

2019 round of TSE EURL EQAs: Results feedback
Turin/Rome, Italy
Online meetings: 16th-18th September 2020

Confirmatory BSE and Scrapie Immunoblotting

Pier Luigi Acutis



TSE EURL

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

NRLs participating in Proficiency Testing 2019



23 laboratories for both PTs

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, 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 $-80(\pm 10)$ °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 8th, 9th and 15th October 2019

The deadlines for submission of results were:

- ✓ **06/12/2019** (distributions on 08 - 09 October 2019)
- ✓ **13/12/2019** (distribution on 15 October 2019)

BSE

Scrapie

Form for Veterinary Laboratories: BSE Confirmatory Immunoblot test - W... Distribution dates: 08-09-11

Form for Veterinary Laboratories: Scrapie Confirmatory Immunoblot test - W... Distribution dates: 08-09-11



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 range 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
WB1901	positive	strong
WB1902	negative	////
WB1903	positive	medium
WB1904	positive	medium
WB1905	negative	///

Sample WB1903 = WB1904; Sample WB1902=WB1905.



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 range 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
WS1901	positive	medium	classical scrapie
WS1902	positive	strong	classical scrapie
WS1903	negative	///	///
WS1904	positive	medium	classical scrapie
WS1905	negative	///	///

Sample WS1901 = WS1904; Sample WS1903=WS1905.



TSE EURL

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

EURL EQA round 2019: Quality Control

Confirmatory BSE and Scrapie Immunoblotting



ISO 17034
ISO 17043

Homogeneity

Stability



TSE EURL

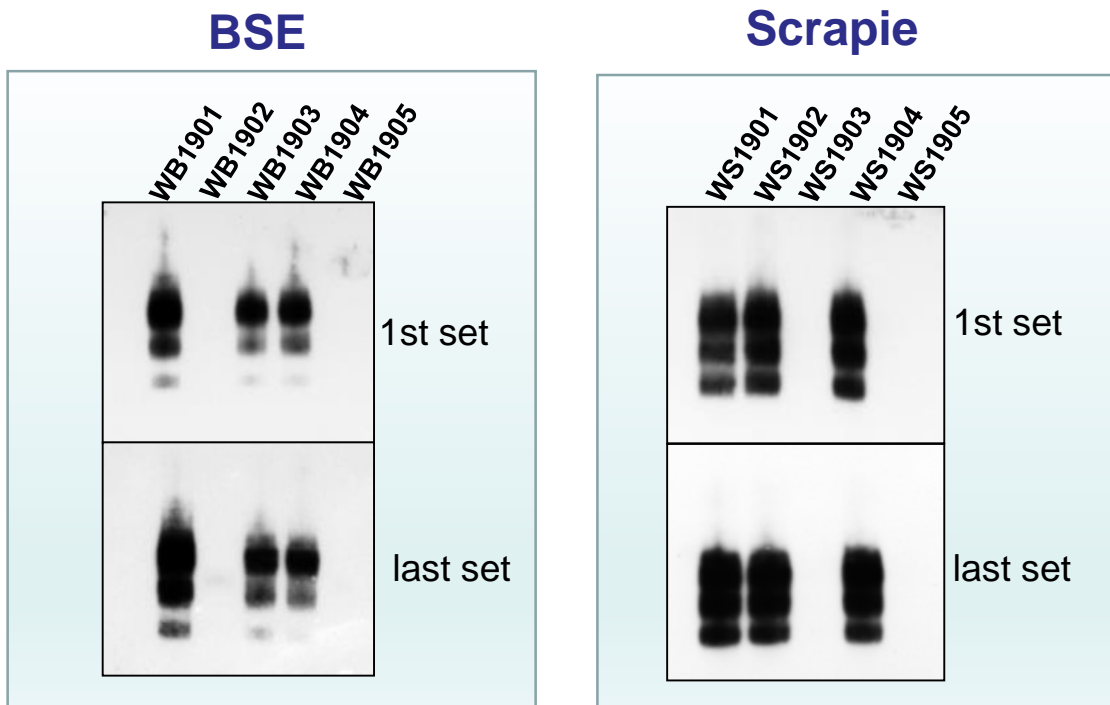
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

EURL EQA round 2019: Quality Control

Confirmatory BSE and Scrapie Immunoblotting



Homogeneity



TSE EURL

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

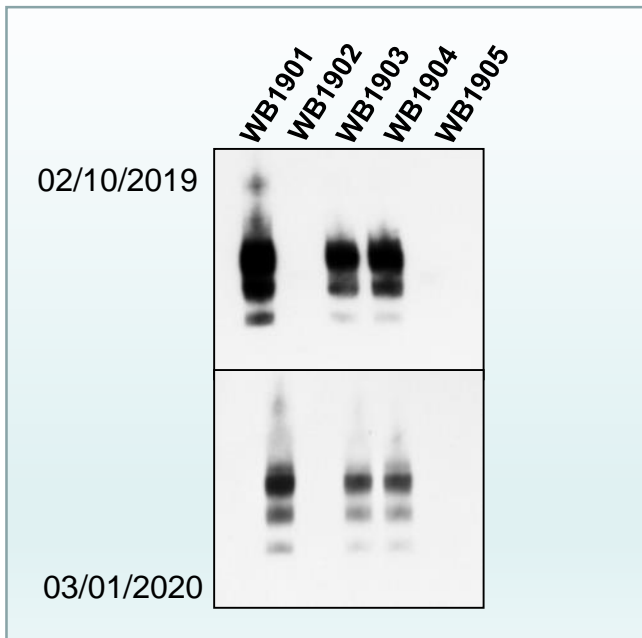
EURL EQA round 2019: Quality Control

Confirmatory BSE and Scrapie Immunoblotting

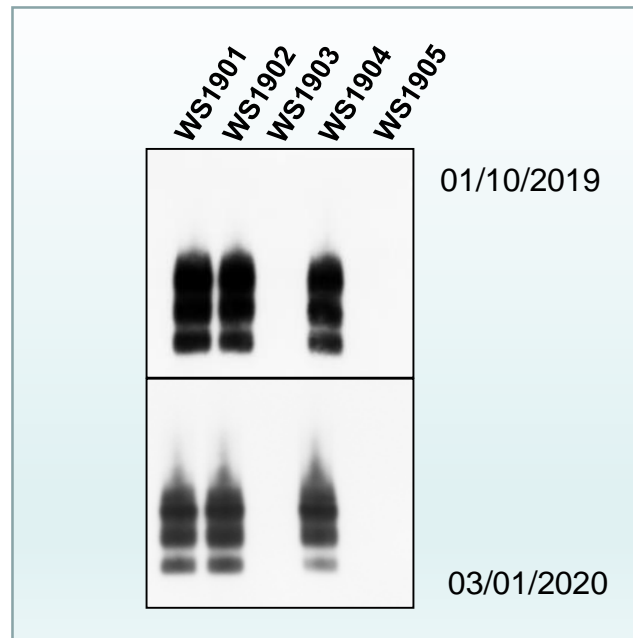


Stability

BSE



Scrapie



TSE EURL

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

Transmission of the results

Submission PTs results by e-mail



- All NRLs submitted the results of both PTs within the two deadlines:
06 and 13 December 2020
- 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: WB19**

Primary Tests	No. NRLs
TeSeE Western Blot Bio-Rad	12
PrioWestern BSE Kit ThermoFisher Scientific	5
APHA Bio-Rad Hybrid §	1
PrioWestern BSE Kit ThermoFisher Scientific (modified)*	1
TeSeE Western Blot Bio-Rad (modified) **	1
OIE SAF immunoblot	1
In house methods***	2



TSE EURL

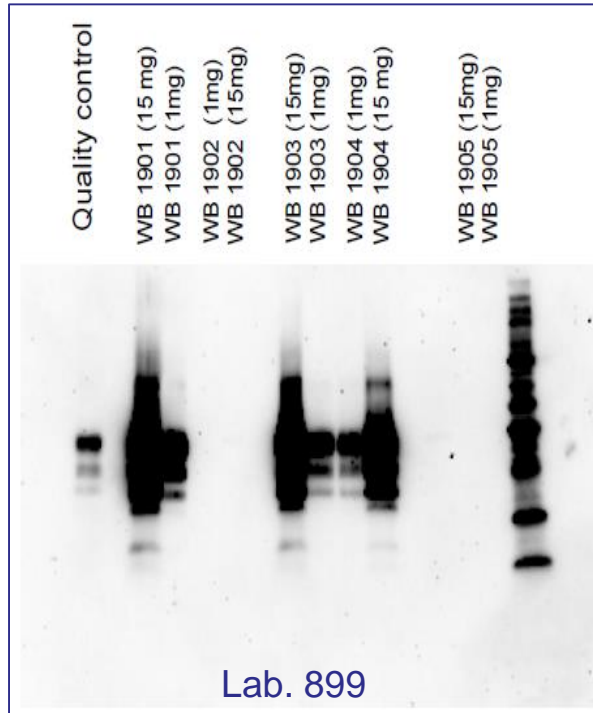
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

BSE confirmatory Immunoblot – Scheme WB19

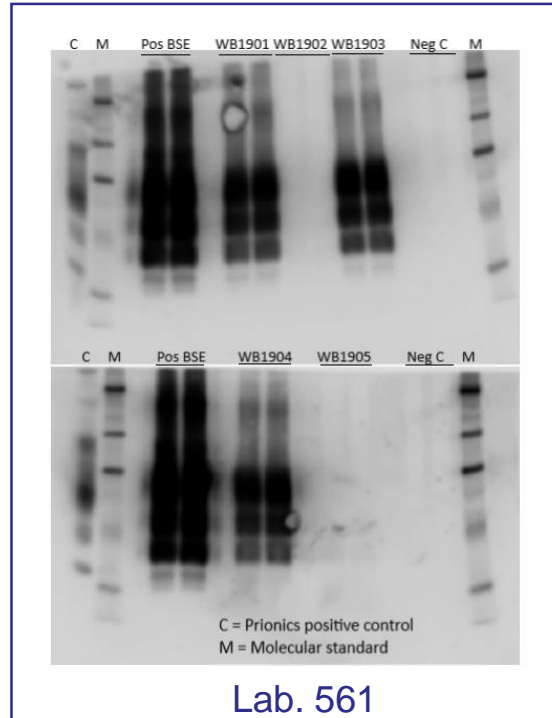


All laboratories have passed this PT round

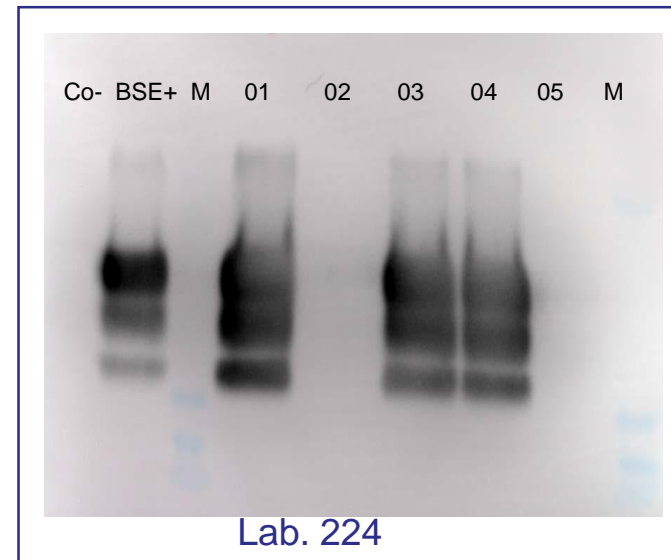
TeSeE Biorad



PrioWestern



In house method



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: WS19**

Primary Tests	No. NRLs
TeSeE Western Blot Bio-Rad	12
APHA Bio-Rad Hybrid §	3
PrioWestern BSE Kit ThermoFisher Scientific (modified)*	5
OIE SAF immunoblot	1
In house methods	2



TSE EURL

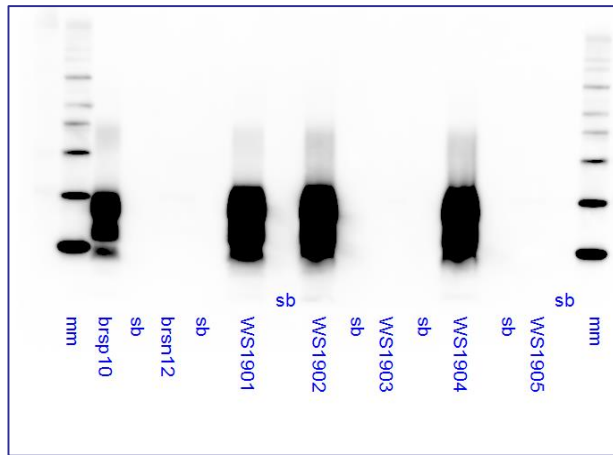
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

Scrapie confirmatory Immunoblot – Scheme WS19



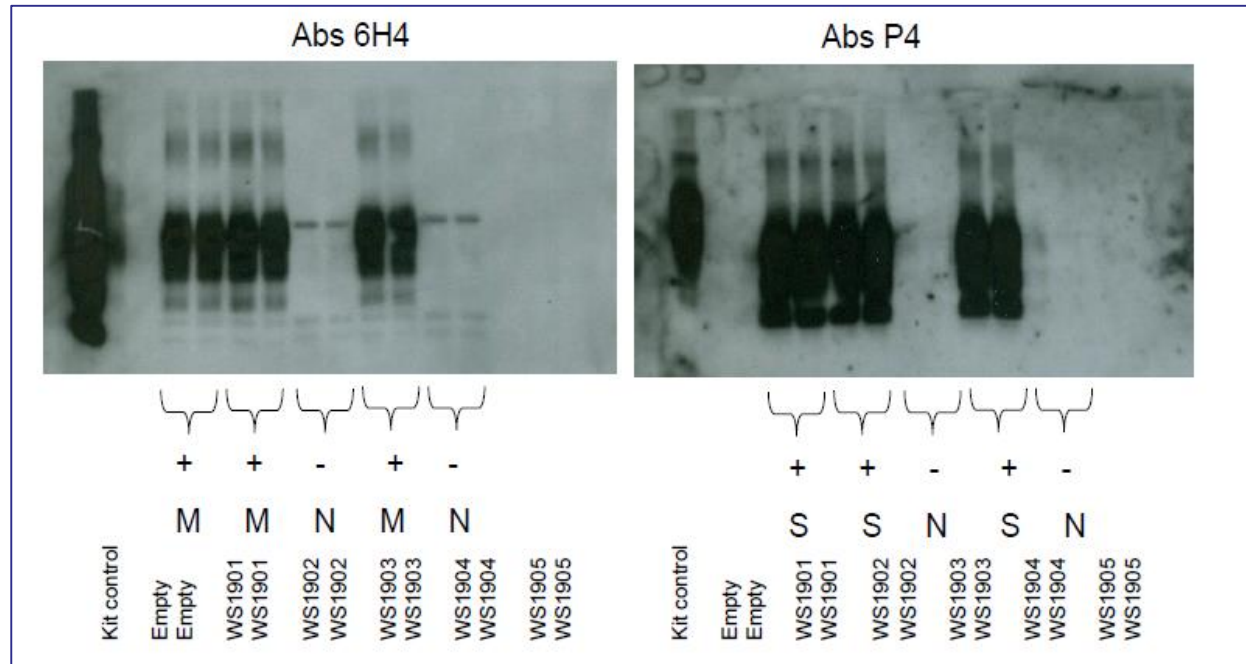
All laboratories have passed this PT round
except lab. 673

TeSeE Biorad



Lab. 401

PrioWestern



Lab. 669



TSE EURL

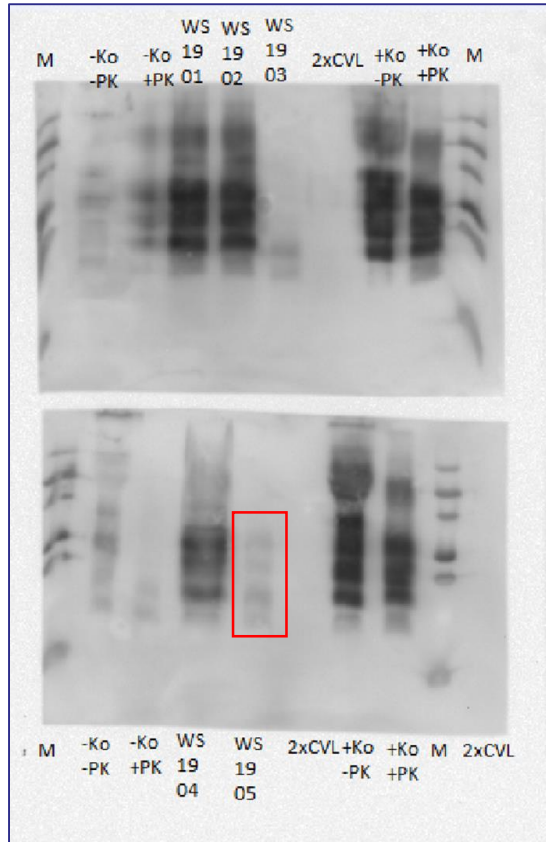
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

Scrapie confirmatory Immunoblot – Scheme WS19

Non-compliant results for lab. 673

1st round

WS1905 false positive



TSE EURL

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

Scrapie confirmatory Immunoblot – Scheme WS19



Lab. 673

2nd round

Sample No	Result	Interpretation	Characterisation
WS1906	negative	////	////
WS1907	positive	strong	classical scrapie
WS1908	negative	////	////
WS1909	positive	medium	classical scrapie
WS1910	positive	medium	classical scrapie

Sample WS1906 = WS1908; Sample S1909 = WS1910.



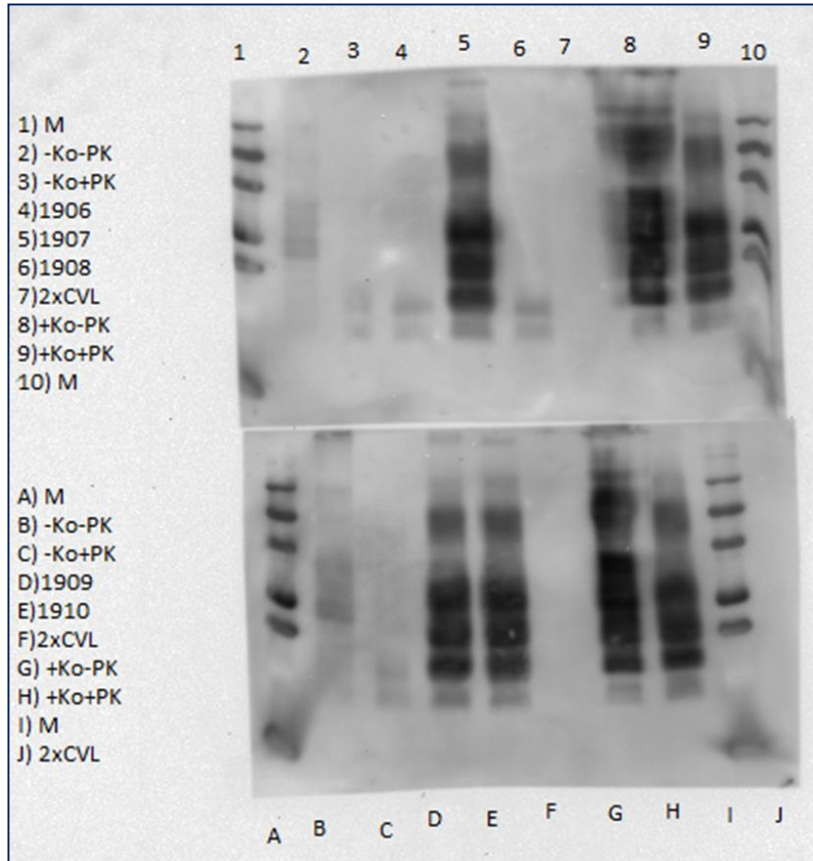
TSE EURL

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

Scrapie confirmatory Immunoblot – Scheme WS19

Lab. 673

2nd round



the laboratory 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 2020 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 the end of October
- 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



TSE EURL

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

Submission PTs results

- Longer times for the analysis and submission of results (1-2 months)
- The results submitted after the deadline will not be evaluated
- The submission of the results has to be defined
- 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 for Confirmatory PTs



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