

19th Annual Meeting of the TSE EURL Torino 17th-18th October 2022

Rapid Tests EQA (RB & RS): results of the 2021 round and forthcoming 2022 round

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Results of the 2021 EQA round

BSE



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Results of the 2021 EQA round

BSE

25 Participating NRLs:

Austria

Belgium

Bulgaria

Croatia

Cyprus

Czech Republic

Estonia

Finland

France

Germany

Greece

Hungary

Italy

Latvia

Lithuania

Netherlands

Norway

Poland

Portugal

Romania

Slovakia

Slovenia

Spain

Sweden

Switzerland



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Methods of the 2021 EQA round

BSE

Samples included in the PT

Sample ID	Gold Standard	Dilution	Bovine Tissue Homogenate
RB2101	NEG	.	NEGATIVE
RB2102	NEG	.	NEGATIVE
RB2103	POS	1:4	POSITIVE
RB2104	NEG	.	NEGATIVE
RB2105	NEG	.	NEGATIVE
RB2106	NEG	.	NEGATIVE
RB2107	NEG	.	NEGATIVE



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Methods of the 2021 EQA round - BSE

During preparation the samples were tested using:

TeSeE SAP Combi Kit

Batch 1H0083 EXP 24/06/2022

1/4	D.O.	2.592	2.671
1/8	D.O.	1.893	1.938
1/16	D.O.	0.591	0.615

CUT OFF 0.221

Idexx ultrashort protocol

Batch HT620 EXP 11/08/2022

1/4	D.O.	3.209	3.367
1/8	D.O.	3.244	3.355
1/16	D.O.	3.135	3.170

CUT OFF 0.155

Distribution date: 13-14 December 2021

Deadline 07 February 2022



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All labs but one have passed
this PT round.

Lab 655 failed the first round
of the PT but passed the
second one



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Results of the 2021 round of EQA-BSE

BioRad-Sap Combi: 15 of the participating labs

Three batches used:

Batch #	# of NRL per batch
1C0081	3
1F0082	5
1H0083	7

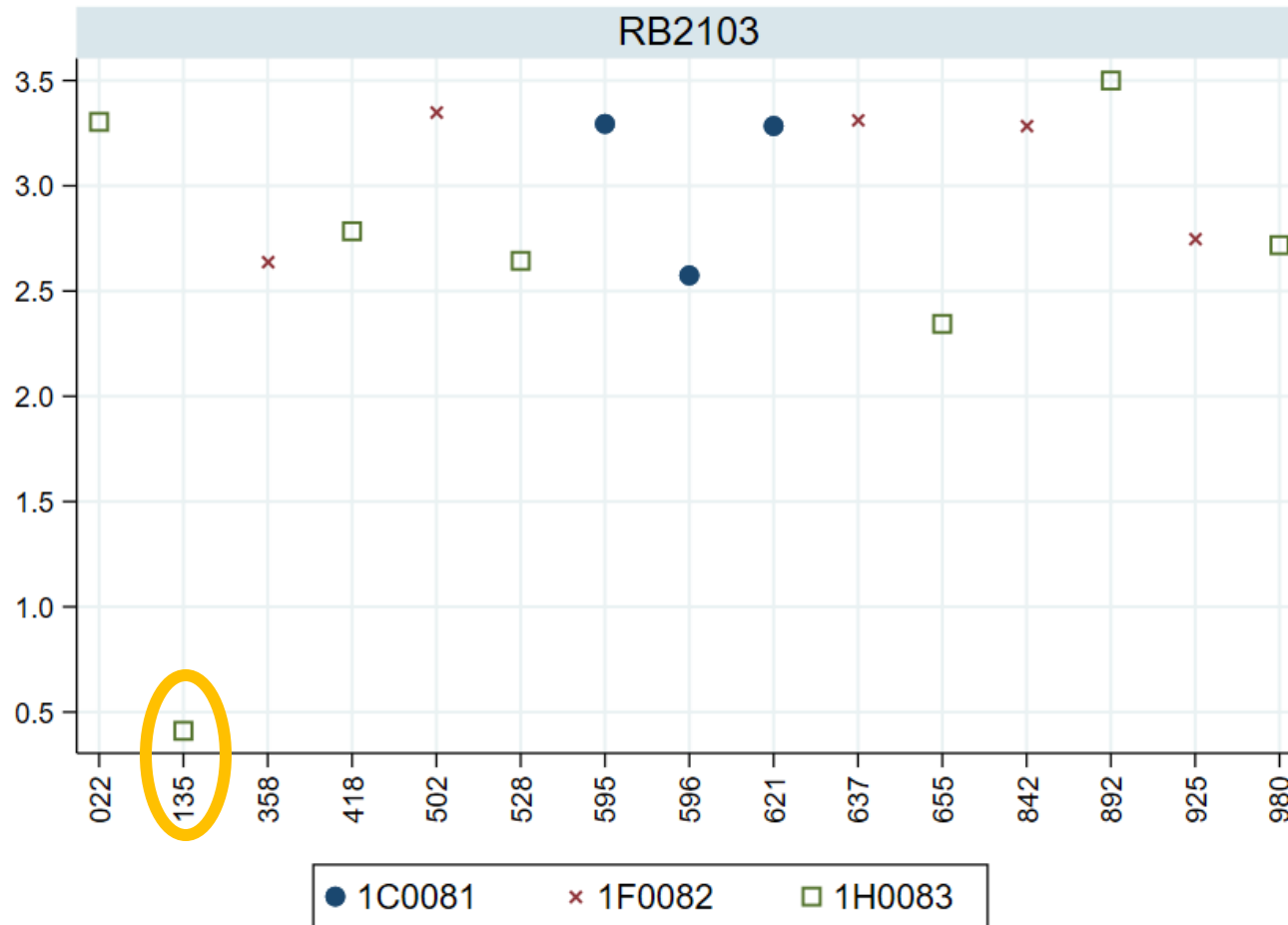


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Results of the 2021 round of EQA-BSE

OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA-BSE

IdexxShort: 10 of the participating labs

Four batches used:

Batch #	# of NRL per batch
AT053	2
CT465	1
GT044	1
HT640	6

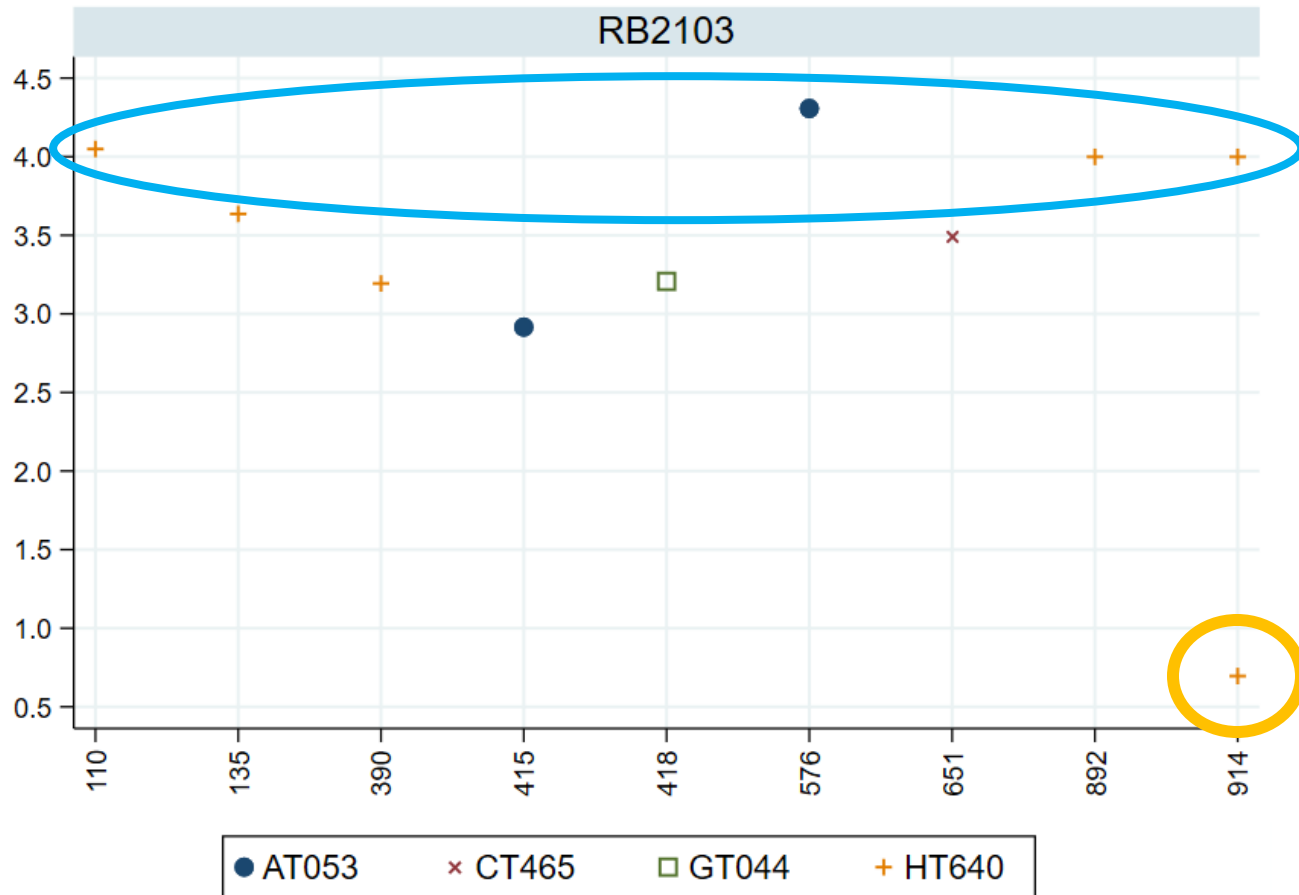


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Results of the 2021 round of EQA-BSE

OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA-BSE

IdexxUltrashort: 7 of the participating labs

Three batches used:

Batch #	# of NRL per batch
CT465	1
GT044	3
HT640	3

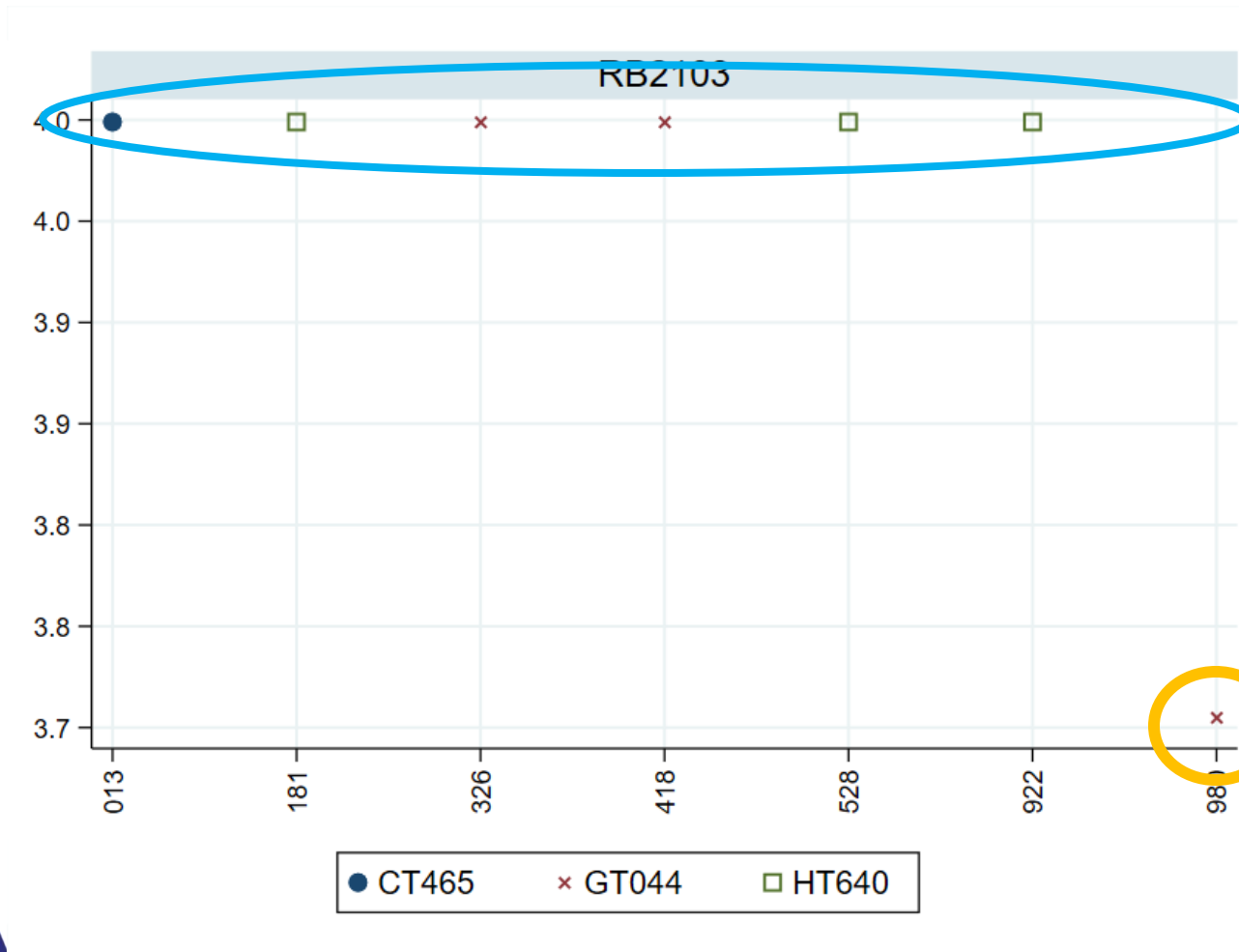


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Results of the 2021 round of EQA-BSE

OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA-BSE

BETAPRION BSE: one of the participating labs

One batch used:

Batch #	# of NRL per batch
0321-01	1

PRIOSTRIP BSE: one of the participating labs

One batch used:

Batch #	# of NRL per batch
S200101G	1



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Results of the 2021 round of EQA-BSE

THERMOFISHER PRIOWESTERN: two of the participating labs

Two batches used:

Batch #	# of NRL per batch
W200101G	1
W200201G	1



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SHORT CONCLUSIONS: PT BSE

BIORAD-SAP COMBI

- It is the first most used test in routine: 48% (12/25) of the laboratories applied BioRad-Sap Combi test in routine. Three labs applied this test as alternative test;
- 135 (batch 1H0083) reported OD lower than other labs for the only positive sample RB2103 (average OD between 1st and 2nd run = 0.411)
- No statistical significant differences among participants for the only positive sample RB2103: $p = 0.4478$;
- No statistical significant differences among batches for the only positive sample RB2103: $p = 0.4489$.



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SHORT CONCLUSIONS: PT BSE

IDEXXSHORT

- It was the second most used test in routine: 28% (7/25) of the laboratories applied IdexxShort test in routine. Three labs applied this test as alternative test;
- Many labs (ID: 110, 576, 892, 914) reported overflow values (>3.999);
- Lab 914 (batch HT640) reported OD lower than other labs for the only positive sample RB2103 (average OD between 1st and 2nd run = 0.696);
- No statistical significant differences among participants for the positive sample
No statistical significant differences among batches for the positive sample;

IDEXXULTRASHORT

- It was the last test used in routine: 24% (6/25) of the laboratories applied IdexxUltraShort test in routine. One lab applied this test as alternative test;
- Six labs out of 7 participants that applied IdexxUltraShort (ID: 013, 181, 326, 418, 528, 922) reported overflow values (>3.999);
- No statistical significant differences among participants for the positive sample
- No statistical significant differences among batches for the only positive sample



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SHORT CONCLUSIONS: PT BSE

THERMOFISHER PRIOWESTERN

- Only 2 labs apply this test as alternative test;
- No statistically significant ($p < 0.05$) differences among participants.

PRIOSTRIP BSE

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

BETAPRION BSE

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



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Results of the 2021 EQA round

Scrapie



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Results of the 2021 EQA round- Scrapie

26 Participating NRL:

Austria

Belgium

Bulgaria

Croatia

Cyprus

Czech Republic

Estonia

Finland

France

Germany

Greece

Hungary

Iceland

Italy

Latvia

Lithuania

Netherlands

Norway

Poland

Portugal

Romania

Slovakia

Slovenia

Spain

Sweden

Switzerland



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Methods of the 2021 round of EQA-Scrapie

Samples included in the PT

Sample ID	Gold Standard	Dilution	Ovine Tissue Homogenate
RS2101	NEG	.	TSE Negative
RS2102	NEG	.	TSE Negative
RS2103	NEG	.	TSE Negative
RS2104	POS	1:10	Classical SCRAPIE POSITIVE
RS2105	NEG	.	TSE Negative
RS2106	NEG	.	TSE Negative
RS2107	POS	1:10	Classical SCRAPIE POSITIVE



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Methods of the 2021 round of EQA-Scrapie

During preparation the samples were tested using:

TeSeE SAP Combi Kit

batch 1H0083 EXP 24/06/2022

1/10 D.O. 1.549 1.539

1/20 D.O. 1.032 1.014

1/40 D.O. 0.337 0.290

CUT-OFF 0.221

Idexx Ultrashort

Batch HT620 EXP 11/08/2022

1/10 D.O. 3.257 3.408

1/20 D.O. 3.259 3.321

1/40 D.O. 3.121 3.220

CUT-OFF 0.209

Distribution date: 13-14 Dec 2021

Deadline 07 February 2022



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All labs but one have passed
the PT round.

Lab 424 failed the first round
of the PT but passed the
second one



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Results of the 2021 round of EQA- Scrapie

TeSeE SAP Combi Kit : 16 of the participating labs

Three batches used:

Batch #	# of NRL per batch
1C0081	3
1F0082	6
1H0083	7

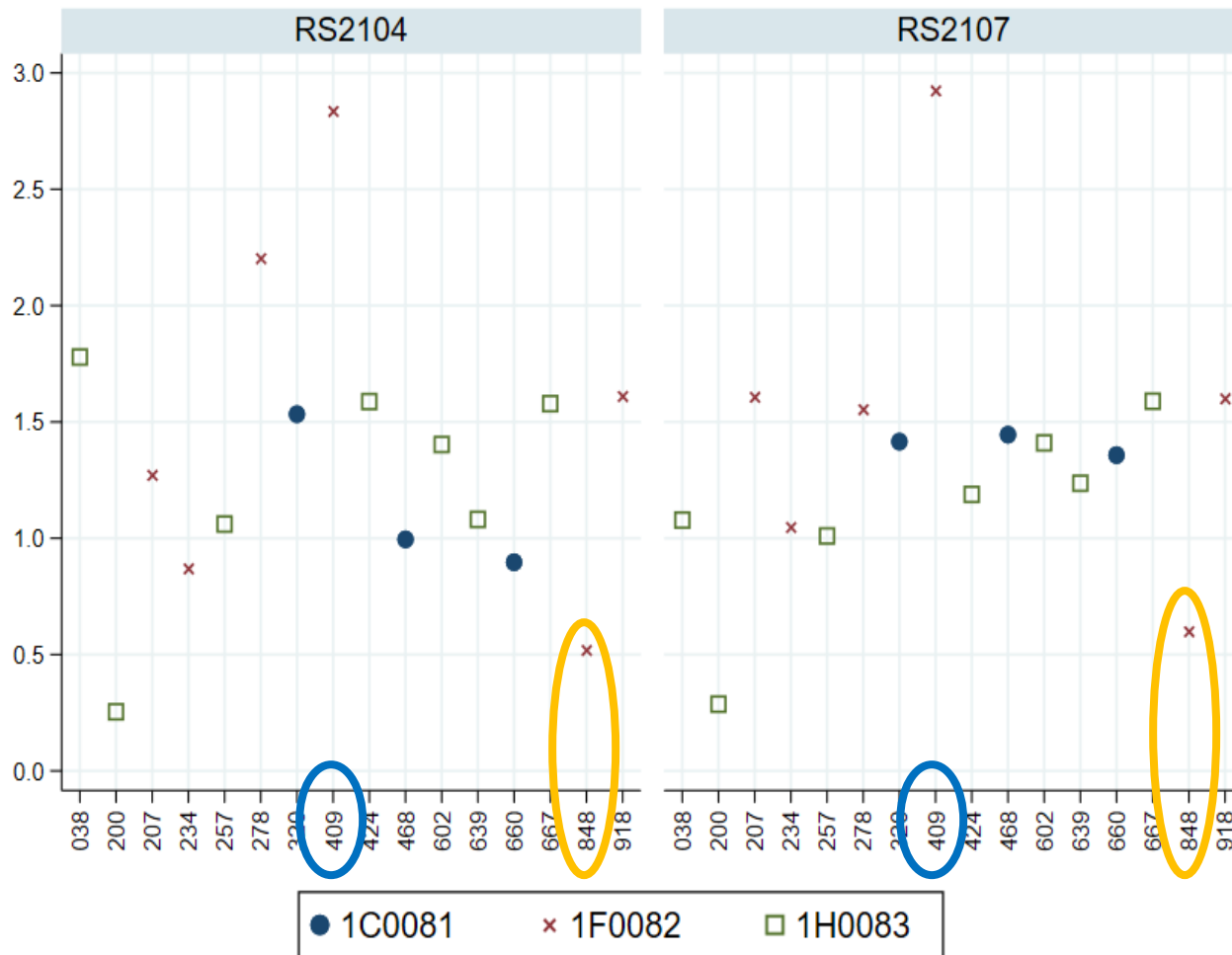


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Results of the 2021 round of EQA- Scrapie

TeSeE SAP Combi Kit :OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA- Scrapie

IDEXXSHORT: 12 of the participating labs

Four batches used:

Batch #	# of NRL per batch
AT053	2
CT465	2
GT044	1
HT640	7

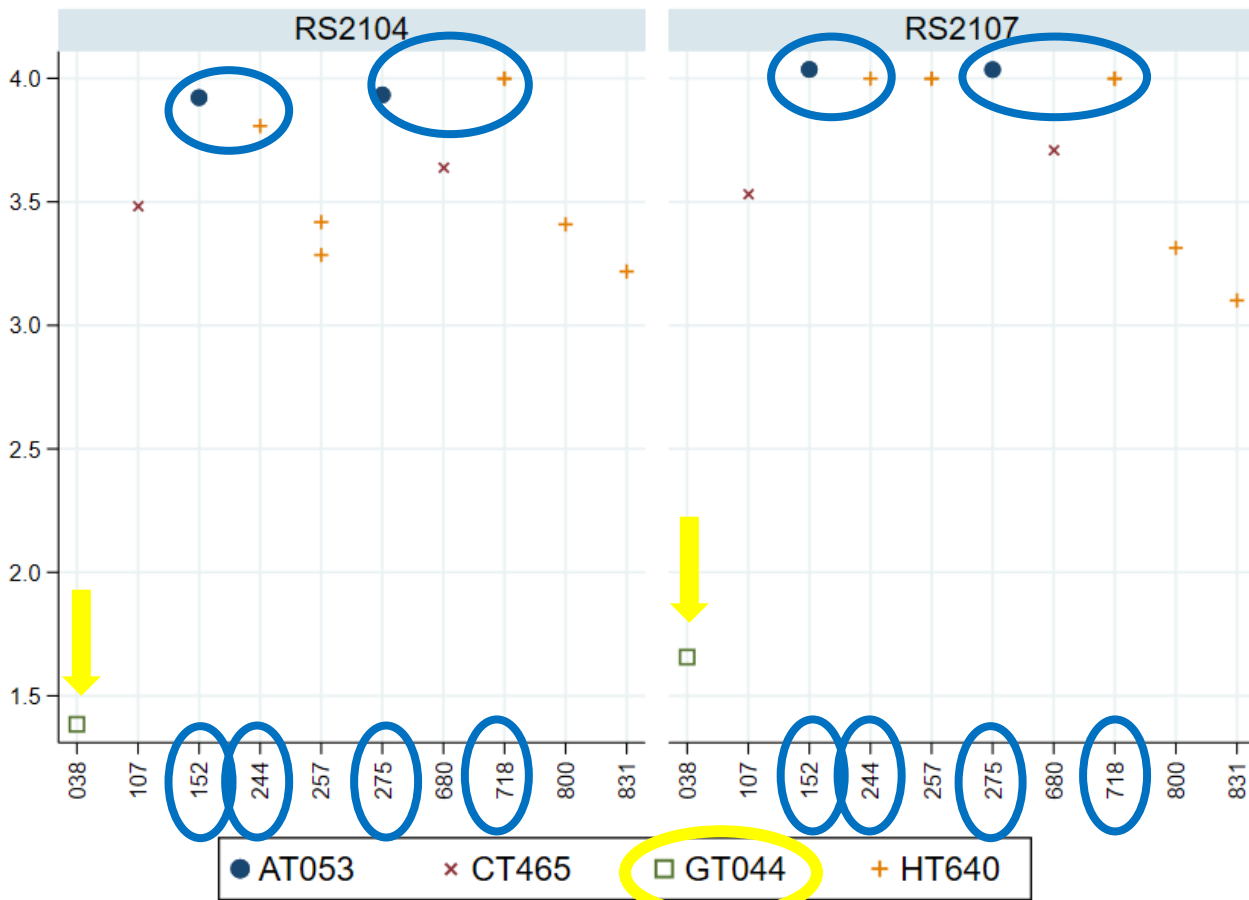


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Results of the 2021 round of EQA- Scrapie

IDEXXSHORT: OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA- Scrapie

IdexxUltrashort: 6 of the participating labs

Two batches used:

Batch #	# of NRL per batch
GT044	3
HT640	3



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Results of the 2021 round of EQA- Scrapie

IdexxUltrashort: OD reported from the Labs for each batch applied and positive sample



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Results of the 2021 round of EQA- Scrapie

BIORAD-SHEEP/GOAT: only 1 of the participating labs
(alternative)

Batch #	# of NRL per batch
1C0045/1K0046	1

PrioWESTERN-BSE-KIT: only 1 of the participating labs
(alternative)

Batch #	# of NRL per batch
W200201G	1



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SHORT CONCLUSIONS: PT SCRAPIE

TeSeE SAP Combi Kit

- It is the first most used test in routine: 54% (14/26) of the laboratories applies test in routine. Two labs apply this test as alternative test;
- Lab 409 (batch: 1F0082) reports ODs higher than others for the positive samples; labs 200 (batch: 1H0083) and 848 (batch: 1F0082) report ODs lower than others;
- No statistical significant differences among participants: $p = 0.4514$ for RS2104; $p = 0.4514$ for RS2107;
- No statistical significant differences among batches: $p = 0.6416$ for RS2104; $p = 0.2424$ for RS2107



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SHORT CONCLUSIONS: PT SCRAPIE

IDEXX SHORT

- It is the second most used test in routine: 31% (8/26) of the laboratories applies IdexxShort test in routine. Four labs apply this test as alternative test;
- Lab 152, 275 (batch AT053), 244 and 718 (batch: HT640) usually reported ODs higher than other labs. Many labs (2 for sample RS2104, 7 for sample RS2107) reported overflow values (>3.999);
- No statistical significant differences among participants: $p = 0.2891$ for RS2104; $p = 0.3321$ for RS2107;
- No statistical significant differences among batches: $p = 0.2940$ for RS2104; $p = 0.0789$, border-line, for RS2107.
- Batch GT044 showed lower ODs than other batches, but only one participant applied it.



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SHORT CONCLUSIONS: PT SCRAPIE

IDEXX ULTRASHORT

- Four laboratories, 15% (4/26) of participants, apply IdexxUltraShort test in routine. Two labs apply this test as alternative test;
- Lab 447 and 667 (batch: HT640) usually report ODs higher than other labs. Lab 896 (batch GT044) usually report lowest ODs;
- No statistical significant differences among participants: $p = 0.4159$ for RS2104; $p = 0.4159$ for RS2107;
- No statistical significant differences among batches for RS2104 ($p = 0.2752$) but border-line significant differences among batches for RS2107: $p = 0.0495$.
- Batch GT044 showed lower ODs than other batches.

BIORAD-SHEEP/GOAT and PrioWESTERN-BSE-KIT

- Only one participant uses this test as alternative test to routine test;
- No statistical evaluation can be performed.



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The forthcoming 2022 round of EQA Rapid Tests



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Proficiency Testing – 1

PARTICIPANTS

BSE

Austria	Italy
Belgium	Latvia
Bulgaria	Lithuania
Croatia	Netherlands
Cyprus	Norway
Czech Republic	Poland
Estonia	Portugal
Finland	Romania
France	Slovakia
Germany	Slovenia
Greece	Spain
Hungary	Sweden
	Switzerland

SCRAPIE

Austria	Italy
Belgium	Latvia
Bulgaria	Lithuania
Croatia	Netherlands
Cyprus	Norway
Czech Republic	Poland
Estonia	Portugal
Finland	Romania
FranceGermany	Slovakia
Greece	Slovenia
Hungary	Spain
Iceland	Sweden
	Switzerland



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Proficiency Testing - 2

- Homogenisation, dilution and creation of aliquots of brain tissue for preparation of Proficiency Samples;
- Analysis by Rapid Tests (IDEXX HerdChek BSE– Scrapie Ag Test Kit and Bio-Rad TeSeE SAP Combi Kit) and Western Blot;
- Interpretation of the results;
- Homogeneity and Stability assessment



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Proficiency Testing - 3

- Packaging and dispatch (dry ice - UN3373 – Import permit);
- Scrapie and BSE Rapid Test and Confirmatory Western Blot schemes: same package (delivery);
- Documenting of results: EURL web site should be available, more details will be provided;



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Proficiency Testing - 4

Timescale

- Dispatch of PT samples: before 15th November;
- Less time (3 weeks) to perform the PTs and send back the results to EURL than the last PT
- Deadline to perform the PTs and send back the results to EURL: 10 December 2022
- Results obtained twice by the same operator (on the same plate, i.e. the same day) will be required



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RAPID and CONFIRMATORY TESTS: **issues and news**

Elena Bozzetta
IZSPLVA Turin



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SOME ISSUES ON PTs

- Please complete all the fields of the forms supplied with the PT sets and include the raw data and WB images;
- there is some confusion on what «alternative test» means;
- for 2023 we will schedule PTs in different periods (the program will be available for the beginning of the year) in order to facilitate your activities (Rapid tests and confirmatory WBs expected for June-July 2023)



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NEWS: rapid test list

- Thermofisher withdrew from the market the PrioStrip and PrioWestern: as a matter of fact from 2022 it will not be possible to adopt the Reg 999/2001 solution (Annex X) of confirming a rapid test with another rapid test
- According to our data all of you can run a confirmatory WB (either Bio-Rad or internal method)



The next amendment of the Regulation will provide this update



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BATCH TESTING PROTOCOL FOR BATCH RELEASE OF TSE RAPID TEST KITS

**To be used by all participating EU TSE National Reference
Laboratories and TSE rapid test kit manufacturers**

REVISION PROPOSAL



Version 02



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BATCH TESTING PROTOCOL FOR BATCH RELEASE OF TSE RAPID TEST KITS

- The aim of this protocol is to provide a single batch release testing procedure which is acceptable to all NRLs.
- It details the approval process by which bovine and small ruminants TSE rapid test kit batches can be released onto the European market and provides reassurance that commercially available TSE rapid tests kits in the European Union (EU) are fit for the purpose of testing bovine and small ruminant samples



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BATCH TESTING PROTOCOL FOR BATCH RELEASE OF TSE RAPID TEST KITS

- Every test kit manufacturer carries out internal Quality Control testing of kit batches and when the manufacturer is satisfied that the batch is suitable for release, the manufacturer shall provide it to the appropriate NRL.
- NRLs carry out the batch testing using the EURL set of material prepared for BSE and scrapie.
- Originally batch tests were carried out using both manufacturers (3 positive, one negative) and EURL (3 positive, one negative) control samples.



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PROPOSAL FOR THE REVISION OF BATCH TESTING PROTOCOL

Three significant changes:

1

- Reduction of number of positive samples** in the sample set for batch testing from 3 to 2 (withdrawing the strong positive)

This to meet the need of reducing the use of TSE positive reference material (scarcity of reference materials, biosafety issues)



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PROPOSAL FOR THE REVISION OF BATCH TESTING PROTOCOL

❑ **Removal of the manufacturer samples set**

2 originally included in order to verify possible issues deriving from the use of water as diluent instead of the specific manufacturer buffer (problems were related to Prionics tests, those showed lower sensitivity with nervous tissue prepared in water; after the withdrawal of Prionics tests from the market this doesn't represent a problem anymore)

Already discussed with manufacturers and NRLs in charge of batch testing



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PROPOSAL FOR THE REVISION OF BATCH TESTING PROTOCOL

3

Addition of a set of **small ruminants** TSE positive and negative samples

in order to evaluate the reliability of batches released also for TSE monitoring in sheep and goats



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NEWS: miscellaneous

- Atypical reference material can be included in future PT exercises thanks to Portugal contribution
- for BSE atypical forms the experimental infection in German premises has been approved and will be funded from EC in order to obtain in 2-3 years reference materials



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ANY OTHER ISSUE?



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