		
3		Istituto Superiore di Sanità – Roma		
4		PROFICIENCY TESTING FOR VETERINARY LABORATORIES		
5	SCHEME:	Discriminatory western blot in small ruminant		
6	ID:	DS20		
7	DISTRIBUTION DATE:	07/12/2020		
8	Please, fill ONLY the grey cells in tables below			
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14	Test method used			
15	Sample No.	Result	Comments	
16	DS2001			
17	DS2002			
18	DS2003			
19	DS2004			
20	DS2005			
21				
22	Test method used	Kit Manufacturer	Batch	Expiry date
23				
24	Antibodies used	Batch	Expiry date	Date of testing
25				
26				
27				
28				
29	1st alternative test			
30	Sample No.	Result	Comments	
31	DS2001			
32	DS2002			
33	DS2003			
34	DS2004			
35	DS2005			
36				
37	1st alternative test method used	Kit Manufacturer	Batch	Expiry date
38				

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

2. Submission file (excel format):

- Identification of the PT scheme
- Laboratory ID
- Test method info
- Alternative tests

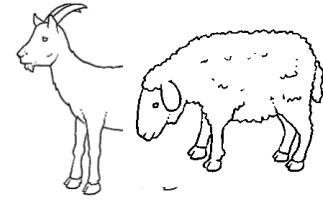
	A	B	C	D
1		TSE EURL		
2		Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Torino		
3		Istituto Superiore di Sanità – Roma		
4		PROFICIENCY TESTING FOR VETERINARY LABORATORIES		
5	SCHEME:	Discriminatory western blot in small ruminant		
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11	Laboratory ID	Date of receipt		
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13				
14	Test method used			
15	Sample No.	Result	Comments	
16	DS2001			
17	DS2002			
18	DS2003			
19	DS2004			
20	DS2005			
21				
22	Test method used	Kit Manufacturer	Batch	Expiry date
23				
24	Antibodies used	Batch	Expiry date	Date of testing
25				
26				
27				
28				
29	1st alternative test			
30	Sample No.	Result	Comments	
31	DS2001			
32	DS2002			
33	DS2003			
34	DS2004			
35	DS2005			
36				
37	1st alternative test method used	Kit Manufacturer	Batch	Expiry date
38				



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Samples dispatched

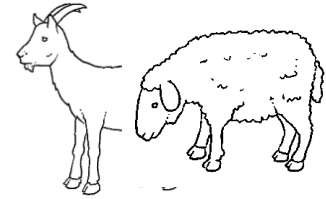
Sample ID	Expected result
DS2001	Classical scrapie
DS2002	Classical scrapie
DS2003	BSE not excluded
DS2004	Classical scrapie
DS2005	Classical scrapie



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Number of Participating Laboratories

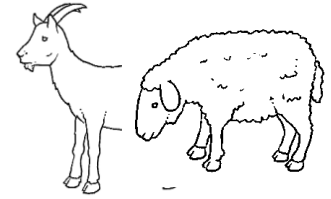
	Total
Laboratories participating with one test method	15
Laboratories participating with two test methods	2
Laboratories participating with three test methods	1
Total participating Laboratories	18



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



Test Method Used

Test method used	Number of laboratories
APHA Bio-Rad TeSeE-based Hybrid Western blotting Method	10
APHA Prionics-based Hybrid Western blot Method	5
Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)	3
FLI Discriminatory Western blot Method	2
ANSES Discriminatory Western blot Method	1
ISS Discriminatory Western blot Method	1

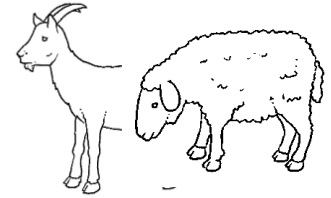


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Proficiency testing for Discriminatory Western Blot in Small Ruminants (PTDS20)

- All Laboratories returned the results in the due time
- All Laboratories reported the correct results



however, EURL contacted 4 laboratories for further information:

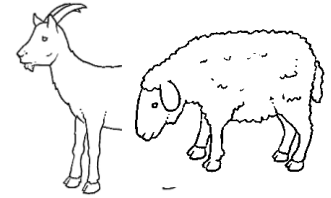
- The excel file compiled with some unexpected modification of the name of the method used (**2 labs**)
- The declared method did not correspond to the reagents and raw data (**1 lab**)
- The declared method did not correspond to the reagents and raw data were incomplete (**1 lab**)



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



Action undertaken:

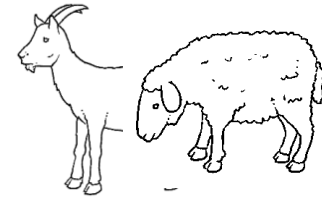
- 3 laboratories were asked to provide clear information on the method/s used.
 - The info provided were satisfactory
- 1 laboratory was asked to provide detailed information (date of testing, antibodies, the correct definition of method used) and provide the complete raw data (images of the blots for each antibody used)
 - The info provided were satisfactory



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



All Participants (18) passed the PT

Belgium

Bulgaria

Czech Republic

France

Germany

Greece

Hungary

Ireland

Italy

Netherlands

Poland

Portugal

Romania

Slovakia

Slovenia

Spain

Switzerland

UK



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Discriminatory Western Blot in Small Ruminants: approved methods

“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

Discriminatory testing must be performed following the protocols and the procedures as reported in the Technical handbook
→ No changes are allowed



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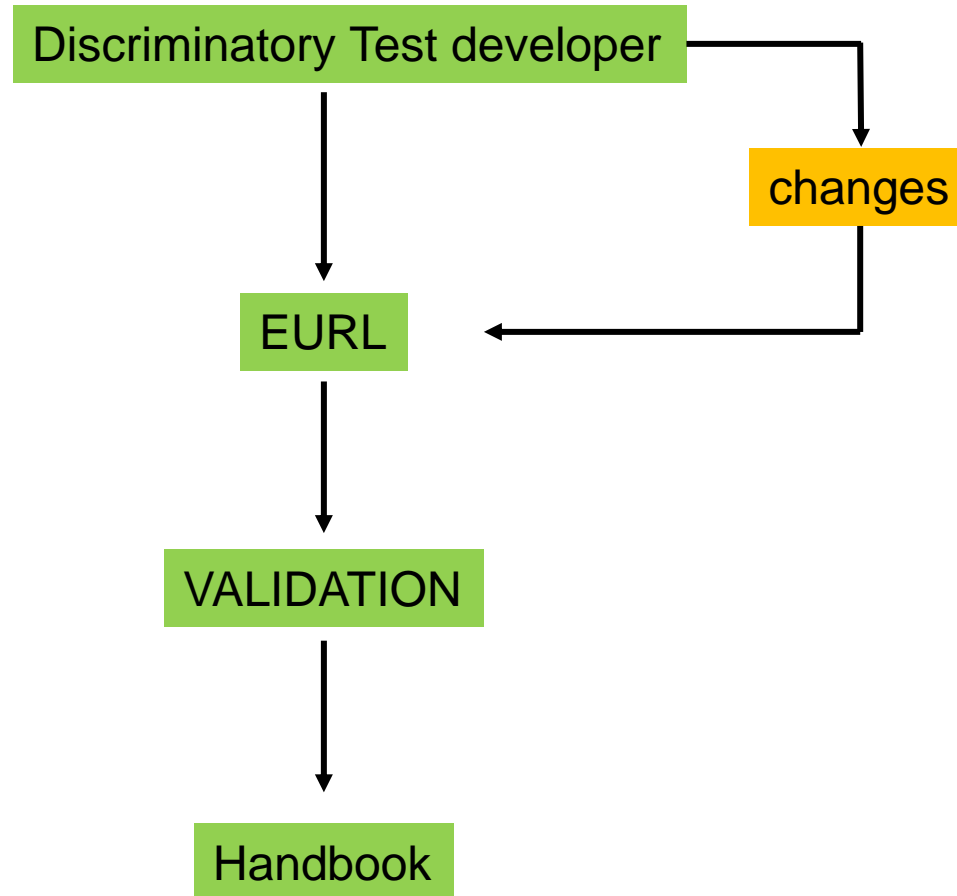
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TSE STRAIN CHARACTERISATION IN SMALL RUMINANTS A TECHNICAL HANDBOOK FOR NATIONAL REFERENCE LABORATORIES IN THE EU

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Discriminatory Western Blot in Small Ruminants: changes in the protocol



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Discriminatory Western Blot in Small Ruminants: changes in the protocol

A test developer may wish to vary the protocol of its approved method (words, reagents, times/temperatures, automated systems...)

→ It is necessary to refer to EURL before implementation of any changes

The previous EURL provided the procedure for change the TSE Discriminatory Tests

The revised guidelines will be soon available



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TSE EU Reference Laboratory



Change Management Procedure for TSE Discriminatory Tests (listed in the European Reference Laboratory (EURL) Discriminatory Testing Handbook)

1. Introduction

Discriminatory tests are used for testing confirmed scrapie positive samples within EU countries (Based on Regulations (EC) No 253/2006 and (EC) No 1053/2003 amending Regulation (EC) No 999/2001.

The methods are approved for use within the EU and are included in the EURL Discriminatory Testing Handbook available on the TSE and EURL website <http://www.tse-lab-net.eu/documents/tse-oie-rl-handbook.pdf>

The approval of a given method is linked to the testing protocol that was used to generate the validation data. In the case of discriminatory tests, this was the successful completion of a ring trial organised by the Strain Typing Expert Group (STEG) of the TSE EURL. If the test developer wished to vary this protocol in any way, approval must be obtained in writing from the TSE European Reference Laboratory (EURL), before implementation of any changes.

This document aims to provide guidelines within which EURL approval may be given. The purpose of this document is to explain what types of changes may be made, how these changes may be made and to outline the types of supporting data required for approval of such changes prior to their incorporation into the Handbook. This document is aimed primarily at the developers of TSE Discriminatory tests, but is also available to NRLs and testing labs so that they know what is required of test developers.

2. Guidance

The EURL should be advised of ALL types of proposed changes to the protocol including:

- Changes of wording
- Changes to the reagents used in the test
- Changes to buffers
- Amendments to how reagents are stored or prepared
- Changes to shelf life
- Changes to how the test is carried out including different incubation times or temperatures
- Use of automated systems
- Changes to interpretation of results or use of controls

Change management procedure for TSE discriminatory tests
Version 2 December 2014

TSE EURL Reviewed: 22/01/2018

EQA rounds

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

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

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Forthcoming 2021 rounds

1. Discriminatory Western Blot in Small Ruminants (DS21)
2. Discriminatory Western Blot in Bovine (DB21)
3. PRNP Genotyping in sheep (GS21)
4. PRNP Genotyping in goats (GG21)

Info about shipment and deadlines → Gabriele's presentation



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ISS

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Gabriele Vaccari
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Elena Esposito
Geraldina Riccardi

IZPLVdA

Giuseppe Ru
Francesco Ingravalle
Elena Bozzetta
Maria Mazza

Thanks to all participants



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