

Torino/Rome, Italy

Online meetings: 16th-18th September 2020

Testing in practice, let's refresh TSE testing schemes

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TSE data collection

- From 1st January 2018 the **TSE data collection activities have been transferred from the EC to EFSA.**
- The **technical requirements** for this TSE data collection were defined in 2016 by an EFSA ad hoc WG.
- Subsequently, EFSA developed and maintained **a dedicated data model using its SSD2 standard.**



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Field data and schemes

- Data reported to EFSA by the MS are used to describe application and results of the national surveillance activities on TSE
- Surveillance includes data on subsequent steps i.e. screening, confirmatory, discriminatory testing and genotyping.
- In R 999/2001 **testing schemes** describe how to decide what to do step by step (and further indications are in the EURL guidance documents or the OIE Manual)



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Data and testing schemes

- In principle, **surveillance data reported** by MS should show the consistency with the **testing schemes** applied in the field based on **the official provisions** of Regulation (EC) 999/2001.
- However, the analysis of reported data shows that this is not always the case...



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Few examples from past data

- Discriminatory testing was not applied on some classical scrapie (CS) cases
- Confirmatory testing was skipped for BSE cases and discriminatory testing was applied directly after a positive screening test
- Genotyping was not carried out on all CS cases
- Etc.



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An EFSA revision

Early this year, a discussion between EURL, EFSA and EC was triggered by the **revision of the EFSA Technical Report on reporting TSE surveillance data** (*Guidelines for reporting surveillance data on Transmissible Spongiform Encephalopathies (TSE) in the EU within the framework of Regulation (EC) No 999/2001. EFSA 2018*)



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On the basis of the revision...

Need of agreeing on:

- the consolidated testing schemes by species (cattle, small ruminants, cervids, other species) and disease type (C-, H-, L-BSE; CS, AS; CWD). The schemes can be summarised through flow charts.
- the list of analytical methods that are expected to be used in each individual diagnostic testing step (i.e. screening, confirmatory, discriminatory, genotyping);



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Let's have a look to the testing schemes and methods to be used

Cattle, small ruminants, cervids



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cattle



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Screening rapid testing

(active surveillance)

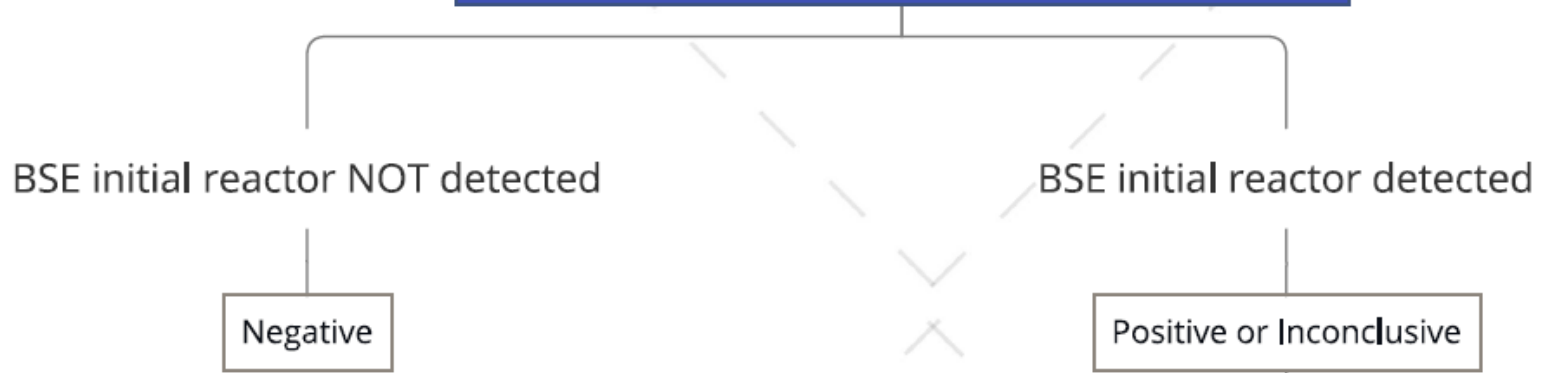
- Testing by one of the authorised tests listed in R999:
- Prionics-Check Western test
 - Bio-Rad TeSeE SAP rapid test
 - Prionics-Check LIA test
 - IDEXX HerdChek BSE Antigen Test Kit, EIA
 - IDEXX HerdChek BSE-Scrapie Antigen
 - Prionics Check PrioSTRIP
 - Roboscreen Beta Prion BSE EIA Test Kit

A positive list

cattle

NB in case of a **clinical suspect (passive surveillance)**, samples shall immediately be subjected to confirmatory examinations

Screening rapid testing



cattle

Screening rapid testing

BSE initial reactor NOT detected

Negative

BSE initial reactor detected

Positive or Inconclusive

Confirmatory testing

TSE not detected

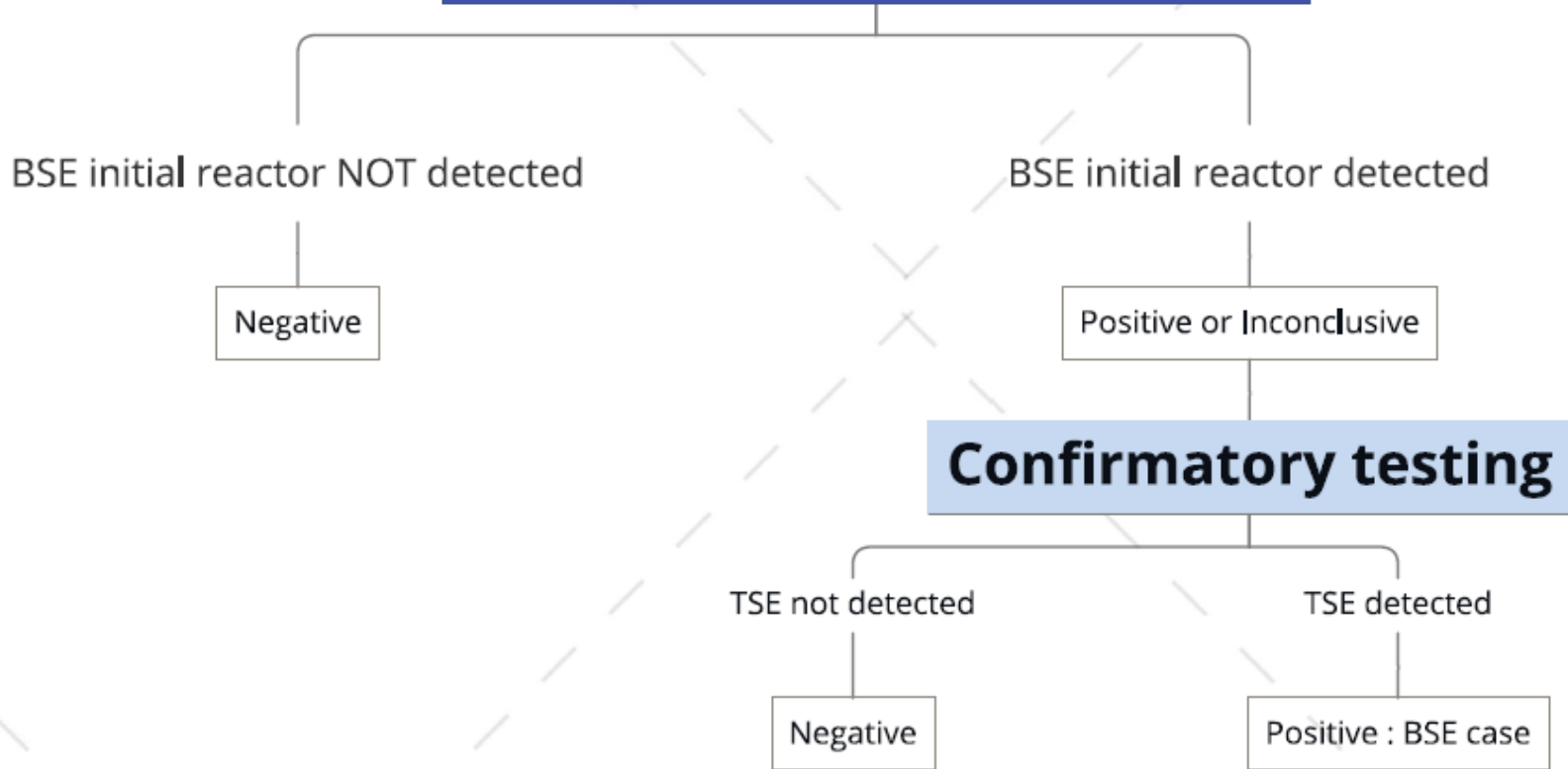
Negative

TSE detected

Positive : BSE case

cattle

Screening rapid testing



cattle

- At least one of these methods:
- Histopathological examination
 - Immunohistochemical (IHC) method
 - Western blot
 - Demonstration of characteristic fibrils by electron microscopy

Or: →

Screening rapid testing

BSE initial reactor NOT detected

Negative

BSE initial reactor detected

Positive or Inconclusive

Confirmatory testing

TSE not detected

Negative

TSE detected

Positive : BSE case

cattle

Or a second rapid test
(following the OIE indications
for rapid test combination)

**OIE rules for the official confirmation of BSE in bovines
(based on an initial reactive result in an approved rapid test)
by using a second rapid test**

		Second test allowed under certain conditions for confirmation				
		IDEXX HerdChek® BSE	Roboscreen Beta Prion®	Prionics® Check Western	Prionics® Check PrioSTRIP	Bio-Rad TeSeE®
First test used	IDEXX HerdChek® BSE		-	+	-	-
	Roboscreen Beta Prion®	-		+	-	-
	Prionics® Check Western	+	+		+	+
	Prionics® Check PrioSTRIP	-	-	+		-
	Bio-Rad TeSeE®	-	-	+	-	

Screening rapid testing

BSE initial reactor NOT detected

Negative

BSE initial reactor detected

Positive or Inconclusive

Confirmatory testing

TSE not detected

Negative

TSE detected

Positive : BSE case



Discriminatory testing

C-type detected

C-Type

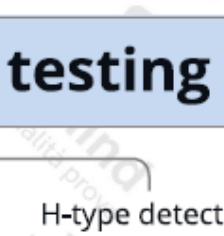
L-type detected

L-Type

H-type detected

H-type

cattle



Discriminatory testing (carried out by NRL that passed the PT) is an ad hoc western blot carried out accordingly with the guidance document available on the EURL website or the FTP repository

BSE initial reactor NOT detected

Two options:

- In house western blot
- APHA Bio-Rad TeSeE-based Hybrid Western blotting Method



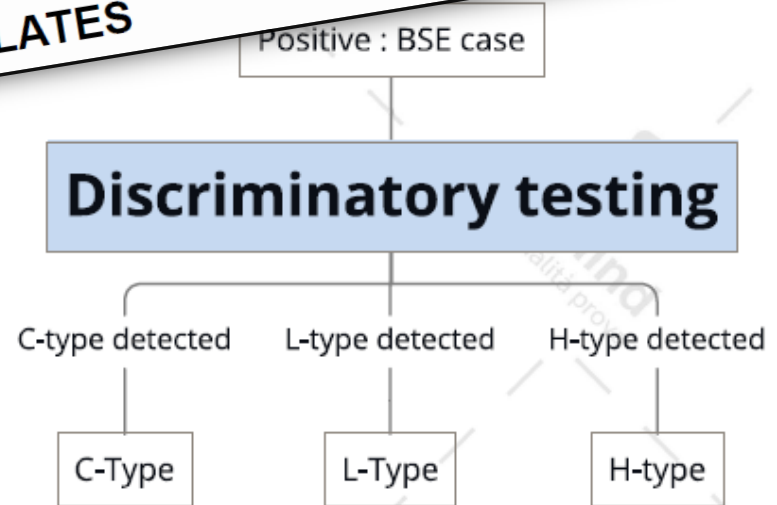
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APPROACH FOR THE PROVISIONAL CLASSIFICATION OF BOVINE TSE ISOLATES

cattle



small ruminants



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Screening - Rapid Testing

Scrapie initial reactor NOT detected

Negative

Scrapie initial reactor detected

Positive or Inconclusive

small
ruminants

Screening - Rapid Testing

Scrapie initial reactor NOT detected

Negative

Scrapie initial reactor detected

Positive or Inconclusive

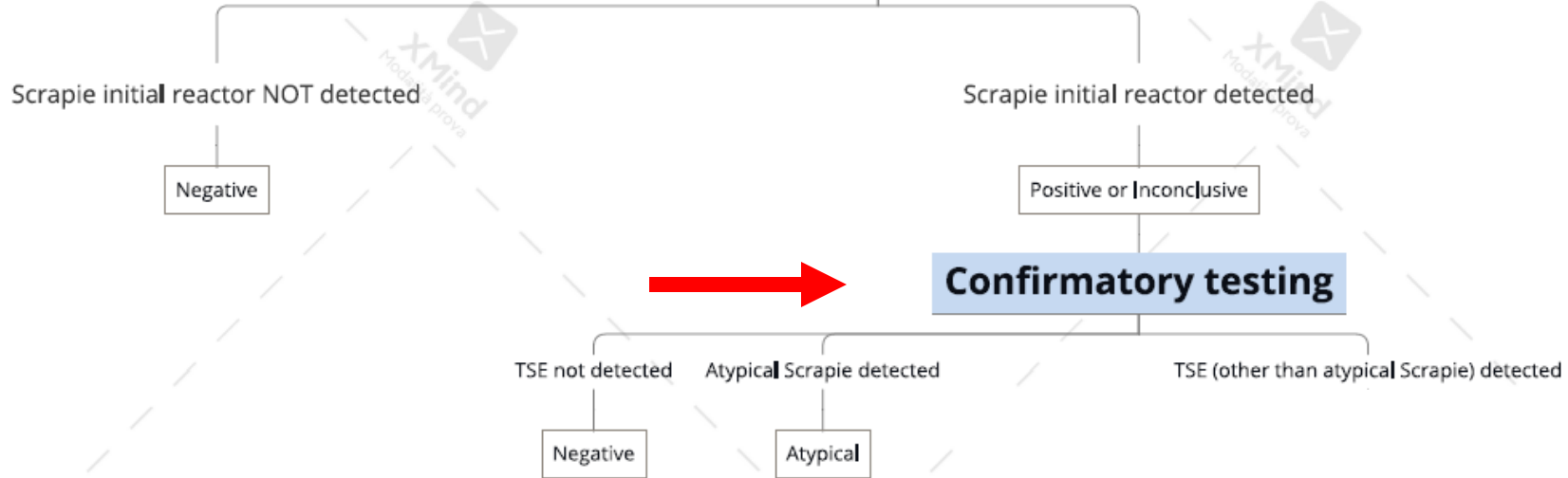
Testing by one of the authorised tests listed in
R999

- Bio-Rad TeSeE SAP
- Bio-Rad TeSeE Sheep/Goat
- IDEXX HerdChek BSE-Scrapie Antigen

small
ruminants

Again a positive list

Screening - Rapid Testing

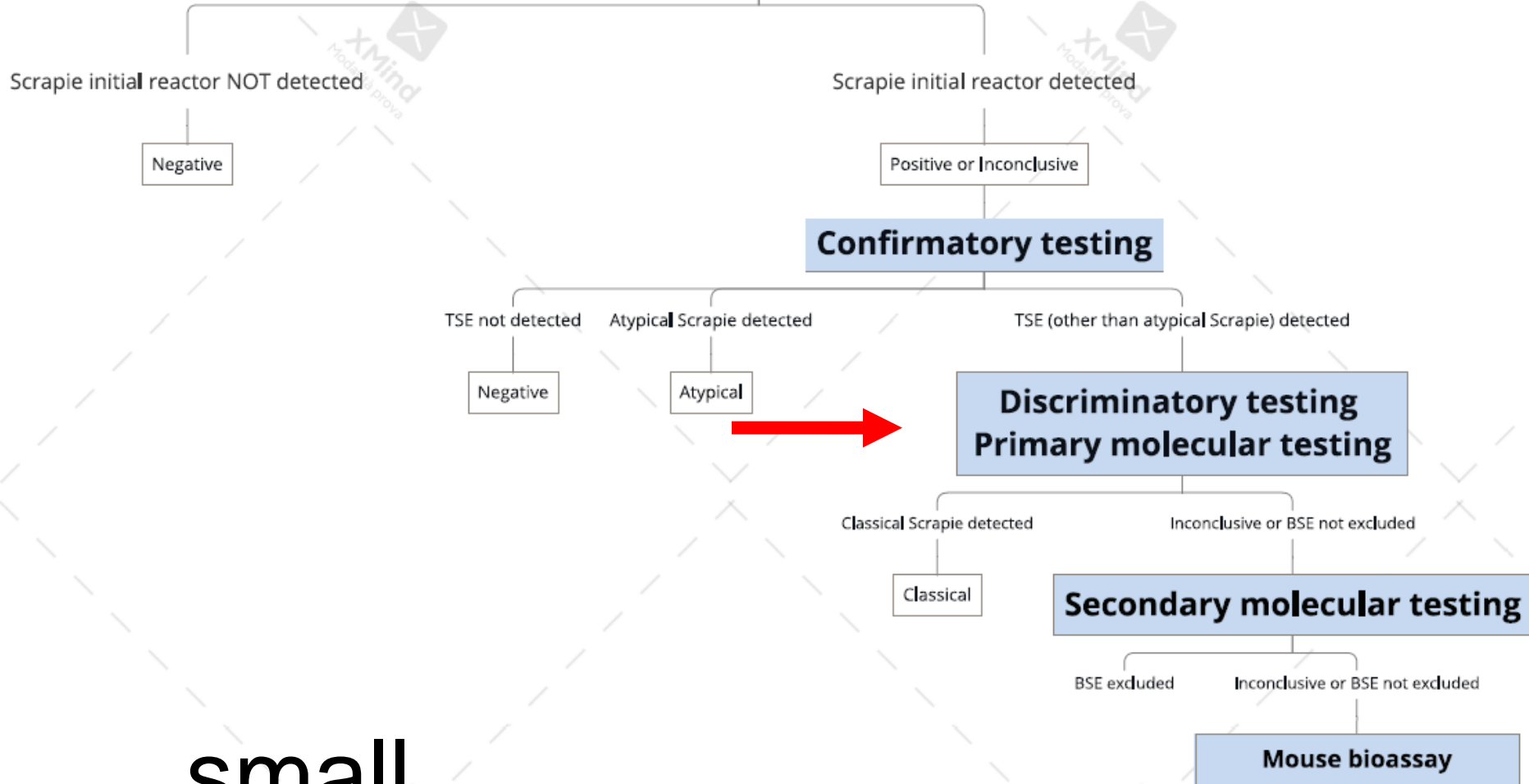


- At least one of these methods:
- Histopathological examination
 - Immunohistochemical (IHC) method
 - Western blot
 - Demonstration of characteristic fibrils by electron microscopy

small
ruminants

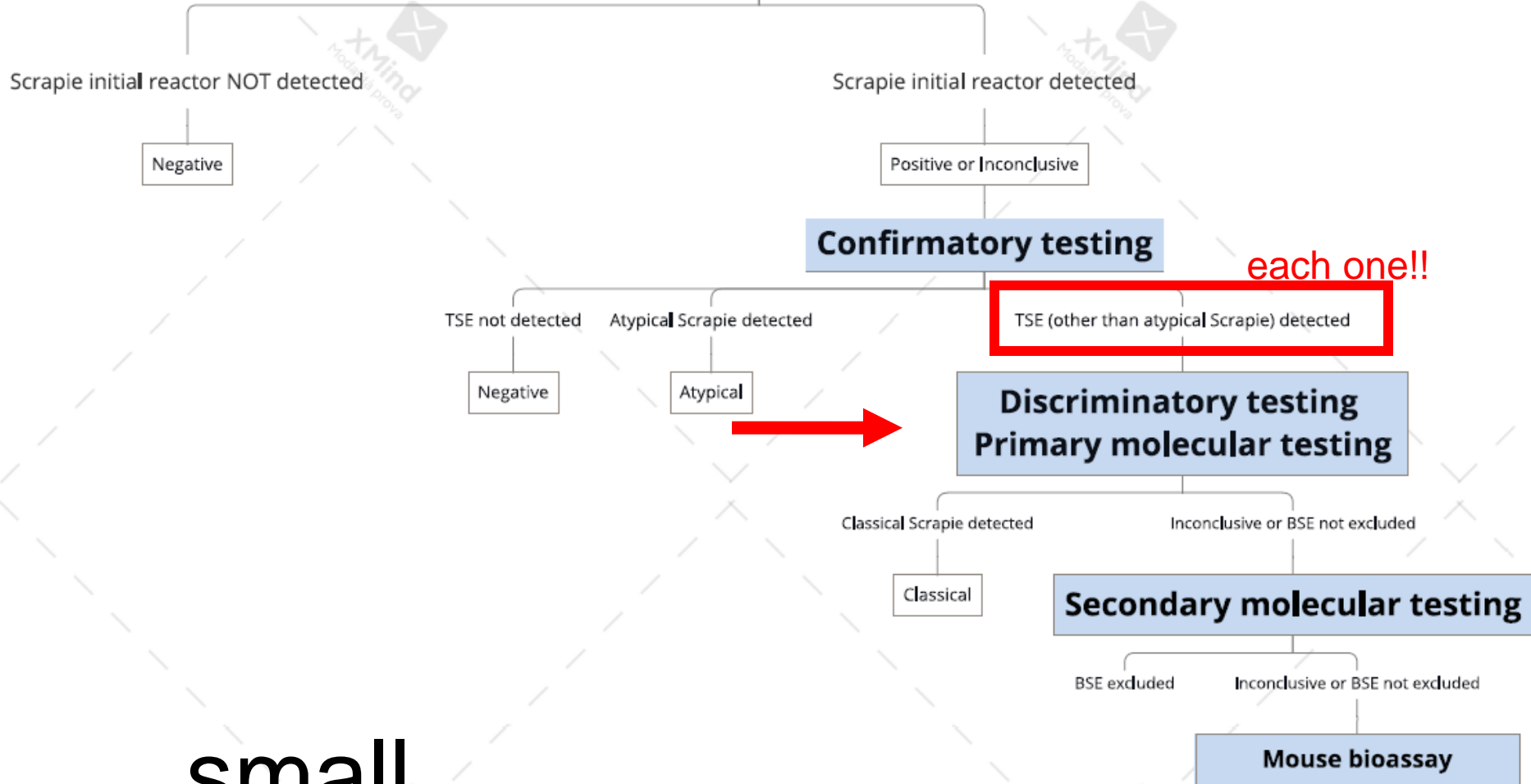
Not allowed in these species the confirmation through a combination of rapid tests

Screening - Rapid Testing



small
ruminants

Screening - Rapid Testing



small
ruminants

Screening - Rapid Testing

Scrapie initial reactor NOT detected

Negative

Scrapie initial reactor detected

Positive or Inconclusive

Again a positive list

TSE not detected Atypica

Negative

Atypical

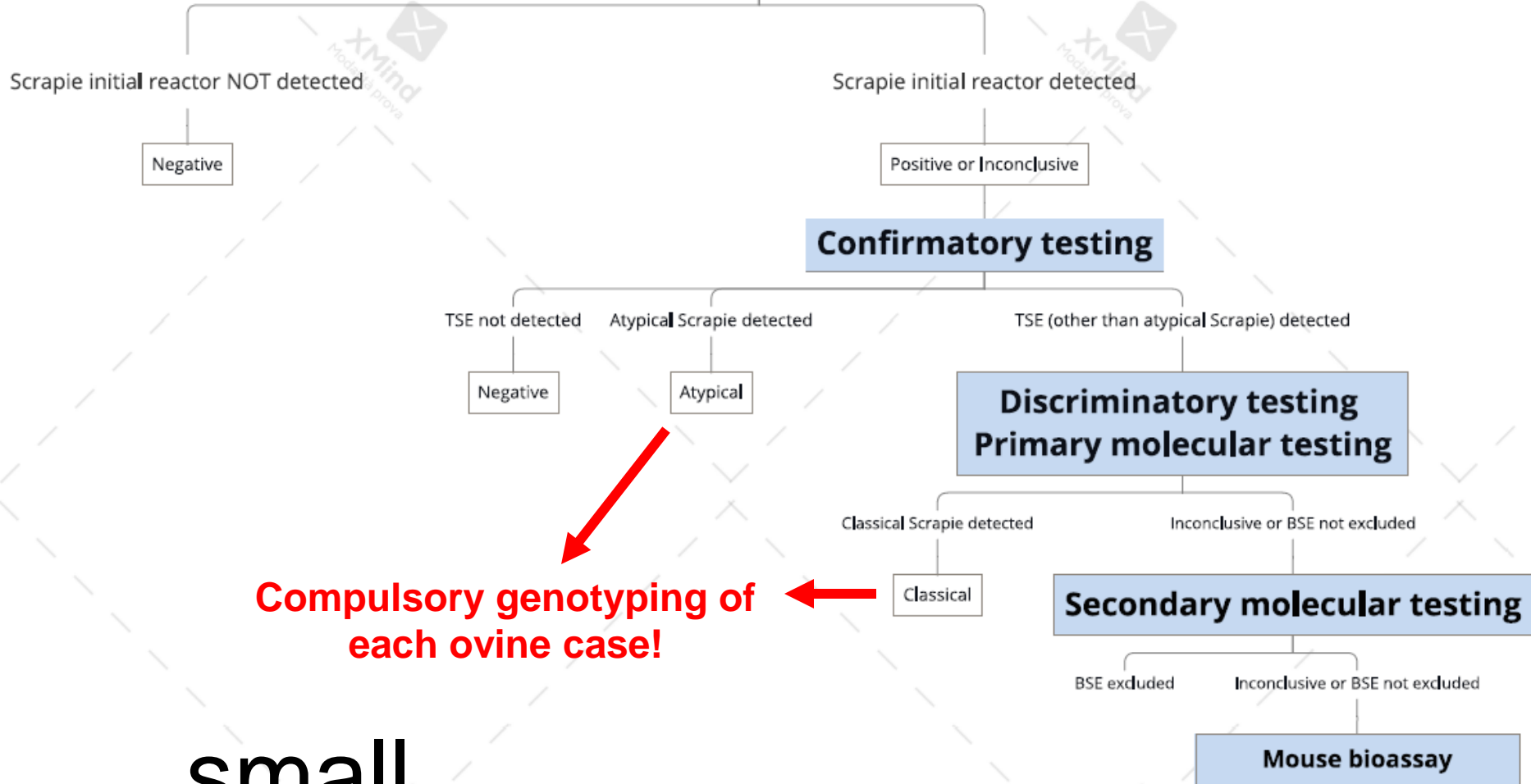
**Discriminatory testing
Primary molecular testing**

Classical Scrapie detected

small
ruminants

- One of the WB methods listed in the EURL Handbook
- APHA Bio-Rad TeSeE-based Hybrid Western blotting Method
 - APHA Prionics-based Hybrid Western blot Method
 - ANSES Discriminatory Western blot Method
 - Bio-Rad Discriminatory Test
 - CIDC-Lelystad Discriminatory Western blot method
 - FLI Discriminatory Western blot method
 - ISS-Discriminatory Western blot method

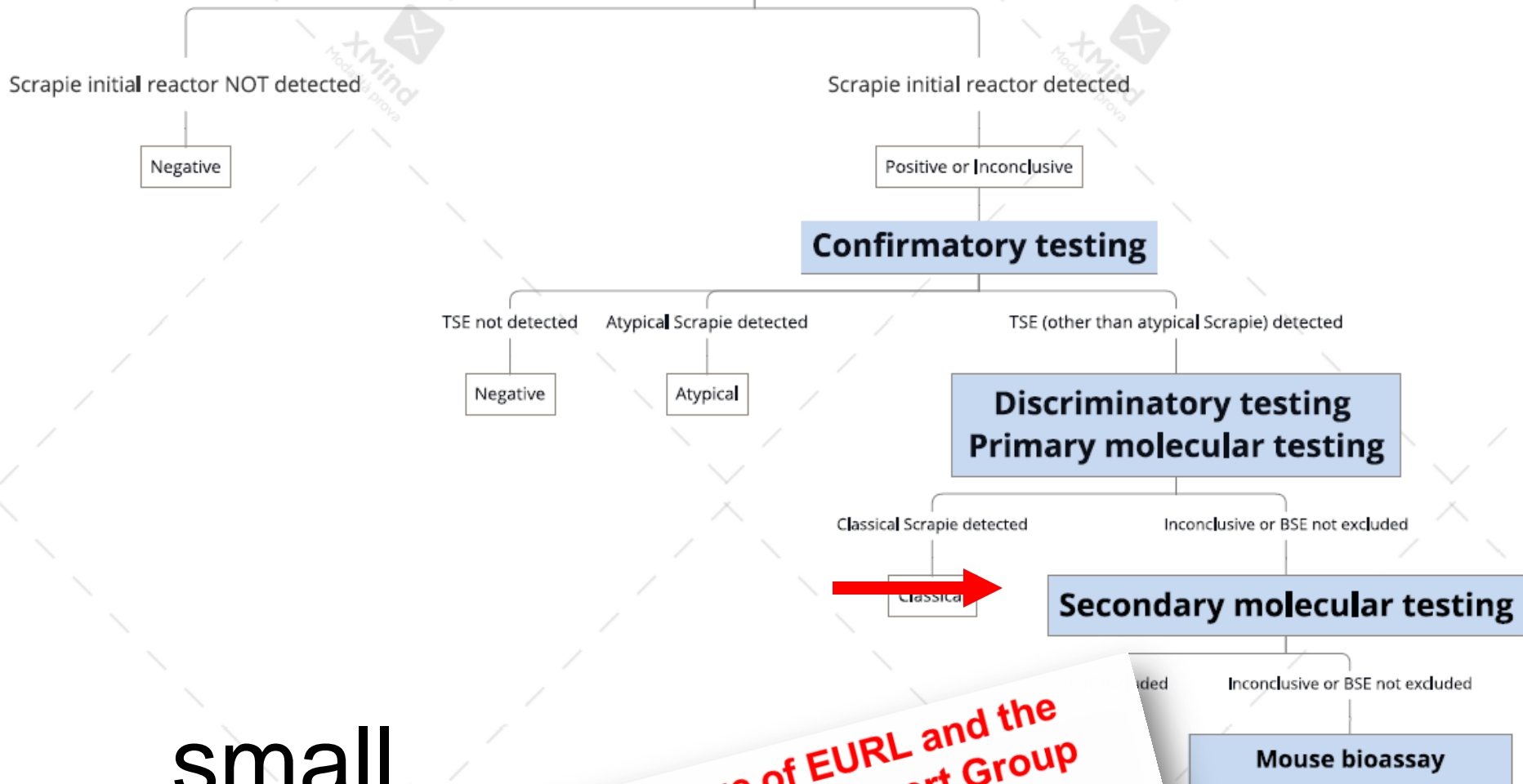
Screening - Rapid Testing



Compulsory genotyping of each ovine case!

small
ruminants

Screening - Rapid Testing



small
ruminants

**In charge of EURL and the
Strain Typing Expert Group
(STEG)**

cervids



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Screening - Rapid Testing

- Based on the EURL guidance document:
- Bio-Rad TeSeE SAP rapid test (using the CWD protocol)
 - IDEXX HerdChek BSE-Scrapie Antigen (using CWD protocol)

Again a positive list

cervids

Screening - Rapid Testing

CWD initial reactor NOT detected

Negative

CWD initial reactor detected

Positive or Inconclusive

cervids

Screening - Rapid Testing

CWD initial reactor NOT detected

Negative

CWD initial reactor detected

Positive or Inconclusive

Confirmatory testing

TSE not detected

Negative

TSE detected

Positive or inconclusive

cervids

Based on the OIE Manual and R999
at least using:

- IHC or
- WB

Screening - Rapid Testing

CWD initial reactor NOT detected

Negative

CWD initial reactor detected

Positive or Inconclusive

Confirmatory testing

TSE not detected

Negative

TSE detected

Positive or inconclusive

Isolate characterization in consultation with TSE EURL

cervids

There is no discriminatory issues raised in R999 but the need to further characterise (under the EURL supervision)

Analytical methods and diagnostic steps

Let's summarise (1/2) step by step (S,C,D,G)

- (S) Screening: for all species, *positive list* of approved tests (commercial kits); differences by species; in case of clinical suspects, no need of primary screening
- (C) Confirmatory testing: for all species, generic methods (HI, IHC, WB, EM); differences by species (e.g. in cervids the OIE Manual and R999 indicate at least IHC or WB)



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Analytical methods and diagnostic steps

Let's summarise (2/2) step by step
(S,C,D,G)

- (D) Discriminatory testing:
 - Cattle: ad hoc WB (even if in house methods)
 - SR: *positive list* based on the EURL Handbook
 - Cervids: not required; R999 requires isolate characterization carried out in cooperation with the EURL
- (G) Genotyping: no specific methods required

EURL updates on the web

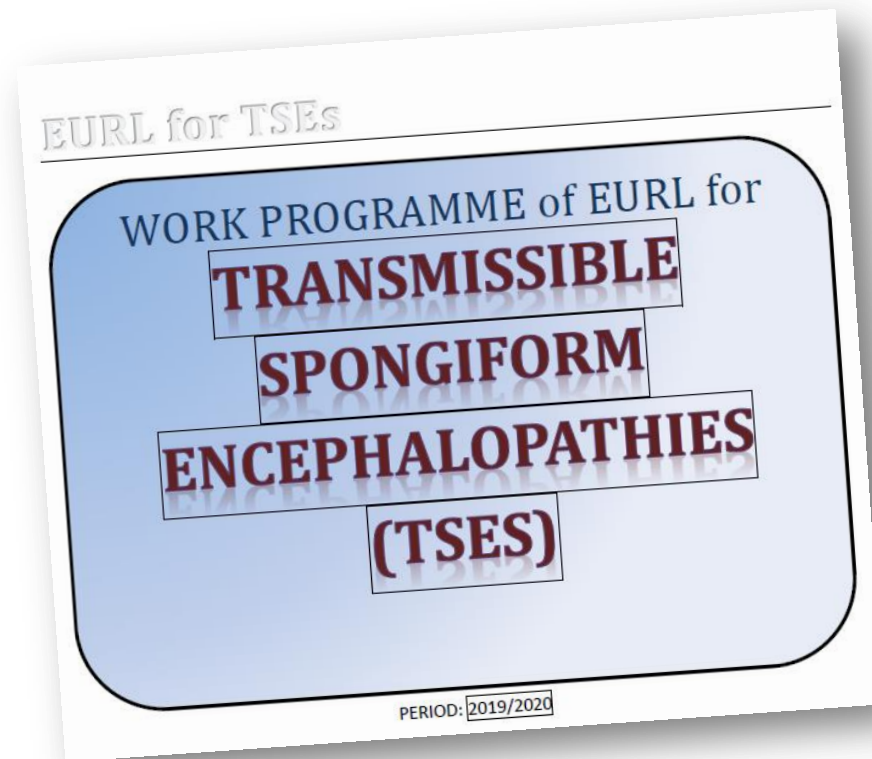
- We have recently made new documentation available.
- The following material can be accessed online in our FTP folder (accessible at <ftp://ftp.izsto.it>) or in the zip folder of our website (http://www.izsto.it/images/stories/EURL_guidance.zip folder):
 - A new document, the official confirmation of BSE in cattle using a second rapid test (*"OIE rules for the official confirmation of BSE in cattle (based on a first reactive result in an approved rapid test) using a second rapid test.pdf"*)
 - A new guidance document for discriminatory tests in cattle (*"EURL bovine discriminatory guidance.pdf"*)
 - A new version of discriminatory tests in small ruminants (*"EURL small ruminants discriminatory guidance.pdf"*)



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Thanks and enjoy our meetings!



Thanks to our outstanding EURL staff!!



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