

19th Annual Meeting of the TSE EURL

Turin, 17th – 18th October 2022

2021 round of TSE EURL EQAs: Results feedback

Confirmatory BSE and Scrapie Immunoblotting

Pier Luigi Acutis
IZSPLVA - Turin



TSE EURL

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

NRLs participating in Proficiency Testing 2021

23 laboratories for Confirmatory BSE

24 laboratories for Confirmatory Scrapie

BSE

Austria, Belgium, Bulgaria,
Croatia, Czech Republic, Finland,
France, Germany, Greece,
Hungary, Ireland, Italy, The
Netherlands, Norway, Poland,
Portugal, Romania, Slovakia,
Slovenia, Spain, Sweden,
Switzerland and United Kingdom

Scrapie

Austria, Belgium, Bulgaria,
Croatia, Czech Republic, Finland,
France, Germany, Greece,
Hungary, Ireland, Iceland, Italy,
The Netherlands, Norway, Poland,
Portugal, Romania, Slovakia,
Slovenia, Spain, Sweden,
Switzerland and United Kingdom



TSE EURL

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

Planning

- Each NRL has been identified with a code, different for the two PTs
- Each round included 5 samples of frozen brainstem homogenates
- The samples were stored at -80(± 10) °C until shipment by the courier on dry ice
- The set of samples included information on their preparation, manipulation and transmission of the results (deadline, forms)



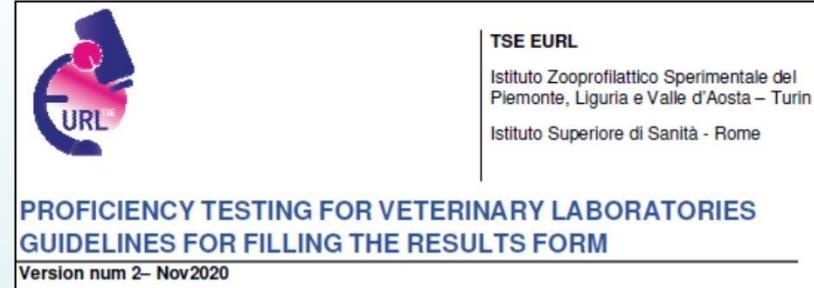
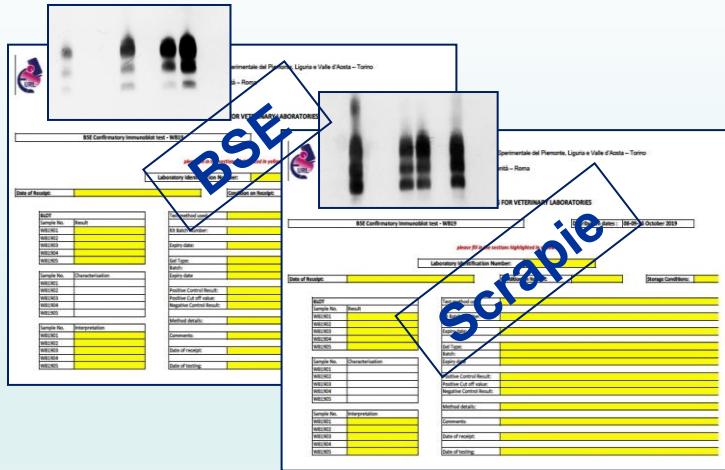
TSE EURL

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

Shipping and Transmission results

The samples were shipped on 13th and 14th December 2021

The deadline for submission of results was on 7th February 2022



TSE EURL

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

BSE CONFIRMATORY IMMUNOBLOTTING

The samples provided for the TSE quality assessment are homogenates of brainstem material from a panel of BSE-positive and BSE-negative animals

The tissue have been homogenised using a 50/50 mix of tissue and distilled water

Sample No	Result	Interpretation
WB2101	positive	strong
WB2102	negative	////
WB2103	negative	////
WB2104	negative	////
WB2105	negative	////

Sample WB2102 = sample WB2103 = sample WB2104 = sample WB2105



TSE EURL

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

SCRAPIE CONFIRMATORY IMMUNOBLOTTING

The samples provided for the TSE quality assessment are homogenates of brainstem material from a panel of Scrapie-positive and Scrapie-negative animals

The tissue have been homogenised using a 50/50 mix of tissue and distilled water

Sample No	Result	Interpretation	Characterisation
WS2101	negative	////	///
WS2102	positive	medium	classical scrapie
WS2103	negative	////	///
WS2104	positive	medium	classical scrapie
WS2105	negative	///	///

Sample WS2101 = sample WS2103 = sample WS2105

Sample WS2102 = sample WS2104



TSE EURL

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

EURL EQA round 2021: Quality Control

Confirmatory BSE and Scrapie Immunoblotting

 **QUALITY**



ISO 17034
ISO 17043

Homogeneity

Stability



TSE EURL

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

Transmission of the results

- All NRLs submitted the results of both PTs by 7th February 2022
- The submission included the raw data (pictures of the blots, readings), the information about the kit batches used for the analysis plus any information considered important for the correct interpretation of the results



TSE EURL

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

Processing of results

Elaboration of a report with the PTs results



Correct results

laboratories suitable to perform confirmatory tests

Incorrect results



analysis on a new set of samples



TSE EURL

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

Results Tabulation for BSE Confirmatory Immunoblot Scheme: WB21

Primary Tests	No. NRLs
TeSeE Western Blot - Bio-Rad	17
PrioWestern BSE Kit - ThermoFisher Scientific	2
In house method	2
OIE SAF immunoblot	1
APHA Bio-Rad Hybrid Western Blot §	1

§ APHA Biorad Hybrid: used mAbs Sha31 e P4



TSE EURL

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

Results Tabulation for BSE Confirmatory Immunoblot Scheme: WB21



**TeSeE Western Blot Bio-Rad used two batches
1C0047 – 0L0046**



**PrioWestern BSE Kit – ThermoFisher Scientific used two batches
W200201G – W200101G**



TSE EURL

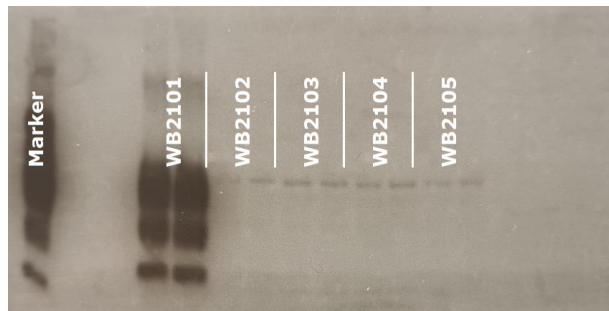
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

WELL DONE!!!

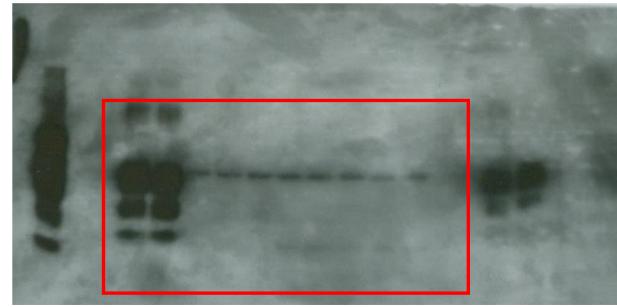
BSE confirmatory Immunoblot – Scheme WB21

All laboratories have passed this PT round

PrioWestern – Lab. 92

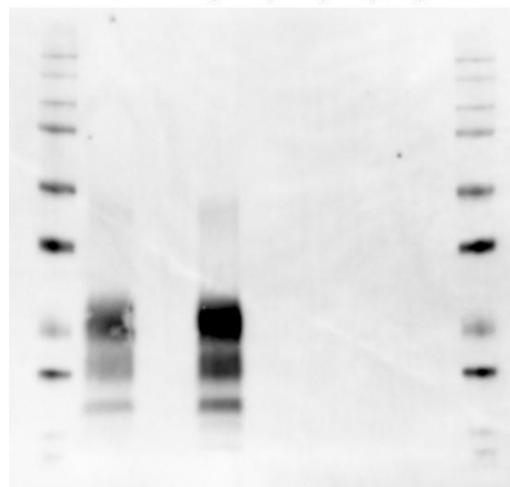


PrioWestern – Lab. 378



TeSeE Western Blot Lab. 564

MW BP35-03 BN26-02 46865 - WB2101: Positive 46866 - WB2102: Negative 46867 - WB2103: Negative 46868 - WB2104: Negative 46869 - WB2105: Negative MW



BP35-03: Internal control BSE Positive
BN26-02: Internal control BSE Negative

OIE IB method – Lab. 648

1 2 3 4 5 6 7 8 9 10

A B C D E F G H I K



TSE EURL

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

Confirmatory BSE and Scrapie Immunoblotting

Results Tabulation for Scrapie Confirmatory Immunoblot Scheme: WS21

Primary Tests	No. NRLs
TeSeE Western Blot Bio-Rad	15
PrioWestern BSE/TeSeE Western Blot	1
APHA Bio-Rad Hybrid §	2
In house method	3
OIE SAF immunoblot	1
TeSeE Western Blot modified*	1
PrioWestern BSE Kit - ThermoFisher Scientific**	1

§ APHA Bio-Rad Hybrid: 2 mAbs Sha31 and P4

* TeSeE Sheep/Goat kit for extraction

**PrioWestern BSE Kit: used 2mAbs 6H4 and P4



TSE EURL

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

Results Tabulation for Scrapie Confirmatory Immunoblot Scheme: WS21



**TeSeE Western Blot Bio-Rad used two batches
1C0047 – 0L0046**



**PrioWestern BSE Kit – ThermoFisher Scientific used two batches
W200101G – W200201G**



TSE EURL

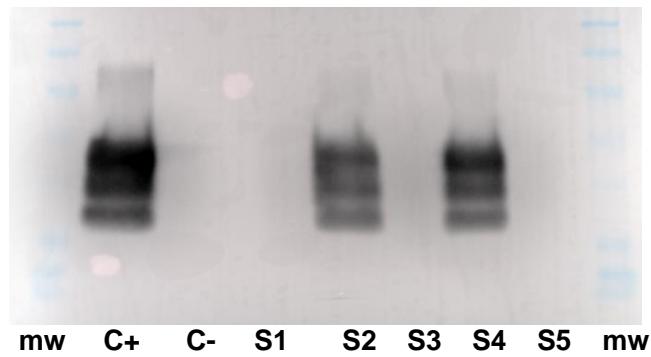
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

Scrapie confirmatory Immunoblot – Scheme WS21

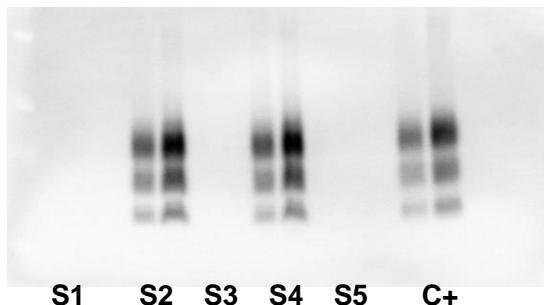
All laboratories have passed this PT round except laboratories 363 and 855 (defined WS2102 as negative)

WELL DONE!!!

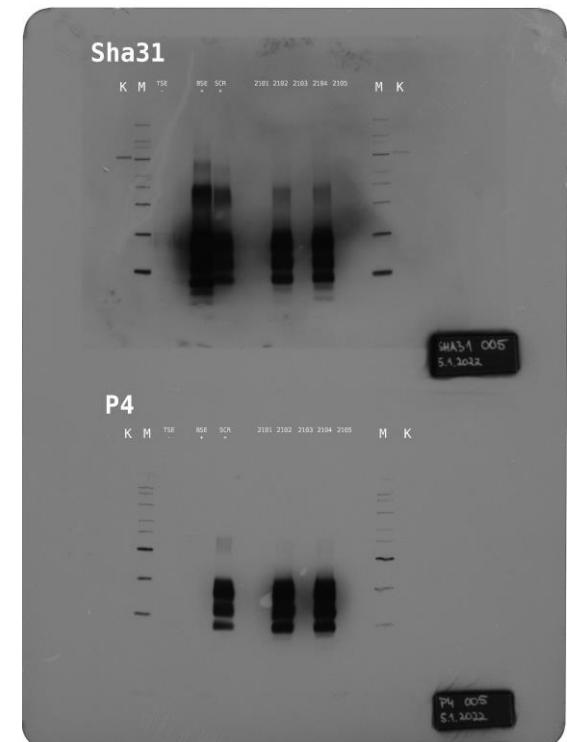
TeSeE Western Blot modified – Lab. 804



TeSeE Western Blot – Lab. 519



APHA Bio-Rad Hybrid – Lab. 164



TSE EURL

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

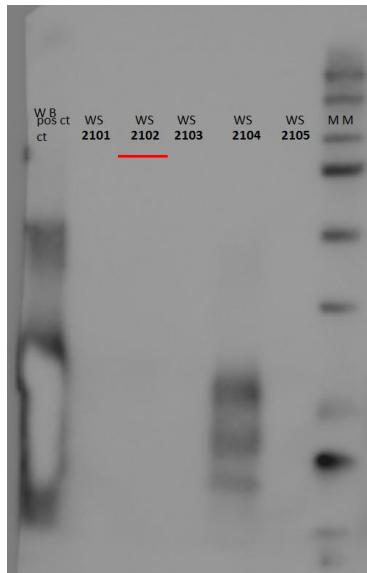
1st round

Non-compliant results for labs. 363 and 855

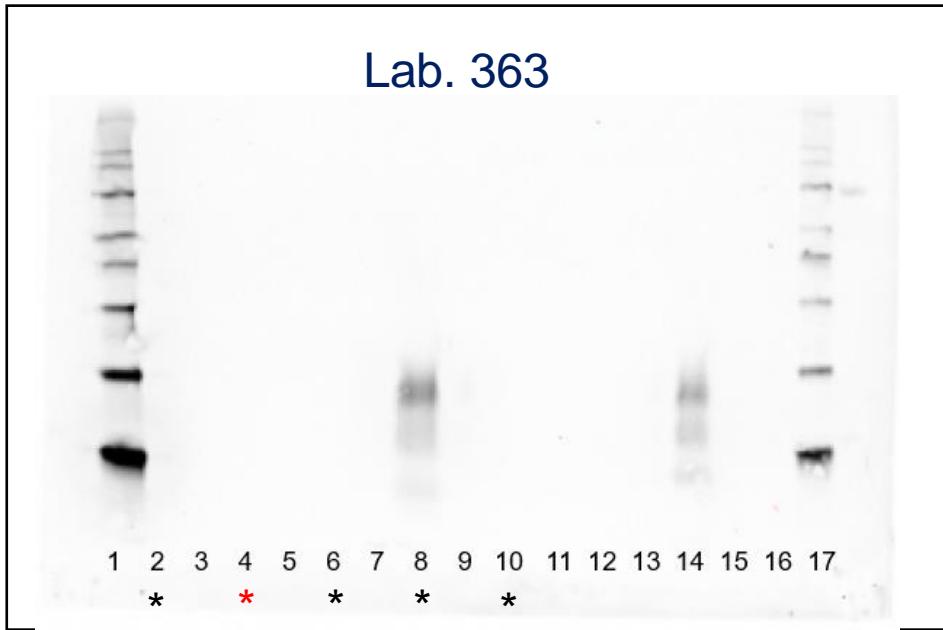


WS2102 defined as negative by both laboratories

Lab. 855



Lab. 363



TSE EURL

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

Laboratories 363 and 855

2nd round

Sample No	Result	Interpretation	Characterisation
WS2106	positive	medium	classical scrapie
WS2107	negative	////	////
WS2108	positive	medium	classical scrapie
WS2109	negative	////	////
WS2110	negative	////	////

Sample WS2107 = sample WS2109 = sample WS2110

Sample WS2106 = sample WS2108

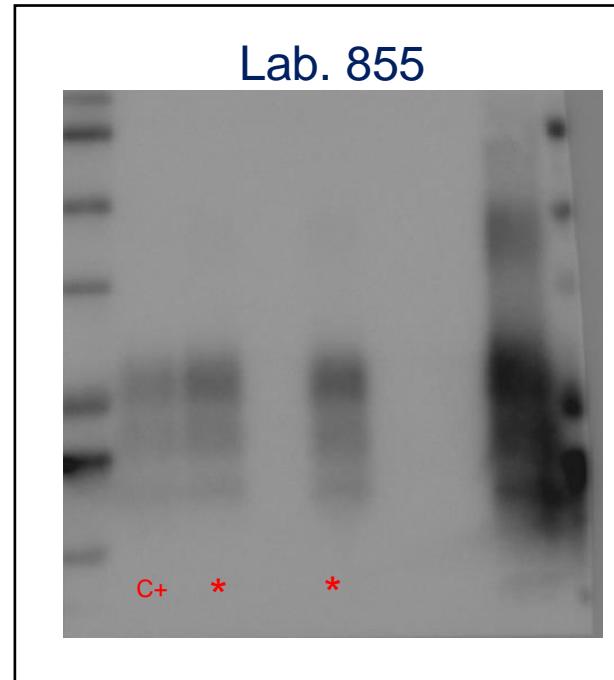
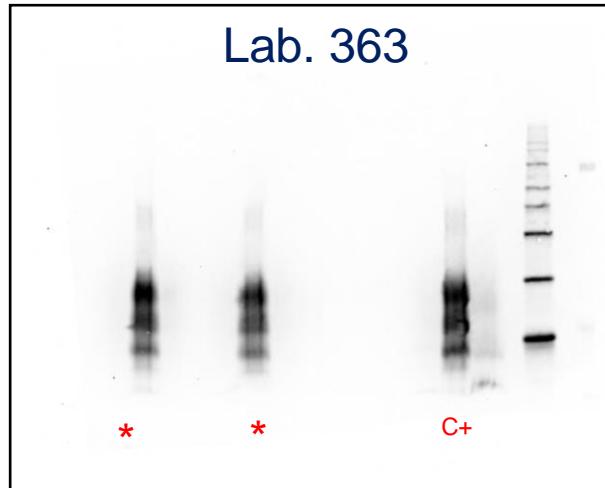


TSE EURL

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

Laboratories 363 and 855

2nd round



both laboratories correctly interpreted all samples from the second round



TSE EURL

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

The forthcoming 2022 round of EQAs

Confirmatory BSE and Scrapie Immunoblotting



TSE EURL

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

About shipping

- The shipment of the samples is scheduled by mid November
- BSE and Scrapie confirmatory PTs will be sent along with sample sets for the rapid test
- The samples will be packed with dry-ice and the shipment will be scheduled at the beginning of the week
- E-mail to communicate the shipment

Please transmit the import permit as soon as possible, if required and expired



TSE EURL

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

Submission PTs results

- The submission of results is expected by 3 weeks after the shipment (by 10th December 2022)
- The results submitted after the deadline will not be evaluated
- The results will be submitted by EURL web site (information will be provided soon)
- Please include in the submission the raw data (pictures of the blots, readings) and all the information about the kit batches used for the analysis
- Report any information considered important for the correct interpretation of the results



TSE EURL

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

EURL Contact

Pier Luigi Acutis

e-mail address: pierluigi.acutis@izsto.it
telephone n.: +39 011 2686324
Fax n.: +39 011 2686322

Maria Mazza

e-mail address: maria.mazza@izsto.it
telephone n.: +39 011 2686282
fax n.: +39 011 2686322



TSE EURL

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

THANKS FOR YOUR ATTENTION



TSE EURL

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