

22nd Annual Meeting of the TSE EURL

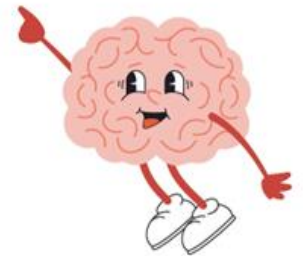
Turin 12th – 13th May 2025



RAPID TESTS AND CONFIRMATORY IMMUNOBLOT 2024 EQA ROUNDS: RESULTS AND FORTHCOMING 2025 ROUNDS



Maria Mazza
IZSPLVA - Turin



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PLANNING

Elena Bozzetta - *Coordinator*

Maria Mazza - *Tech. supervisor*

Daniela Loprevite - *Technician*

Maria Cavarretta - *Technician*

Francesco Ingravalle - *Statistician*

RB24 - RS24

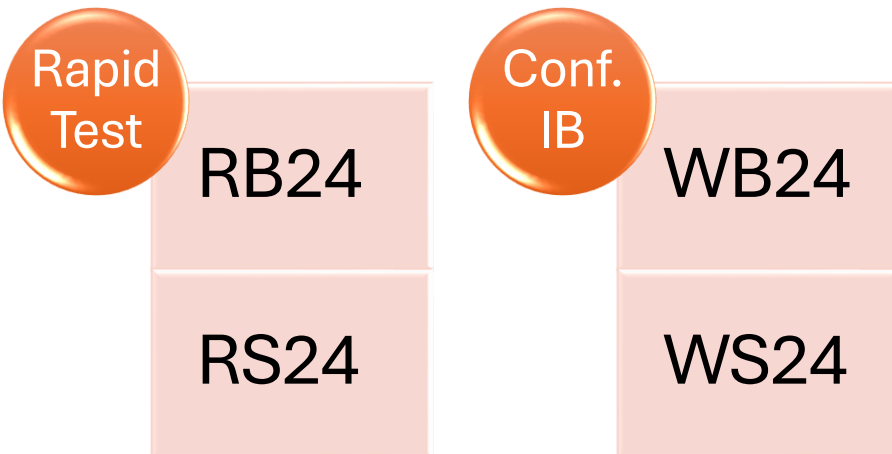
WB24 - WS24



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PLANNING



Shipment 2-3
December 2024



Deadline on 30
December 2024



Submission
results
on website



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PLANNING

Identification NRLs

- Number code for each PT
- Personal number code

Storage samples

- -80 °C until shipment
- Transport on dry ice

Panel samples

- 7 samples for RB24 and RS24*
- 5 samples for WB24 and WS24



* Analysis in duplicate





EURL EQA round 2024



Samples preparation

Homogenates of brainstem material from a panel of natural cases confirmed positive and negative for BSE and Scrapie

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

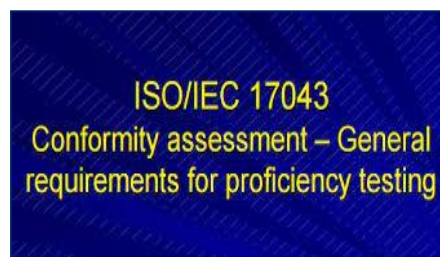
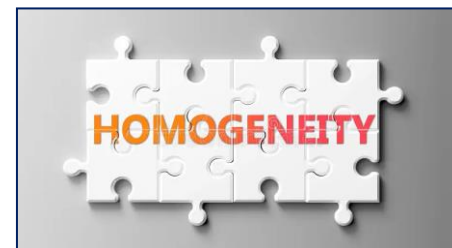


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EURL EQA round 2024 Quality Control



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Processing of results

Elaboration of a report with the PTs results for BSE and Scrapie round



Correct results

Laboratories suitable to perform screening and confirmatory tests

Incorrect results

analysis on a new set of samples

statistical analysis

RB24 – RS24



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2024 round of TSE EURL EQAs: Results feedback



BSE SCREENING TEST RB24



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NRLs participating in Proficiency Testing 2024

BSE SCREENING TEST SCHEME RB24

Austria
Belgium
Bulgaria
Croatia
Cyprus
Czech Republic
Estonia
Finland



France
Germany
Greece
Hungary
Italy
Latvia

Lithuania
Malta
Netherlands
North Macedonia*
Norway
Poland

Portugal
Romania
Slovakia
Slovenia
Spain
Sweden
Switzerland
United Kingdom*

28 NRLs

*Countries extra UE



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Results Tabulation for BSE Screening Test

Scheme: RB24

Primary Tests	No. NRLs
TeSeE SAP COMBI (Bio-Rad)	10
HerdChek BSE/Scrapie Ag - short protocol (IDEXX)	9
HerdChek BSE/Scrapie Ag – ultrashort protocol (IDEXX)	8



Results Tabulation for BSE Screening Test

Scheme: RB24

First alternative Tests	No. NRLs
TeSeE SAP COMBI (Bio-Rad)	3
HerdChek BSE/Scrapie Ag - short protocol (IDEXX)	1

Second alternative Tests	No. NRLs
BetaPrion BSE EIA (Roboscreen)	1
HerdChek BSE/Scrapie Ag - ultrashort protocol (IDEXX)	1



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Results Tabulation for BSE Screening Test

Scheme: RB24



HERDCHEK BSE/SCRAPIE Ag (IDEXX) - Batch

AY185

BY445

CY816

EY700

HY363



TeSeE SAP COMBI (Bio-Rad) - Batch

4C0093

4J0094

4H0095



BetaPrion BSE EIA (Roboscreen) - Batch

0624-01



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BSE SCREENING TEST SCHEME RB24

Sample No	Result
RB2401	TSE negative bovine tissue homogenate
RB2402	Classical positive bovine tissue homogenate (Dilution 1:4)
RB2403	TSE negative bovine tissue homogenate
RB2404	TSE negative bovine tissue homogenate
RB2405	TSE negative bovine tissue homogenate
RB2406	TSE negative bovine tissue homogenate
RB2407	TSE negative bovine tissue homogenate



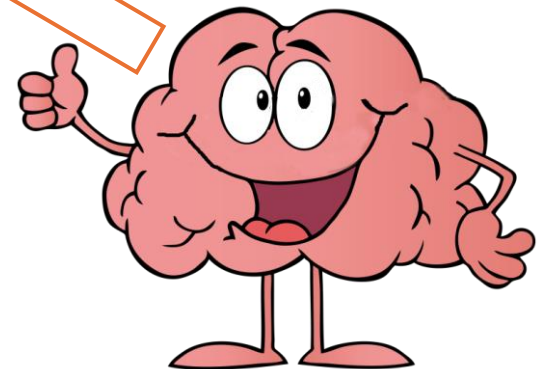
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BSE Screening Test Scheme RB24

ALL THE LABS PASSED THIS
PROFICIENCY TESTING AT THE
FIRST ROUND

Very
good
job!!!



CONGRATULATIONS



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**Statistical
analysis**

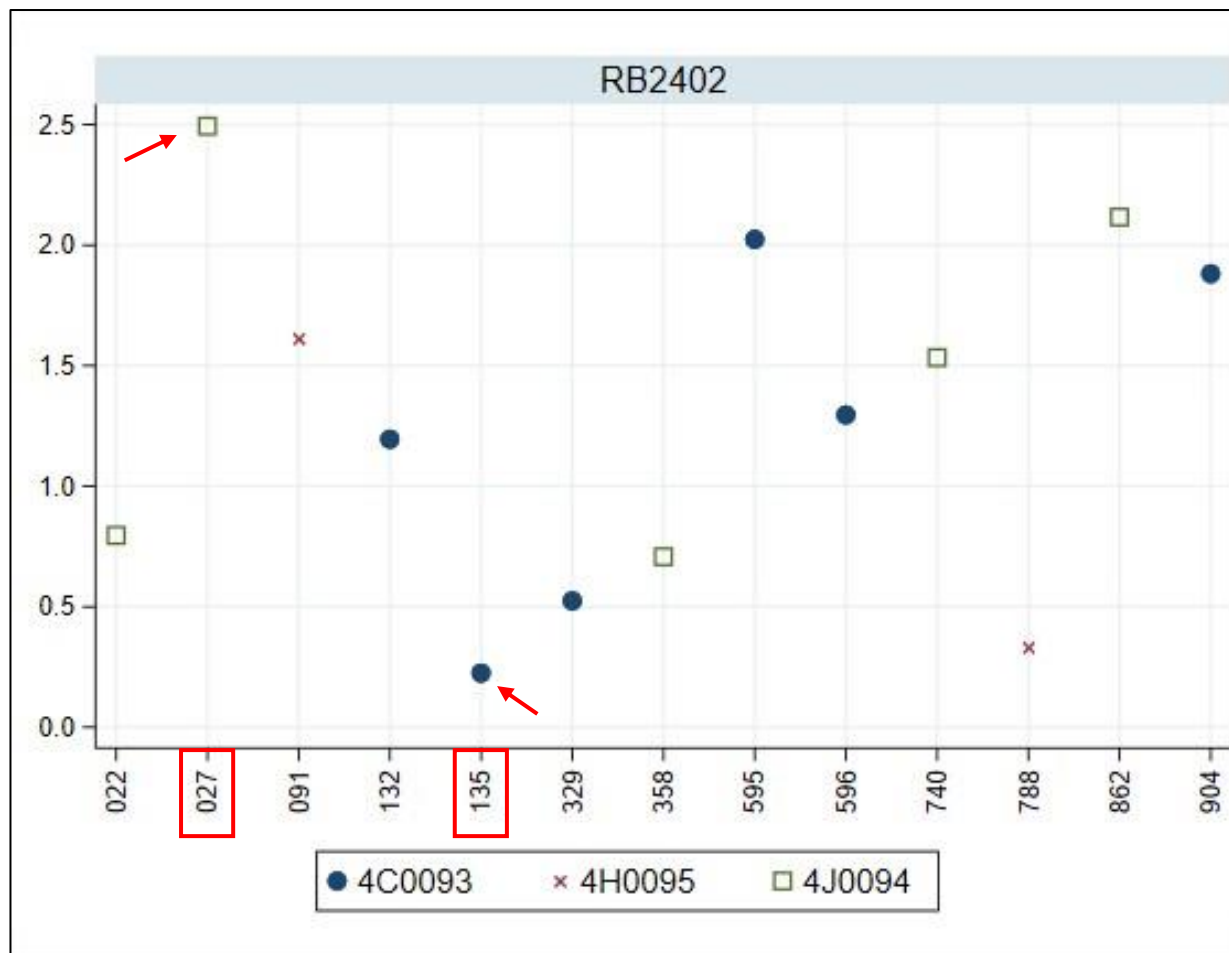
**BSE
Screening Test
Scheme RB24**



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BSE SCREENING TEST - SCHEME RB24

TeSeE SAP COMBI: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample

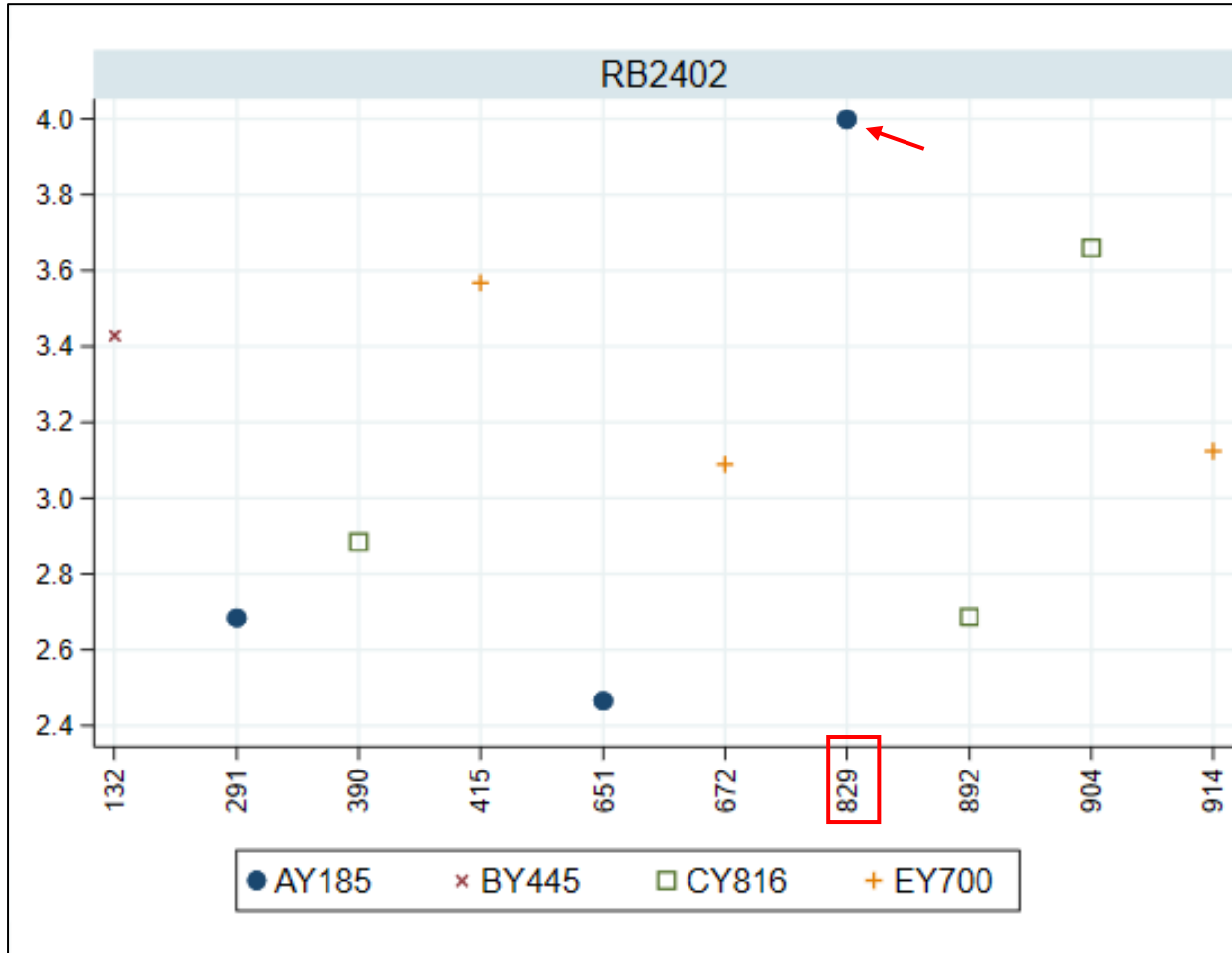


Lab 135 (batch 4C0093) reported OD lower than other labs.

Lab 027 (batch 4J0094) reported OD higher than other labs.

BSE SCREENING TEST - SCHEME RB24

HerdChek BSE-Scrapie Antigen Test-SHORT PROTOCOL: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample



Lab 829 reported overflow values

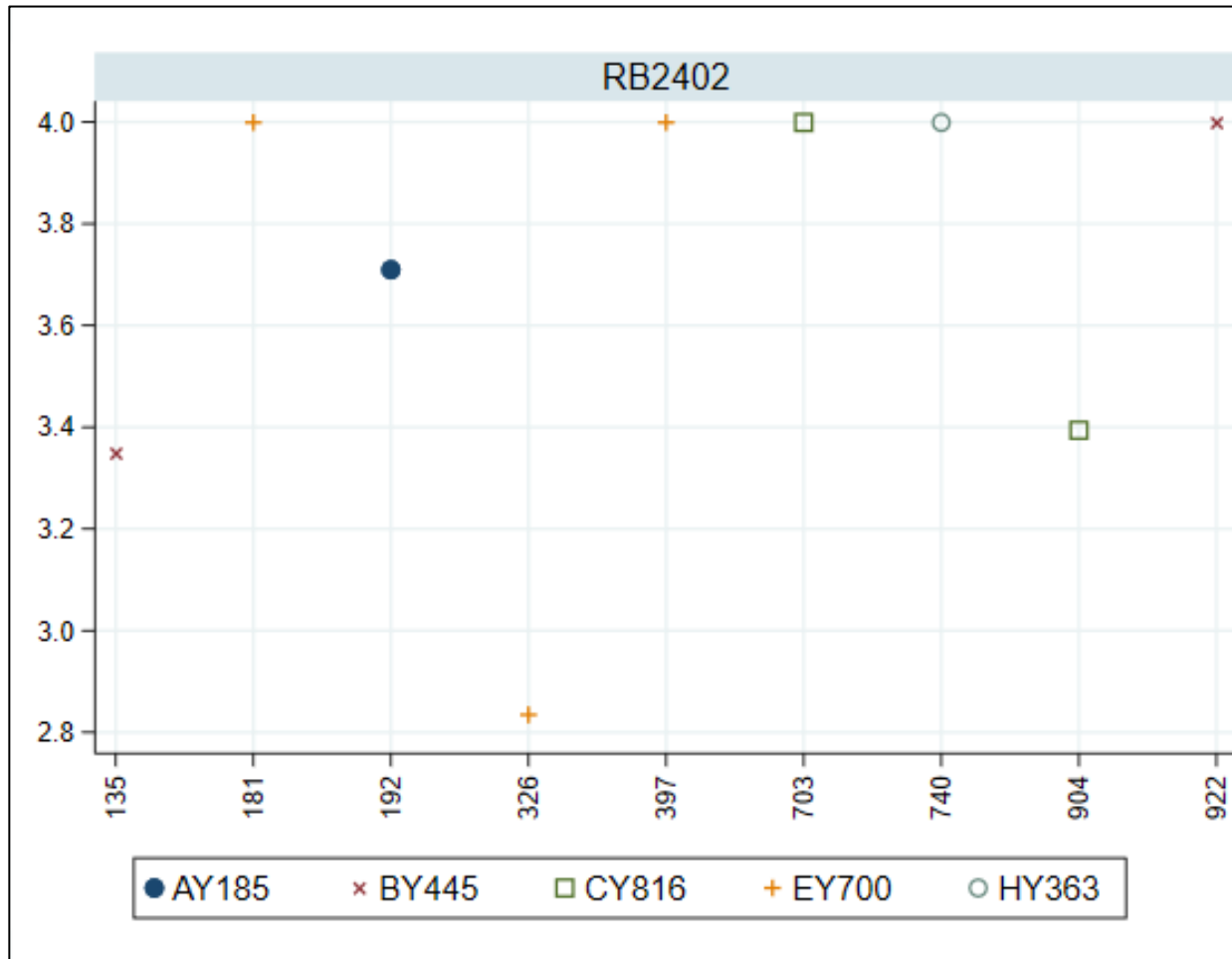


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BSE SCREENING TEST - SCHEME RB24

HerdChek BSE-Scrapie Antigen Test-ULTRASHORT PROTOCOL: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample



Five labs (181, 397, 703, 740, 922) reported overflow values

BSE SCREENING TEST - SCHEME RB24

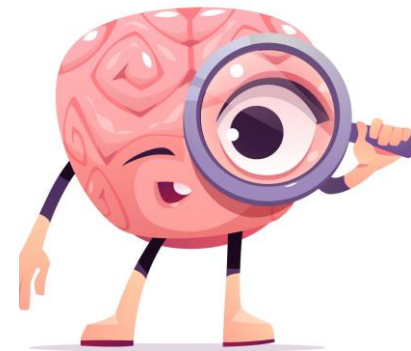
IN CONCLUSION

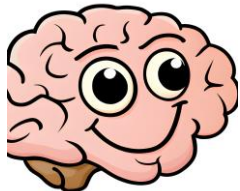
TeSeE SAP COMBI

- It was the most used test in routine: 37% (10/27) of the laboratories applied TeSeE SAP COMBI test in routine.
- Three labs applied this test as alternative test.
- No statistically significant differences in ODs between participants or batches for sample RB2402.



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BSE SCREENING TEST - SCHEME RB24

IN CONCLUSION

IDEXX HerdChek BSE-Scrapie Antigen Test- SHORT PROTOCOL

- It was the second most used test in routine: 33% (9/27) of the laboratories applied IDEXX HerdChek BSE-Scrapie Antigen Test- SHORT PROTOCOL test in routine.
- One lab applied this test as alternative test.
- No statistically significant differences between participants for the positive sample.
- No statistically significant differences between batches for the positive sample.



BSE SCREENING TEST - SCHEME RB24

IN CONCLUSION

IDEXX HerdChek BSE-Scrapie Antigen Test- ULTRASHORT PROTOCOL

- It was the last test used in routine: 30% (8/27) of the laboratories applied IDEXX HerdChek BSE-Scrapie Antigen Test- ULTRASHORT PROTOCOL test in routine.
- Only one lab applied this test as alternative test.
- No statistically significant differences between participants for the positive sample.
- No statistically significant differences between batches for the positive sample.

BSE SCREENING TEST - SCHEME RB24



IN CONCLUSION

BETAPRION BSE EIA TEST KIT

- ☐ Only one participant used this test as alternative test.
- ☐ No statistical evaluation can be performed.



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BSE SCREENING TEST - SCHEME RB24

IN CONCLUSION

Qualitative results (-, +, ++, +++)

- The **general reproducibility** (inter-rater agreement) of the dichotomous results (negative/positive) obtained by laboratories **with routine tests is perfect: Cohen's kappa is 1.**
- The **reproducibility for categorical results** (-, +, ++, +++) is very good, excellent: Cohen's kappa is 0.9121 (95%CI: 0.8794 - 0.9448).



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2024 round of TSE EURL EQAs: Results feedback

SCRAPIE SCREENING TEST SCHEME RS24



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NRLs participating in Proficiency Testing 2024

SCRAPIE SCREENING TEST SCHEME RS24

Austria
Belgium
Bulgaria
Croatia
Cyprus
Czech Republic
Estonia
Finland



France
Germany
Greece
Hungary
Iceland
Italy
Latvia

Lithuania
Malta
Netherlands
North Macedonia*
Norway
Poland

Portugal
Romania
Slovakia
Slovenia
Spain
Sweden
Switzerland
United Kingdom*

29 NRLs

*Countries extra UE



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SCRAPIE SCREENING TEST

SCHEME RS24

Sample No	Result
RS2401	TSE negative ovine tissue homogenate
RS2402	TSE negative ovine tissue homogenate
RS2403	TSE negative ovine tissue homogenate
RS2404	Classical Scrapie ovine tissue homogenate (Dilution 1:10)
RS2405	TSE negative ovine tissue homogenate
RS2406	Classical Scrapie caprine tissue homogenate (Dilution 1:4)
RS2407	TSE negative ovine tissue homogenate



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Results Tabulation for Scrapie Screening Test

Scheme: RS24

Primary Tests	No. NRLs
TeSeE SAP COMBI (Bio-Rad)	11
HerdChek BSE/Scrapie Ag - short protocol (IDEXX)	11
HerdChek BSE/Scrapie Ag – ultrashort protocol (IDEXX)	6



Results Tabulation for Scrapie Screening Test

Scheme: RS24

First alternative Tests	No. NRLs
TeSeE SAP COMBI (Bio-Rad)	3
HerdChek BSE/Scrapie Ag - short protocol (IDEXX)	1
Second alternative Tests	No. NRLs
HerdChek BSE/Scrapie Ag - ultrashort protocol (IDEXX)	1



Results Tabulation for Scrapie Screening Test Scheme: RS24



HERDCHEK BSE/SCRAPIE Ag (IDEXX) - Batch

AY185

BY445

CY816

EY700

HY363



TeSeE SAP COMBI (Bio-Rad) - Batch

4C0093

4J0094

4H0095



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Scrapie

Screening Test

Scheme RS24

**ALL THE LABS PASSED THIS
PROFICIENCY TESTING AT THE
FIRST ROUND**

Well done!!!



SCRAPIE

Screening Test

Scheme RS24

**Statistical
analysis**

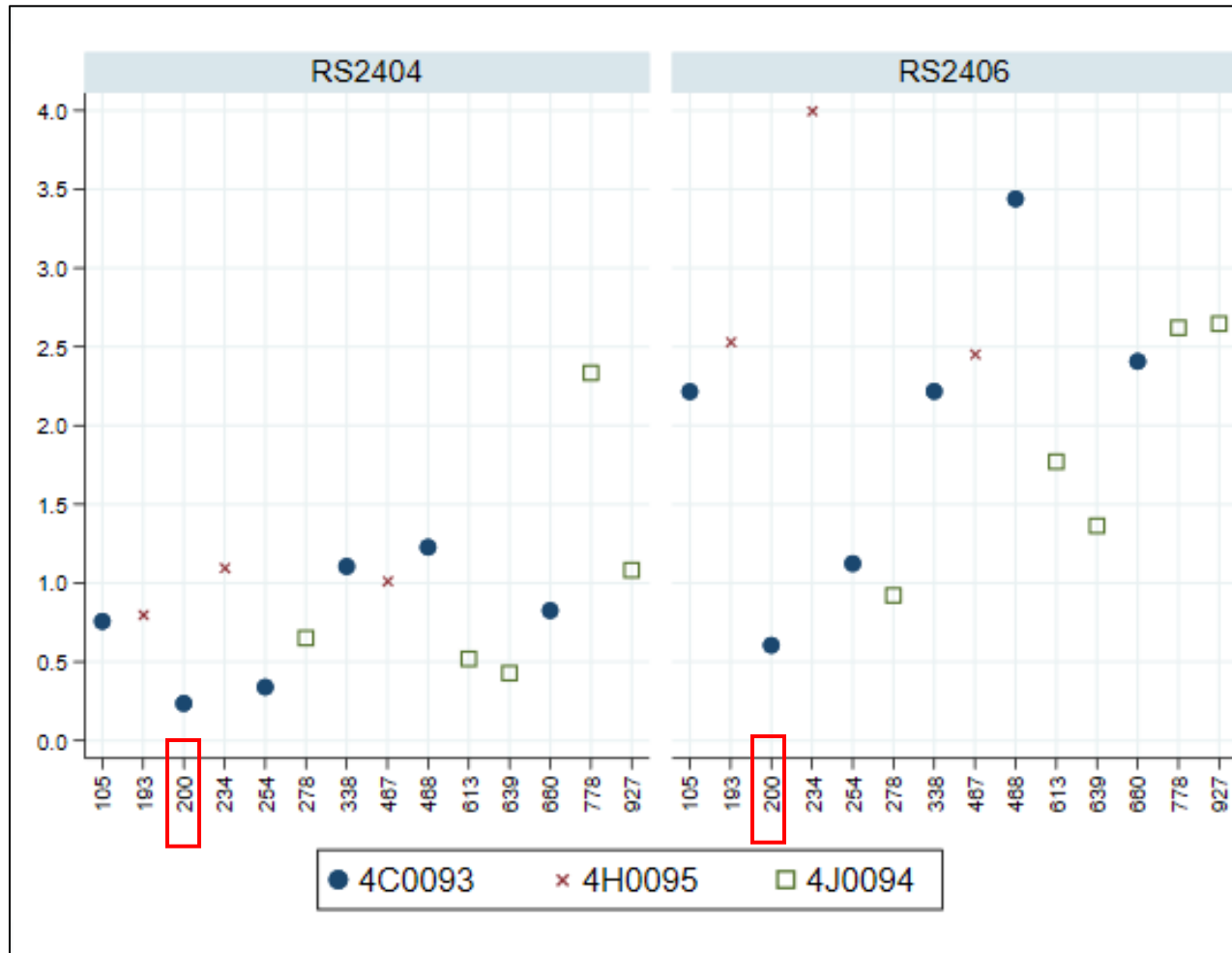


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SCRAPIE SCREENING TEST - SCHEME RS24

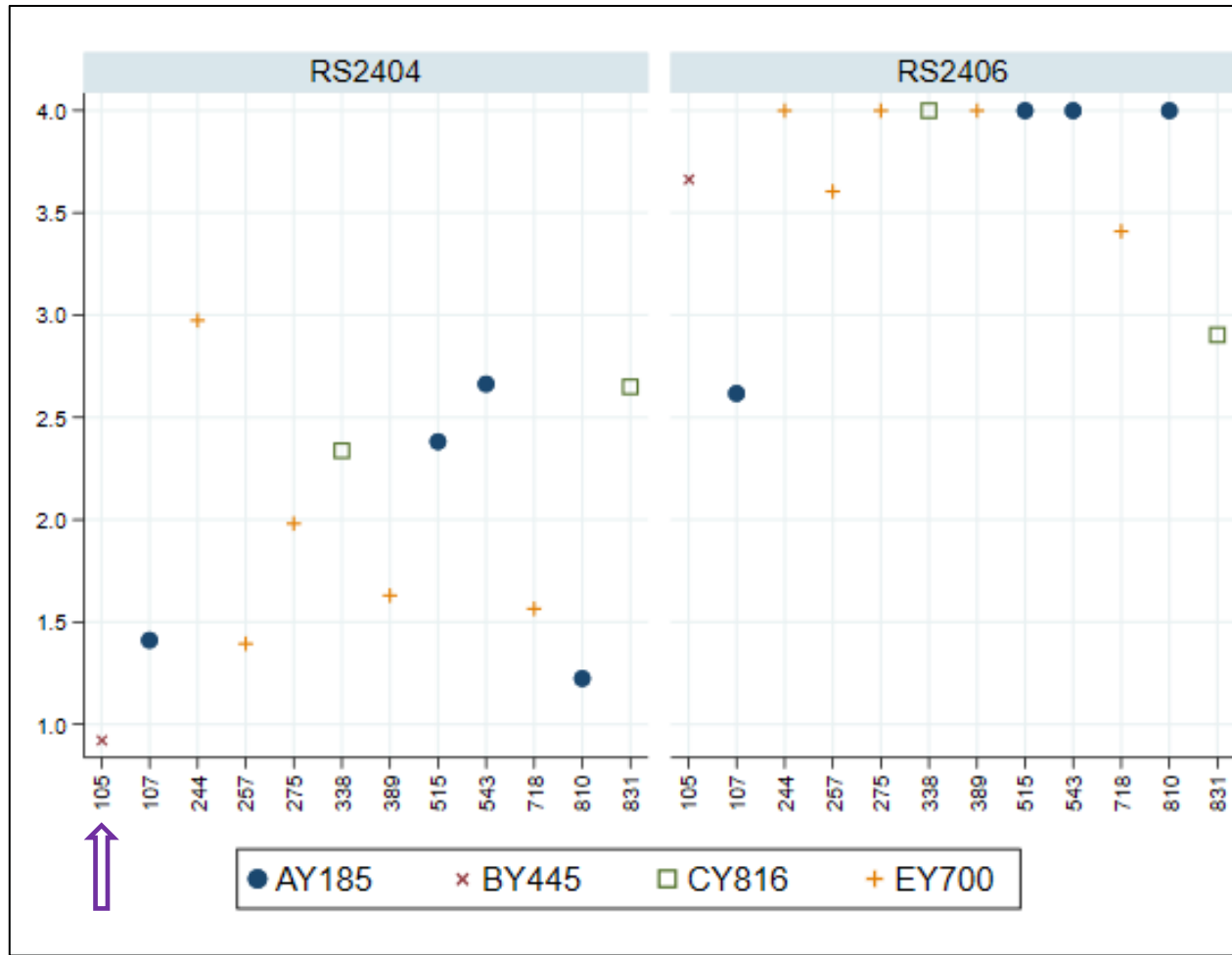
TeSeE SAP COMBI: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample



Lab 200 (batch: 4C0093) reported lower OD values than others for both positive samples

SCRAPIE SCREENING TEST - SCHEME RS24

HerdChek BSE-Scrapie Antigen Test-SHORT PROTOCOL: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample

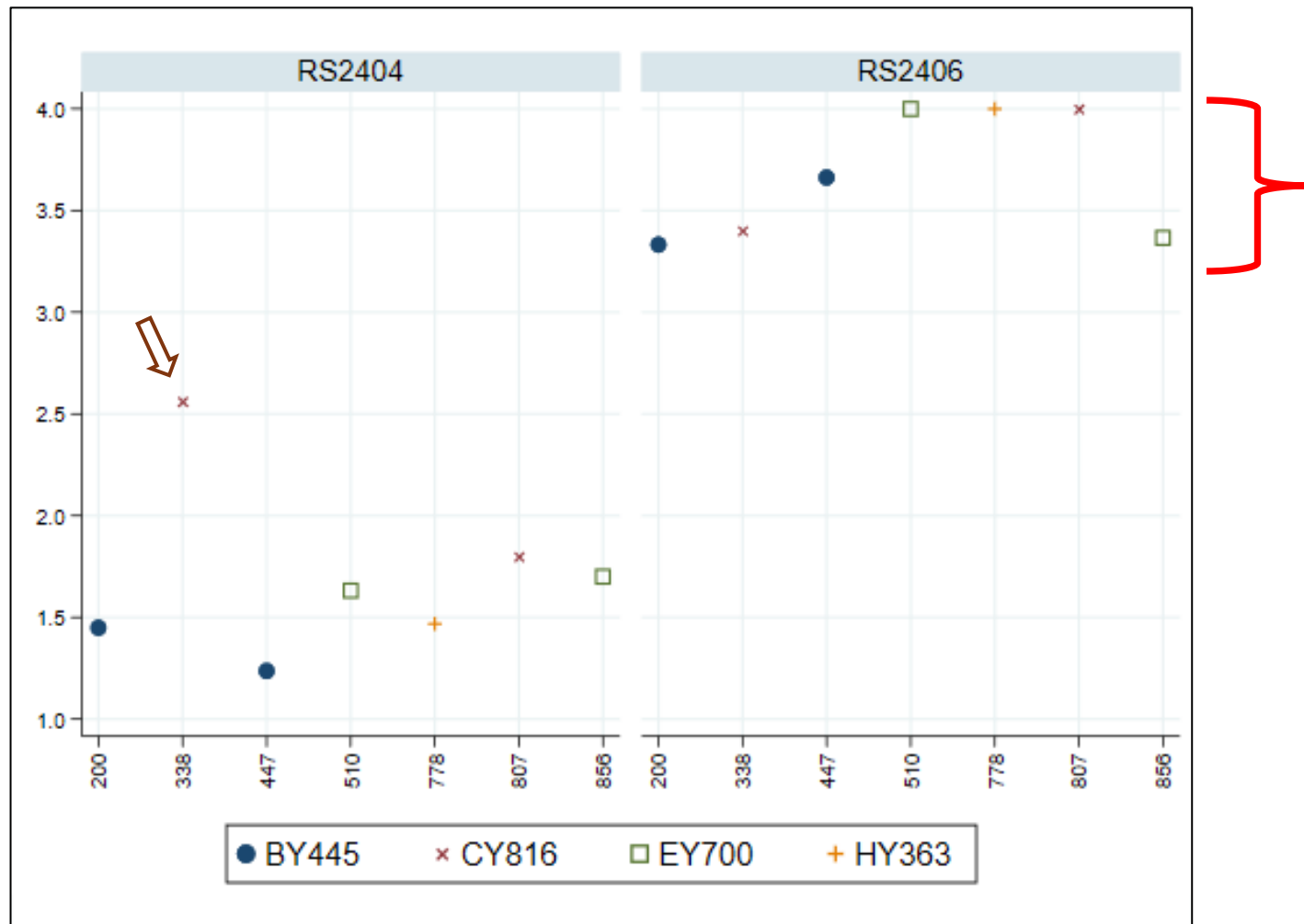


Many labs reported overflow values (>3.999) for RS2406

Participant 105 reported the lowest OD (OD < 1) for RS2404

SCRAPIE SCREENING TEST - SCHEME RS24

HerdChek BSE-Scrapie Antigen Test - ULTRASHORT PROTOCOL: average ODs of 1st and 2nd run reported from the Labs for each batch applied and positive sample



SCRAPIE SCREENING TEST - SCHEME RS24

IN CONCLUSION

TeSeE SAP COMBI

- It was the test first most used in routine: 39% (11/28) of the laboratories applied this test in routine.
- Three labs applied this test as alternative test.
- No statistically significant differences between participants and batches for both positive samples (RS2404, RS2406).



SCRAPIE SCREENING TEST - SCHEME RS24

IN CONCLUSION

IDEXX HerdChek BSE-Scrapie Antigen Test- SHORT PROTOCOL

- It was the first test most used in routine: 39% (11/28) of the laboratories applied this test in routine.
- One lab applied this test as alternative test.
- No statistically significant differences between participants for both positive samples (RS2404, RS2406).
- No statistically significant differences between batches for RS2404 and RS2406 samples.



SCRAPIE SCREENING TEST - SCHEME RS24

IN CONCLUSION

IDEXX HERDCHEK BSE-SCRAPIE ANTIGEN TEST-ULTRASHORT PROTOCOL

- Six laboratories, 21% (6/28) of participants, applied it in routine.
- One lab applied this test as alternative test.
- No statistically significant differences between participants among participants (RS2404, RS2406).
- No statistically significant differences between batches for both positive samples (RS2404, RS2406).



SCRAPIE SCREENING TEST - SCHEME RS24

IN CONCLUSION

Qualitative results (-, +, ++, +++)

- The **general reproducibility** (inter-rater agreement) of the dichotomous results (negative/positive) obtained by laboratories with routine tests is perfect: **Cohen's kappa is 1**.
- The reproducibility for categorical results (-, +, ++, +++) is very good: Cohen's kappa is **0.9006** (95%CI: 0.8060 - 0.9952).
- A slight increase of the level of the reproducibility from the previous PT: in 2023 it was substantial, fair to good (Cohen's kappa was **0.8088** (95%CI: 0.6115 - 1.0000)).





22nd Annual Meeting of the TSE EURL
Turin 12th – 13th May 2025

BSE CONFIRMATORY IMMUNOBLOT SCHEME WB24



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NRLs participating in Proficiency Testing 2024

BSE CONFIRMATORY IMMUNOBLOT SCHEME WB24

Austria
Belgium
Bulgaria
Croatia
Czech Republic
Finland

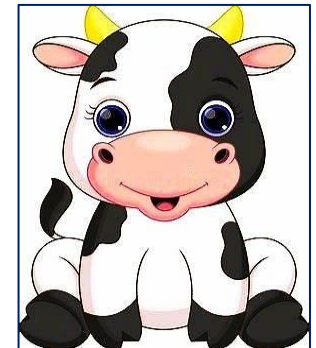


Romania
Slovakia
Spain
Sweden
Switzerland
United Kingdom

France
Germany
Greece
Hungary
Ireland

Italy
Netherlands
Norway
Poland
Portugal

22 NRLs including United Kingdom



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BSE CONFIRMATORY IMMUNOBLOT SCHEME WB24

Sample No	Result
WB2401	negative bovine tissue homogenate
WB2402	negative bovine tissue homogenate
WB2403	C-positive bovine tissue homogenate
WB2404	negative bovine tissue homogenate
WB2405	negative bovine tissue homogenate



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Results Tabulation for BSE Confirmatory Immunoblotting Scheme WB24

Primary Tests	No. NRLs
TeSeE Western Blot - Bio-Rad	20
In house SAF method	1
OIE SAF immunoblot	1

Lab 792 used 1st alternative test – TeSeE WB (Bio-Rad)



Results Tabulation for BSE Confirmatory Immunoblotting Scheme WB24

TeSeE Western Blot Bio-Rad used four batches

3J0053 (7 labs)

4J0055 (6 labs)

3M0054 (4 labs)

3G0129 (1 lab)



No information from 092 and 748 labs



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BSE Confirmatory Immunoblotting Scheme WB24

**ALL THE LABS PASSED
THIS PROFICIENCY
TESTING AT THE FIRST
ROUND**

EXCEPT.....



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BSE Confirmatory Immunoblotting – Scheme WB24



Sample No	Result	Lab 239	Lab 633
WB2401	negative bovine tissue homogenate	negative	positive
WB2402	negative bovine tissue homogenate	positive	negative
WB2403	C-positive bovine tissue homogenate	negative	positive
WB2404	negative bovine tissue homogenate	negative	negative
WB2405	negative bovine tissue homogenate	negative	negative

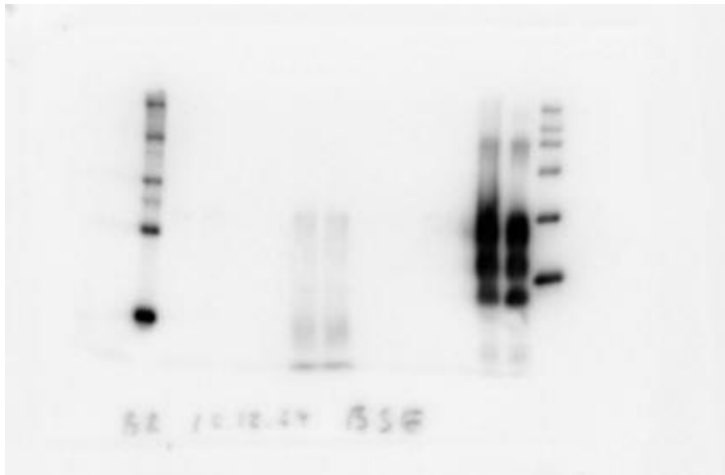


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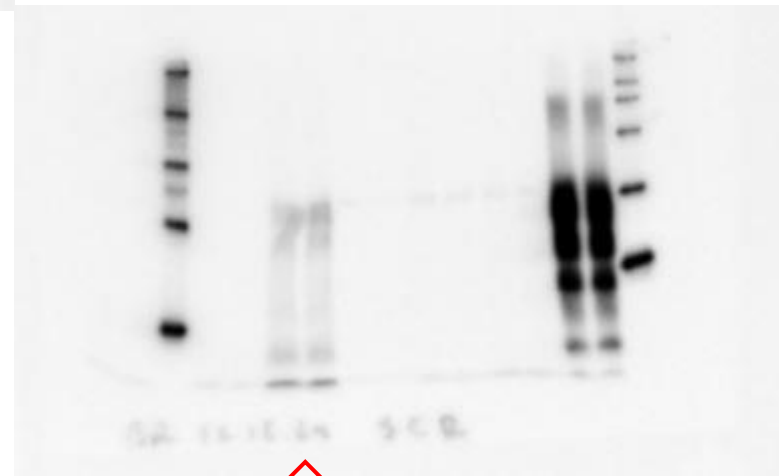
BSE Confirmatory Immunoblotting – Scheme WB24

Lab 239



The submitted results were incorrect because the sample readings were taken on the inverted wb images for the two PTs

WB2403



WS2402



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BSE Confirmatory Immunoblotting – Scheme WB24



Lab 633

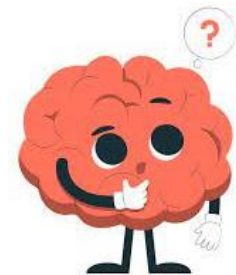
Very weak PrP^{Sc} signals for sample WB2403 but no signals present in this image for sample WB2401

problems linked to the image analyzer



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BSE Confirmatory Immunoblotting – Scheme WB24



**Lab 239 passed the
second round**



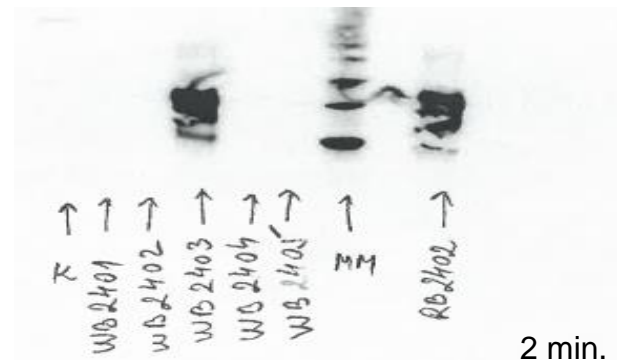
**Lab 633 failed this PT
In the first and
second round**



BSE Confirmatory Immunoblotting – Scheme WB24

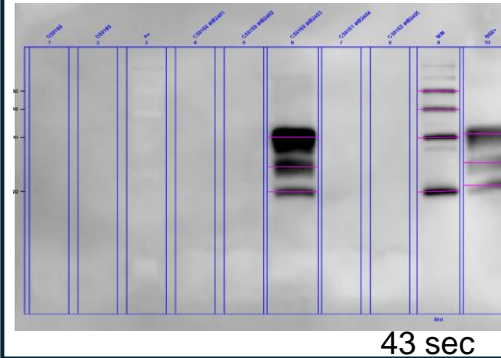
Blot quality

Lab 991

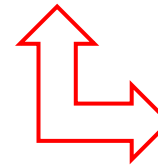


- Non-optimal blotting
- Non-optimal blot coverage with antibodies or chem. sub

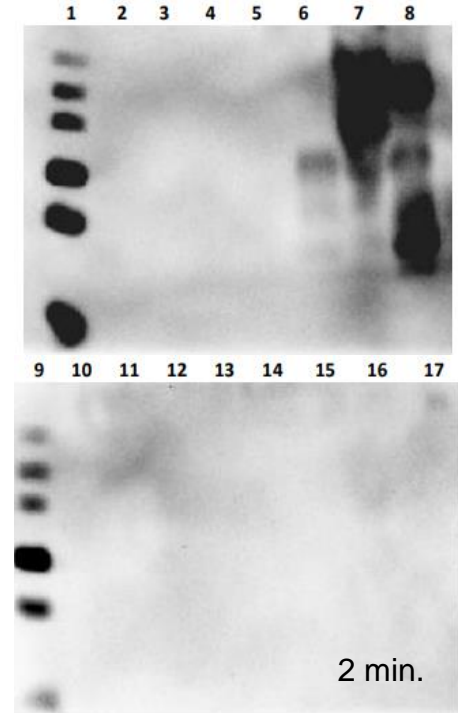
Lab 052



High background



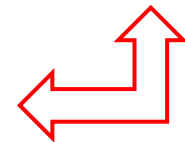
Lab 648



Lab 717



Non-optimal digestion

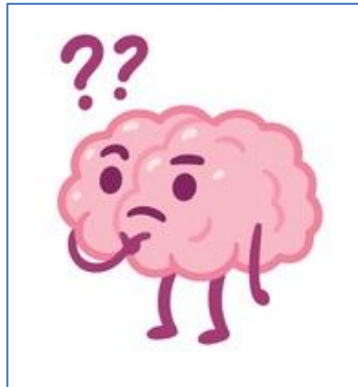


Critical issues



- Lab 450 submitted the results after the deadline and so it is not included in this report.
- Lab 605 entered the incorrect ID code instead of the correct code 196.
- Lab 052 did not provide information on the gel used for this PT.
- Labs 092 and 748 did not report any information on the kit and gel used for this PT.
- Lab 117 did not use any control sample for the analysis.

BSE Confirmatory Immunoblotting Scheme WB24





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Turin 12th – 13th May 2025

SCRAPIE CONFIRMATORY IMMUNOBLOT SCHEME WS24



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NRLs participating in Proficiency Testing 2024

SCRAPIE CONFIRMATORY IMMUNOBLOT SCHEME WS24

Austria
Belgium
Bulgaria
Croatia
Czech Republic
Finland



France
Germany
Greece
Hungary
Iceland

Ireland
Italy
Netherlands
Norway
Poland

Portugal
Romania
Slovakia
Spain
Sweden
Switzerland
United Kingdom

23 NRLs including United Kingdom



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SCRAPIE CONFIRMATORY IMMUNOBLOTTING SCHEME WS24

Sample No	Result
WS2401	negative ovine tissue homogenate
WS2402	C-Scrapie ovine tissue homogenate
WS2403	negative ovine tissue homogenate
WS2404	negative ovine tissue homogenate
WS2405	negative ovine tissue homogenate



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Results Tabulation for Scrapie Confirmatory Immunoblotting

Scheme: WS24

Primary Tests	No. NRLs
TeSeE Western Blot - Bio-Rad	20
In house SAF method	1
OIE SAF immunoblot	1
APHA Bio-Rad TeSeE-based Hybrid Western Blot Method	1

Lab 274 used 1st alternative test – TeSeE WB (Bio-Rad)



Results Tabulation for Scrapie Confirmatory Immunoblotting Scheme WS24

TeSeE Western Blot Bio-Rad used four batches

3J0053 (7 labs)

4J0055 (7 labs)

3M0054 (3 labs)

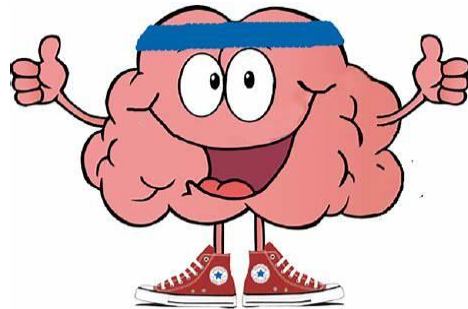
2K0047 (1 lab)



No information from 454 and 674 labs



Scrapie Confirmatory Immunoblotting Scheme WS24



**ALL THE LABS PASSED THIS
PROFICIENCY TESTING AT
THE FIRST ROUND**



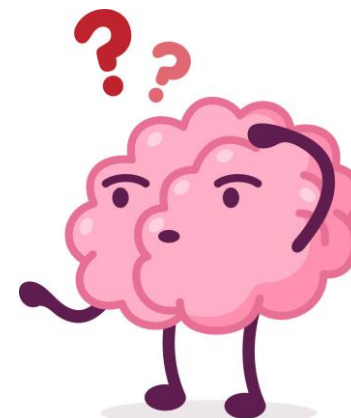
EXCEPT.....



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SCRAPIE Confirmatory

Immunoblotting – Scheme WS24



Labs 034 and 509 – incorrect results

Sample No	Result	Lab 034	Lab 509
WS2401	negative ovine tissue homogenate	negative	positive
WS2402	C-Scrapie ovine tissue homogenate	negative	positive
WS2403	negative ovine tissue homogenate	positive	negative
WS2404	negative bovine tissue homogenate	negative	positive
WS2405	negative bovine tissue homogenate	negative	negative

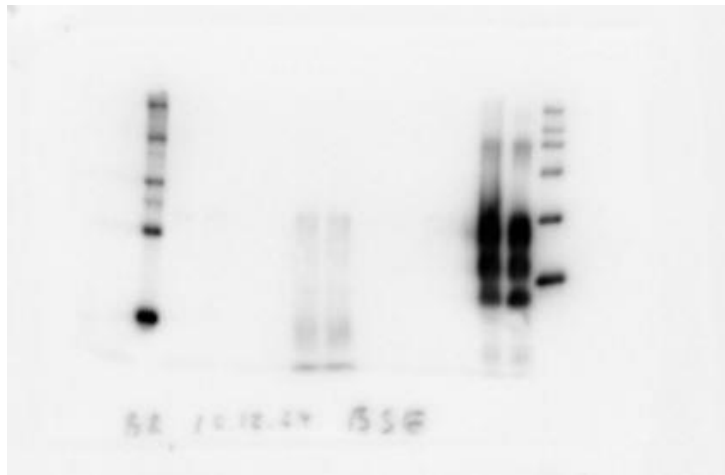


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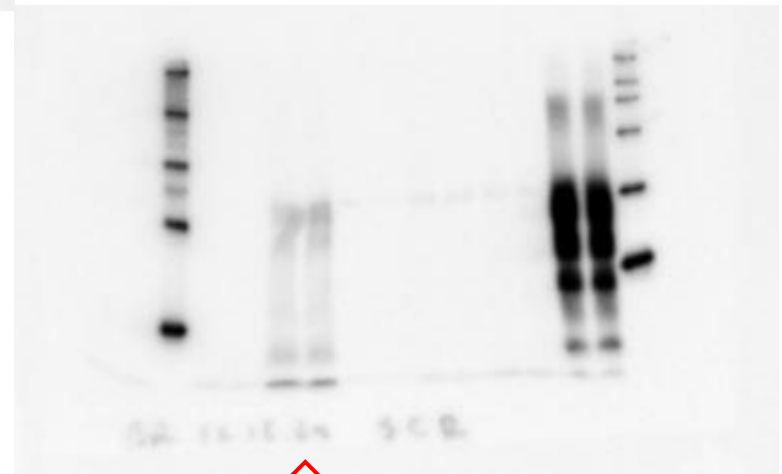
Scrapie Confirmatory Immunoblotting – Scheme WS24

Lab 034



The submitted results were incorrect because the sample readings were taken on the inverted wb images for the two PTs

WB2403



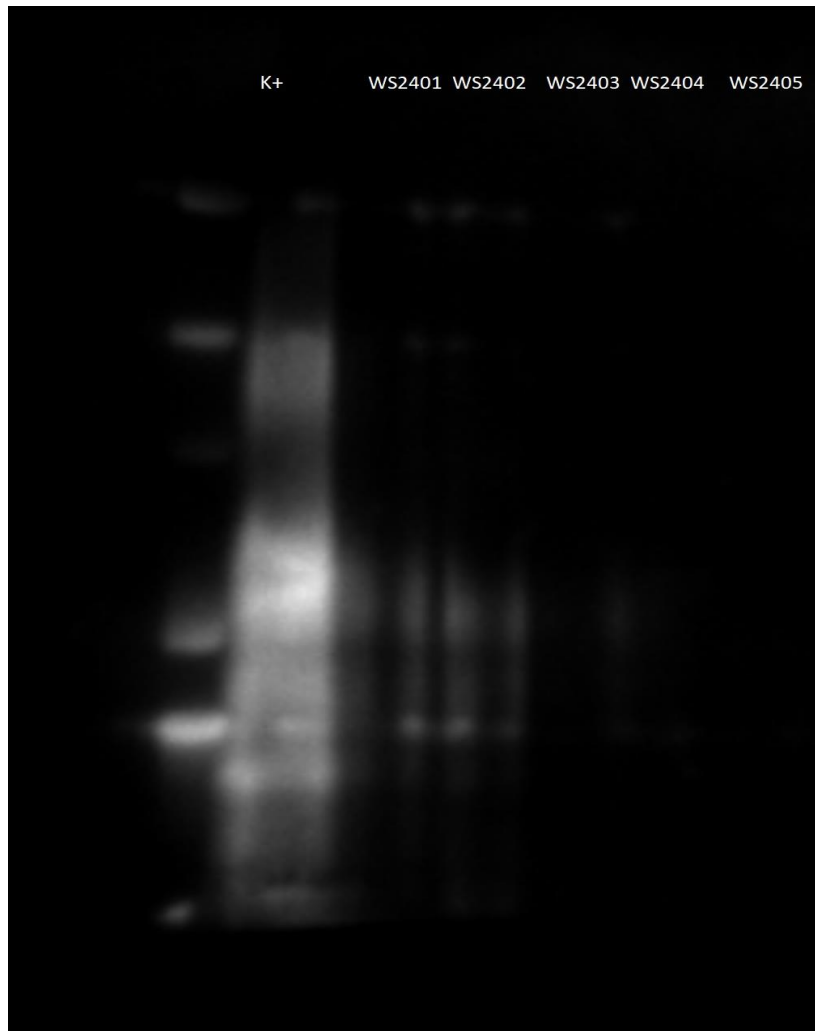
WS2402



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Scrapie Confirmatory Immunoblotting – Scheme WS24



Lab 509

Possible diffusion of the positive control into the two neighbouring wells corresponding to samples WS2401 and WS2402.

No PrP^{Sc} signals are present in the WS2404 sample interpreted as positive.



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Scrapie Confirmatory Immunoblotting – Scheme WS24



WELL DONE!!!

Labs 034 and
509 failed the
first round but
they passed the
second round

BSE Confirmatory Immunoblotting – Scheme WS24

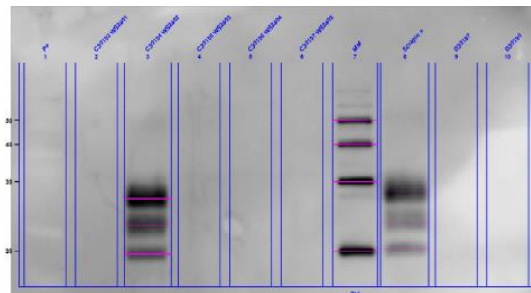
Blot quality

Lab 504

5 min.

↑ ↑ ↑ ↑ ↑ ↑ ↑
K WS2401 WS2402 WS2403 WS2404 WS2405 MM

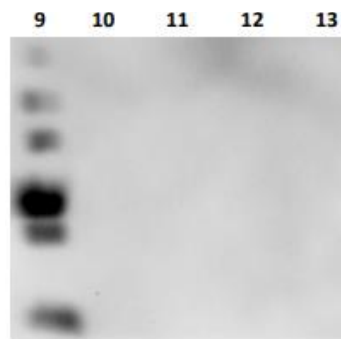
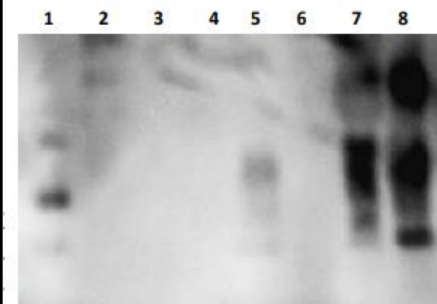
Lab 453



43 sec

High background

Lab 673



2 min.

- Non-optimal blotting
- Non-optimal blot coverage with antibodies or chem. sub

Critical issues



- Lab 414 submitted the results after the deadline, so they are not included in this report.
- Lab 164 entered the incorrect ID code instead of the correct code 251.
- Labs 674 and 454 did not provide any information on the kit and gel used for this PT.
- Lab 453 did not report any information on the gel used for this PT.
- Lab 504 did not use any control sample for the analysis.

Scrapie Confirmatory Immunoblotting – Scheme WS24



The forthcoming EQA 2025 rounds

Rapid tests and confirmatory immunoblotting for BSE and Scrapie



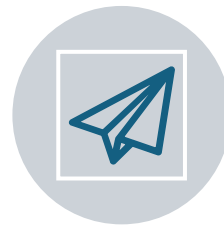
The shipment of the samples is scheduled by half July 2025



RB, RS, WB and WS will be sent all at once



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



PTs results

**The results of the PTs round 2025 will be
reported at the next TSE EURL Meeting**



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The forthcoming EQA 2025 rounds

Rapid tests and confirmatory immunoblotting for BSE and Scrapie

- The results will be submitted by EURL web site
- **Report the correct ID code associated with the laboratory reference person**
- **The submission of results is expected by the deadline**
- **The results submitted after the deadline will not be evaluated**
- **Please include in the submission the raw data (pictures of the blots, readings, time exposure of pictures) and all the information about the kit batches used for the analysis**
- Report any information considered important for the correct interpretation of the results



The forthcoming EQA 2025 rounds

Rapid tests and confirmatory immunoblotting for BSE and Scrapie

SUBMISSION RESULTS ON WEB SITE

- Upload results for each applied test to the Eurl website
- Fill in all fields correctly
- Always indicate if the test used by the laboratory is the one routinely applied or as a first or second alternative test



Thank you!!!

- Elena Bozzetta
- Francesco Ingravalle
- Maria Cavarretta
- Daniela Loprevite

Grazie!!!



**Thank you
for your
attention!!!**

