

2020 round of TSE EURL EQAs: Results feedback
Turin/Rome, Italy
Online meetings: 11 October 2021

Confirmatory BSE and Scrapie Immunoblotting

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NRLs participating in Proficiency Testing 2020

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



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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)



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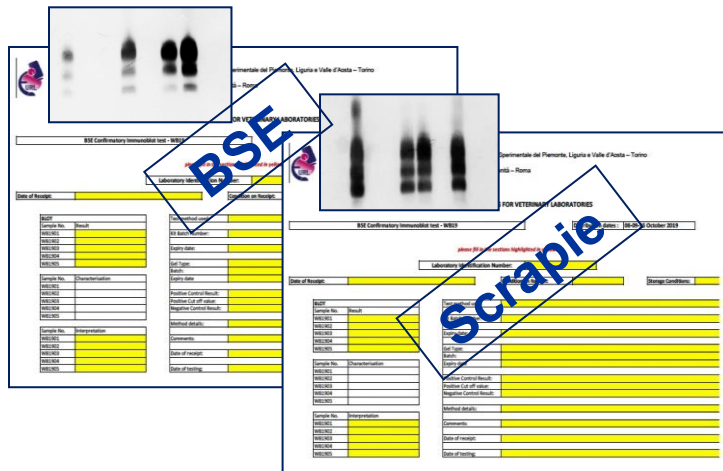
Shipping and Transmission results

The samples were shipped on 21st, 26th and 27th October 2020

The deadlines for submission of results were:

- ✓ **30/11/2020** (distributions on 21st October 2020)
- ✓ **04/12/2020** (distribution on 26th and 27th October 2020)

NEW DEADLINE FOR COVID EMERGENCY EXTENDED TO 31/12/2020



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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
WB2001	negative	////
WB2002	positive	strong
WB2003	negative	////
WB2004	positive	medium
WB2005	positive	medium

Sample WB2001 = WB2003; Sample WB20004=WB2005.



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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
WS2001	negative	////	///
WS2002	positive	strong	classical scrapie
WS2003	positive	medium	classical scrapie
WS2004	negative	////	///
WS2005	positive	medium	classical scrapie

Sample WS2001 = WS2004; Sample WS2003=WS2005.



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EURL EQA round 2020: Quality Control

Confirmatory BSE and Scrapie Immunoblotting



ISO 17034
ISO 17043

Homogeneity

Stability



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Transmission of the results

- All NRLs submitted the results of both PTs by 31 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



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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



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Confirmatory BSE and Scrapie Immunoblotting

Results Tabulation for BSE Confirmatory Immunoblot Scheme: WB20

Primary Tests	No. NRLs
TeSeE Western Blot - Bio-Rad	13
PrioWestern BSE Kit - ThermoFisher Scientific	4
APHA Bio-Rad Hybrid Western Blot §	1
PrioWestern BSE Kit - ThermoFisher Scientific (modified)*	1
TeSeE Western Blot Bio-Rad (modified) **	1
OIE SAF immunoblot	1
In house method	2

§ APHA Biorad Hybrid: used mAbs Sha31 e P4

* PrioWestern modified: used concentration centrifugation and 9A2

** TeSeE WB Biorad modified: used mAbs Sha31 and 12B2



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Results Tabulation for BSE Confirmatory Immunoblot Scheme: WB20



**TeSeE Western Blot Bio-Rad used two batches
0C0045 – 9F0044**



**PrioWestern BSE Kit – ThermoFisher Scientific used one batch
W200101G**



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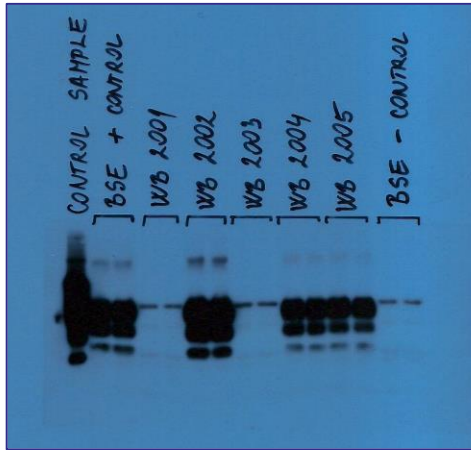
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WELL DONE!!!

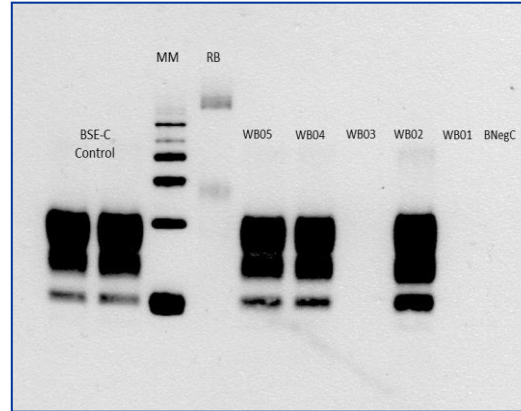
BSE confirmatory Immunoblot – Scheme WB20

All laboratories have passed this PT round

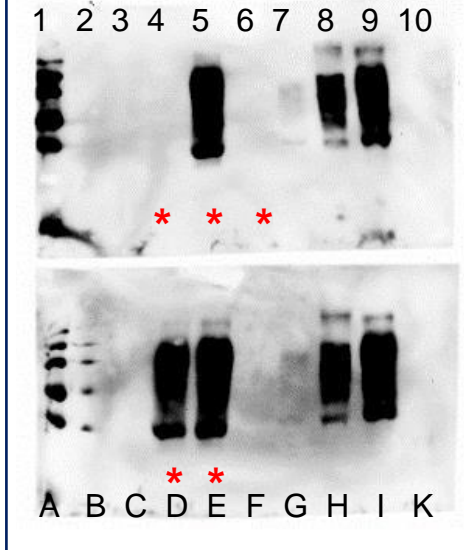
PrioWestern – Lab. 157



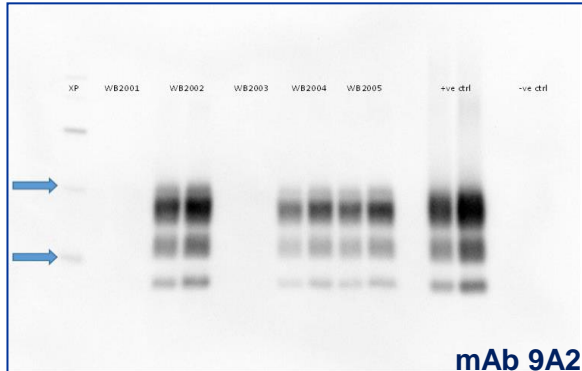
TeSeE BioRad – Lab. 322



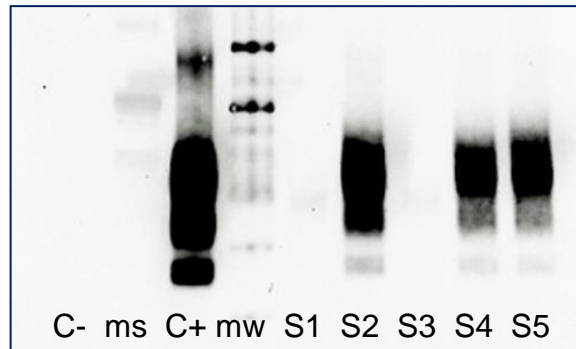
OIE IB method – Lab. 648



PrioWestern modified – Lab. 717



In house method – Lab. 221



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Confirmatory BSE and Scrapie Immunoblotting

Results Tabulation for Scrapie Confirmatory Immunoblot Scheme: WS20

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

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

* PrioWestern modified: 3 mAbs 6H4, P4 and 12B2; used concentration centrifugation



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Results Tabulation for BSE Confirmatory Immunoblot Scheme: WB20



**TeSeE Western Blot Bio-Rad used two batches
0C0045 – 9F0044**



**PrioWestern BSE Kit – ThermoFisher Scientific used one batch
W200101G**



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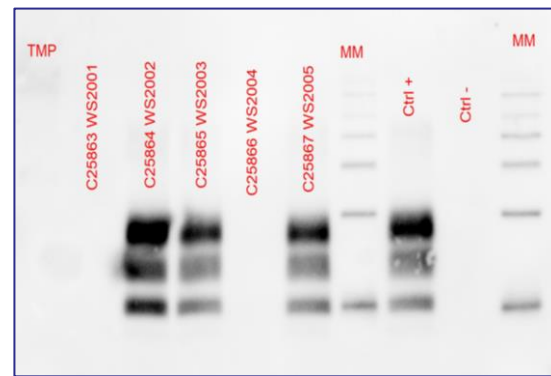
WELL DONE!!!

**All laboratories have passed this PT round
except lab. 164**

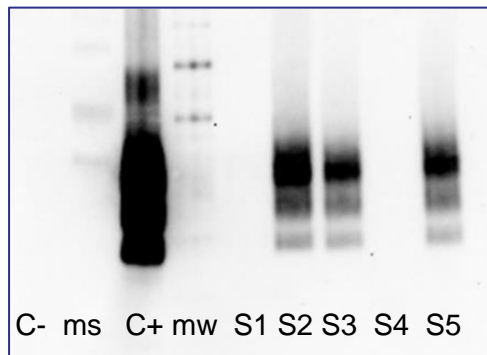
PrioWestern – Lab. 950



TeSeE Biorad - Lab. 225



In house method – Lab. 368

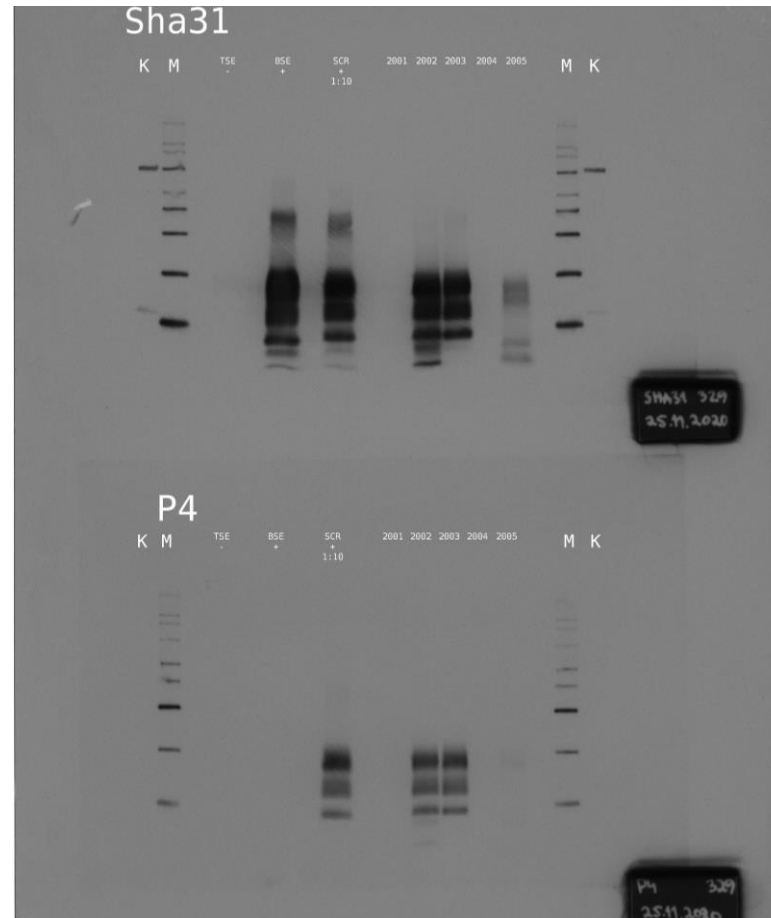


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Non-compliant results for lab. 164

1st round



WS2005 atypical scrapie ⇒



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Lab. 164

2nd round

Sample No	Result	Interpretation	Characterisation
WS2006	positive	strong	classical scrapie
WS2007	negative	////	////
WS2008	positive	strong	atypical scrapie
WS2009	negative	////	////
WS2010	negative	////	////

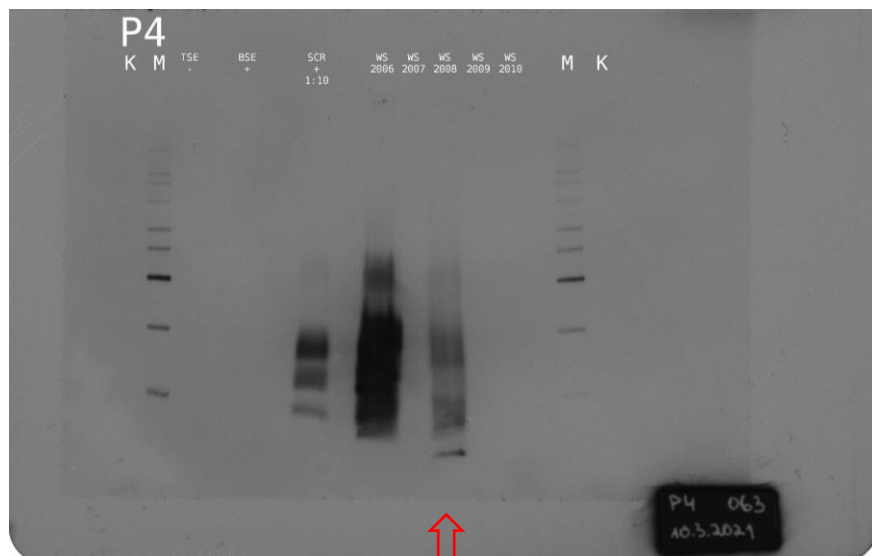


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Lab. 164

2nd round



WS2008 atypical scrapie



the laboratory correctly interpreted all samples from the second round



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The forthcoming 2021 round of EQAs
**Confirmatory BSE and Scrapie
Immunoblotting**



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About shipping

- The shipment of the samples is scheduled by mid December
- 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



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Submission PTs results

- The submission of results is expected by 2-3 weeks after the shipment
- The results submitted after the deadline will not be evaluated
- The results will be submitted in the same way as for the previous PTs
- 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

Please fill the form sent for the submission of results also for alternative tests



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THANKS FOR YOUR ATTENTION



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