

21st Annual Meeting of the TSE EURL

Rome 13rd -15th May 2024

**TSE DIAGNOSIS:
FROM PAST CHALLENGES
TO FUTURE PERSPECTIVES**

**Elena Bozzetta
Laura Pirisinu
Maria Mazza**

EURL for TSEs

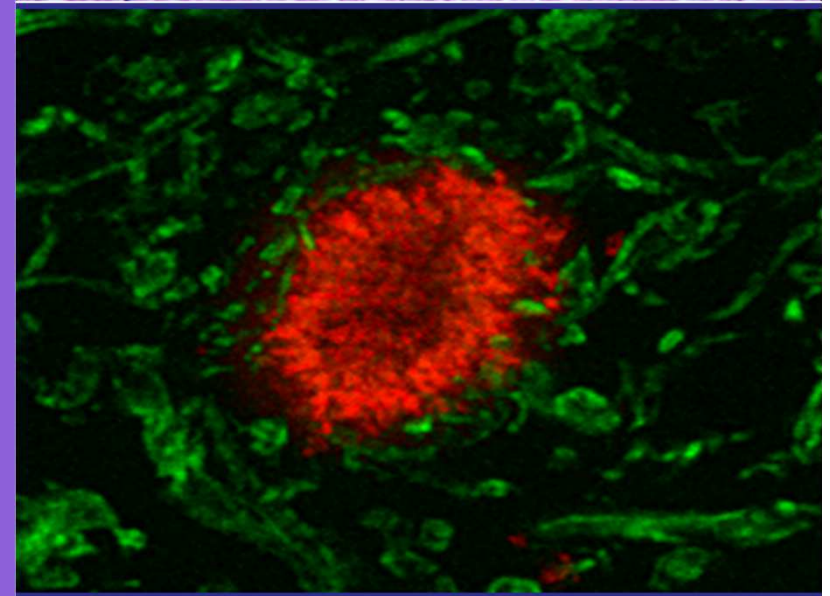
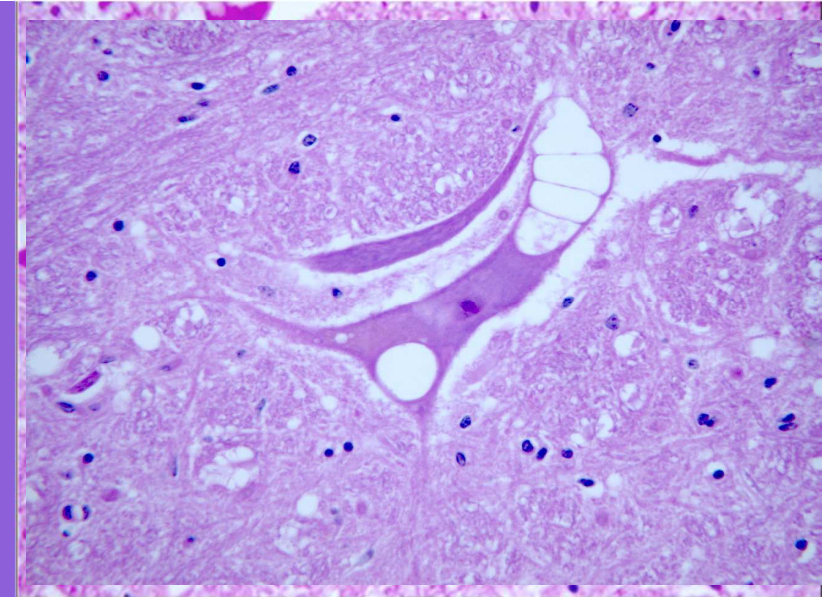


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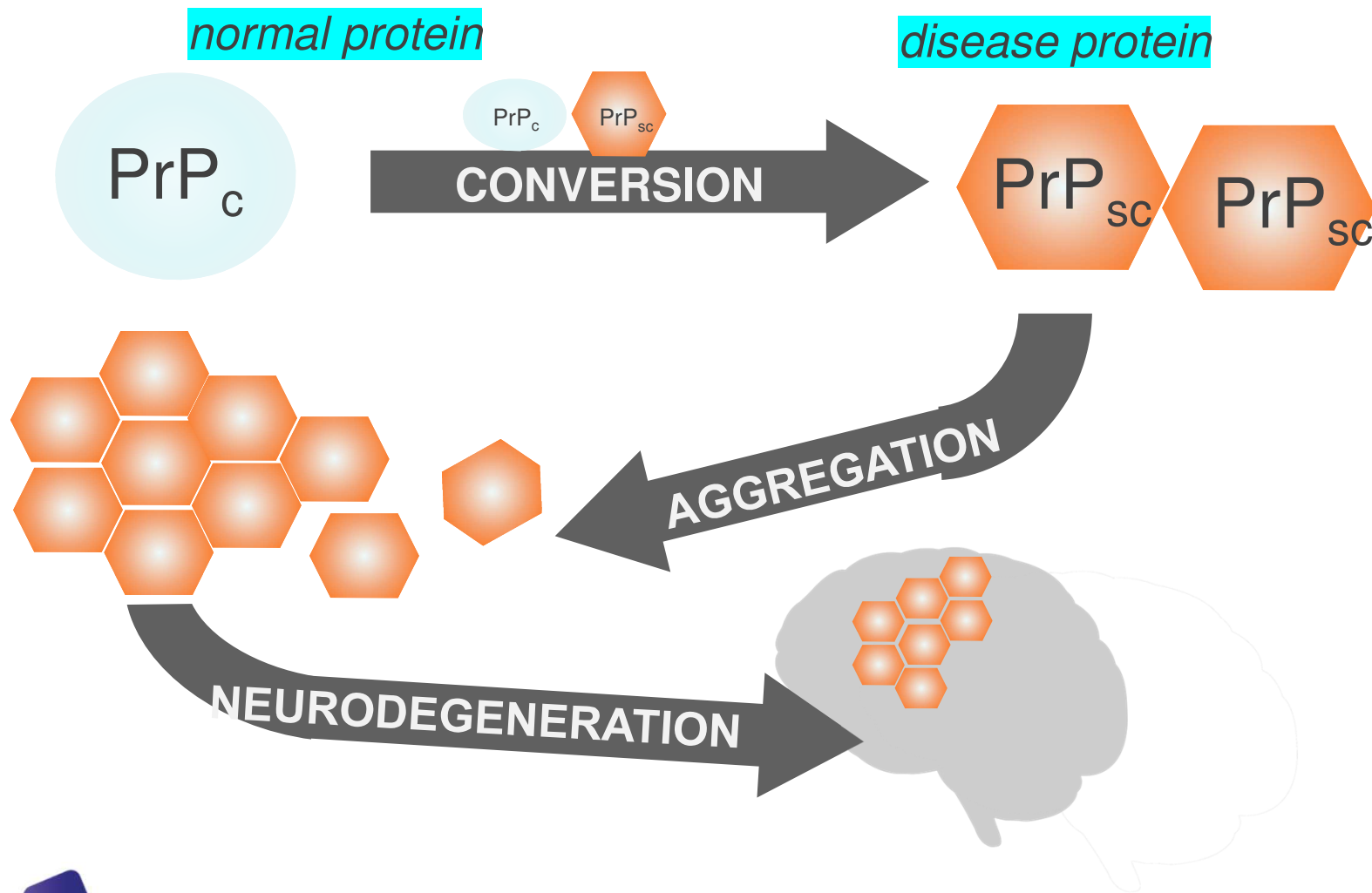
PRION DISEASES OR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE)

- Spongiform degeneration
- Reactive gliosis
-
- Neuronal loss
-
- Formation of amyloid plaques



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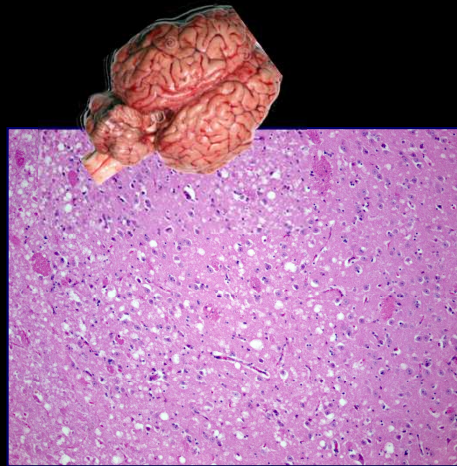
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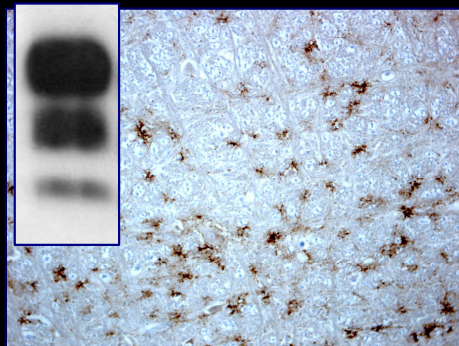
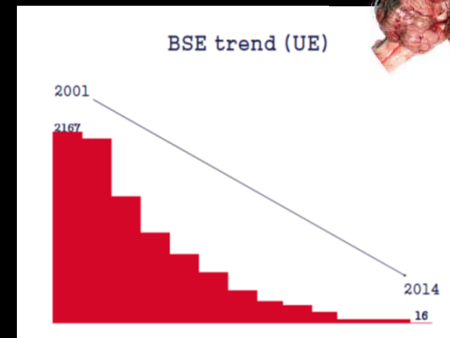
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DIAGNOSIS OF BSE



1986
Histology
3 weeks



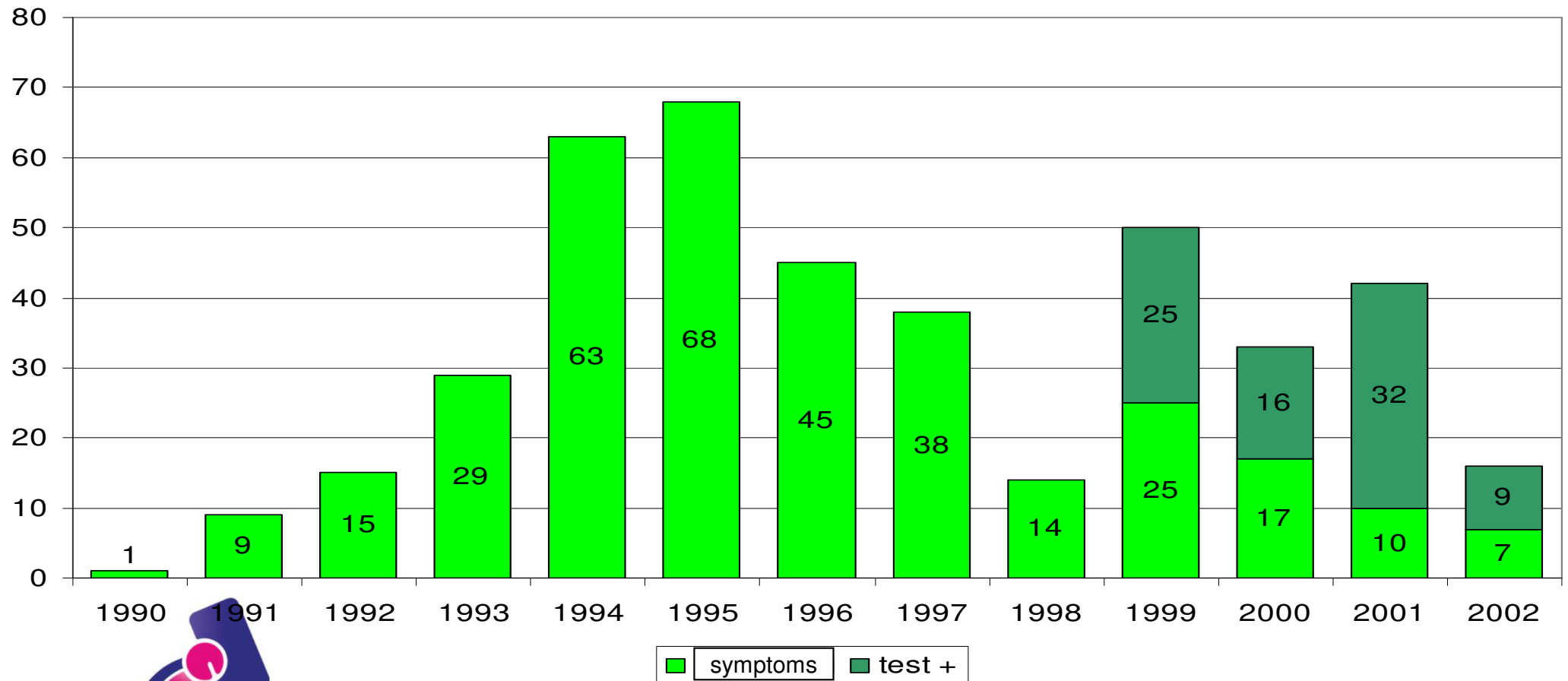
1995
IHC e WB
2 weeks

2001

Rapid Test
1 day



SWITZERLAND



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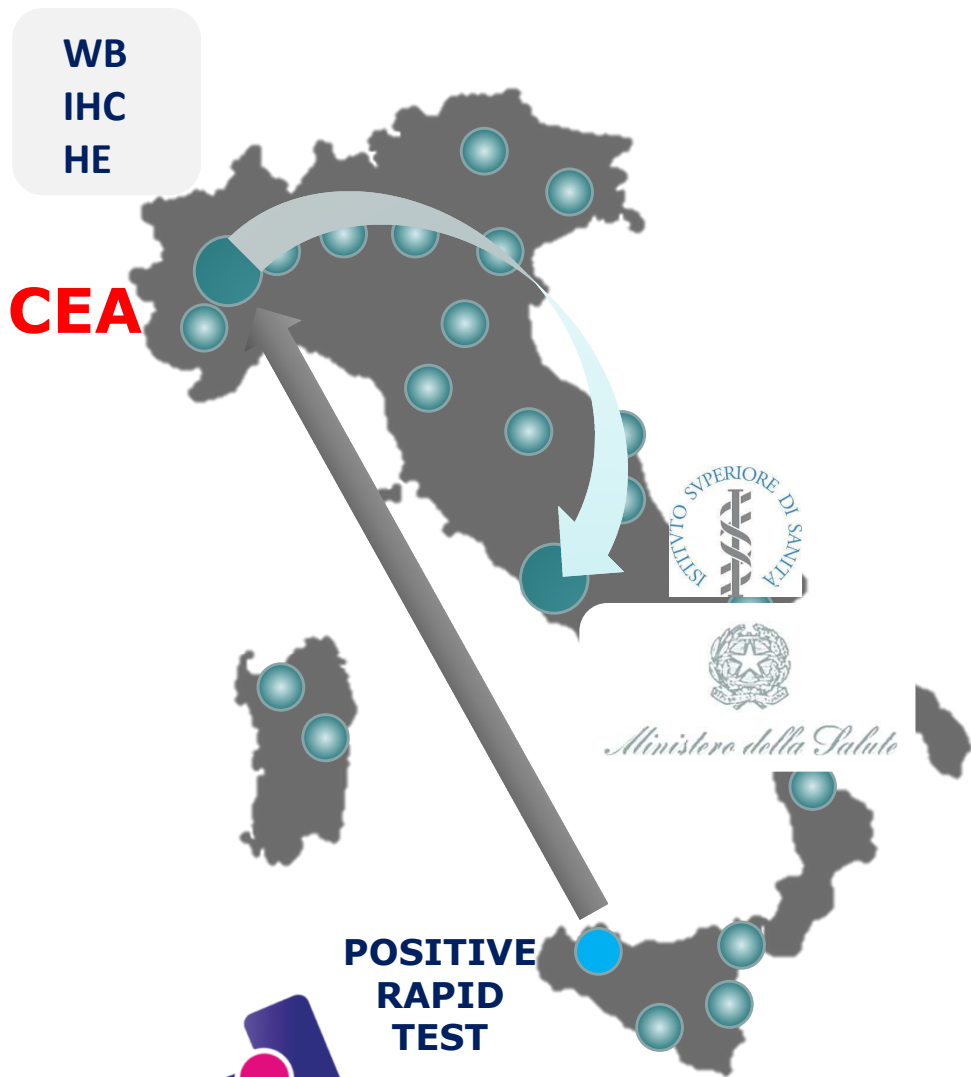
Bse surveillance



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Work flow

RAPID TEST SCREENING



OUTCOME - POSITIVE



SENT TO CEA



CONFIRMATORY TESTS IN
CEA



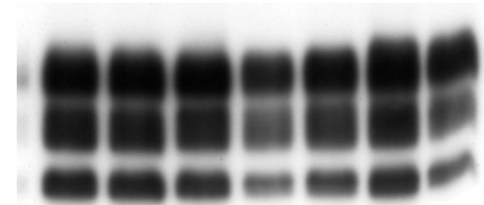
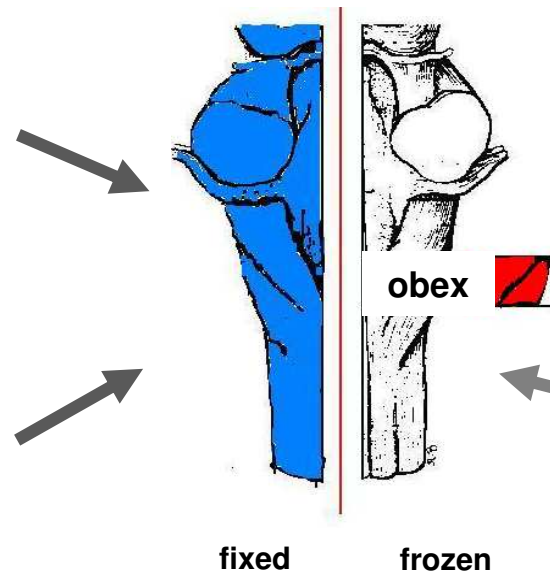
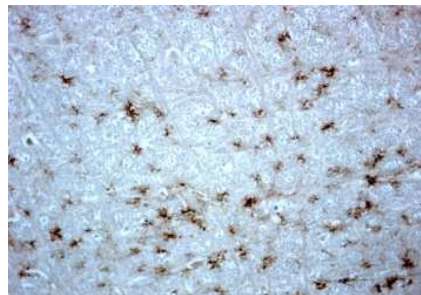
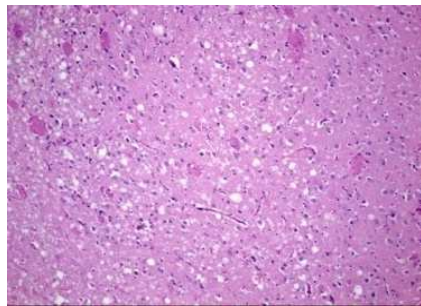
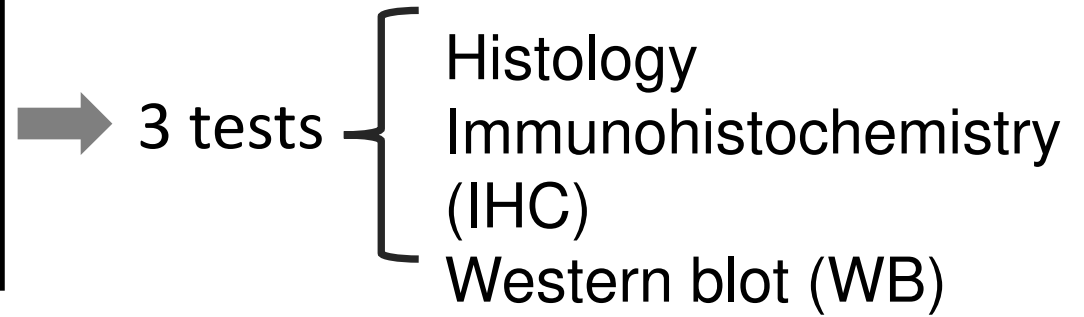
SENT TO ISS - ROME



TRANSMISSION OF DATA TO
ITALIAN MINISTRY OF HEALTH



CONFIRMATORY TESTS



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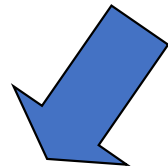
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PASSIVE SURVEILLANCE ACTIVITY

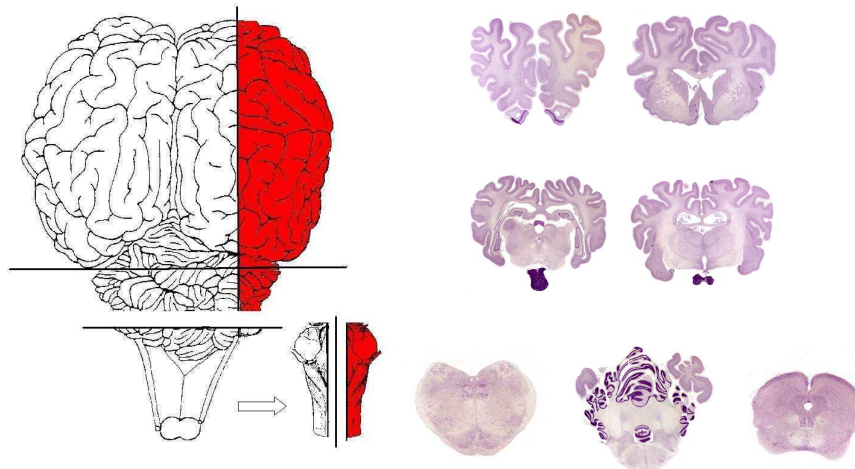
Confirmatory tests (H&E, IHC WB)



In case of negative confirmatory tests for TSE



Differential diagnosis on the whole brain



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Rapid testing in 2001

3 tests validated according to their 100% accuracy:

- Prionics check WB
- Platelia BSE
- Enfer



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COMMISSION REGULATION (EC) No 162/2009

of 26 February 2009

amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies

(Text with EEA relevance)

4. **Rapid tests**

For the purposes of carrying out the rapid tests in accordance with Articles 5(3) and 6(1), the following methods shall be used as rapid tests for the monitoring of BSE in bovine animals:

- the immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrP^{Res} (Prionics-Check Western test),
- the chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer test & Enfer TSE Kit version 2.0, automated sample preparation),
- the microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),
- the sandwich immunoassay for PrP^{Res} detection with the TeSeE SAP Detection kit carried out following denaturation and concentration steps with the TeSeE Purification kit (Bio-Rad TeSeE rapid test),
- the microplate-based immunoassay (ELISA) which detects Proteinase K-resistant PrP^{Res} with monoclonal antibodies (Prionics-Check LIA test),

- the immunoassay using a chemical polymer for selective PrP^{Sc} capture and a monoclonal detection antibody directed against conserved regions of the PrP molecule (IDEXX HerdChek BSE Antigen Test Kit, EIA),
- the lateral-flow immunoassay using two different monoclonal antibodies to detect Proteinase K-resistant PrP fractions (Prionics Check PrioSTRIP),
- the two-sided immunoassay using two different monoclonal antibodies directed against two epitopes presented in a highly unfolded state of bovine PrP^{Sc} (Roboscreen Beta Prion BSE EIA Test Kit),
- the sandwich ELISA for the detection of Proteinase K-resistant PrP^{Sc} (Roche Applied Science PrionScreen).

For the purposes of carrying out the rapid tests in accordance with Articles 5(3) and 6(1), the following methods shall be used as rapid tests for the monitoring of TSE in ovine and caprine animals:

- the sandwich immunoassay for PrP^{Res} detection with the TeSeE SAP Detection kit carried out following denaturation and concentration steps with the TeSeE Purification kit (Bio-Rad TeSeE rapid test),
- the sandwich immunoassay for PrP^{Res} detection with the TeSeE Sheep/Goat Detection kit carried out following denaturation and concentration steps with the TeSeE Sheep/Goat Purification kit (Bio-Rad TeSeE Sheep/Goat rapid test),
- the chemiluminescent ELISA test involving an extraction procedure and an ELISA technique, using an enhanced chemiluminescent reagent (Enfer TSE Kit version 2.0),
- the microplate-based immunoassay for the detection of PrP^{Sc} (Enfer TSE Version 3),
- the immunoassay using a chemical polymer for selective PrP^{Sc} capture and a monoclonal detection antibody directed against conserved regions of the PrP molecule (IDEXX HerdChek BSE-Scrapie Antigen Test Kit, EIA),
- the immuno-blotting test based on a Western blotting procedure for the detection of the Proteinase K-resistant fragment PrP^{Res} (Prionics-Check Western Small Ruminant test),
- the microplate-based chemiluminescent immunoassay for the detection of Proteinase K-resistant PrP^{Sc} (Prionics Check LIA Small Ruminants).



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But in the meanwhile...

- Evaluation of rapid tests performances in comparison with the best performing test (analytical sensitivity not $< 2 \log 10$)
- Evaluation of rapid tests on atypical scrapie



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Atypical scrapie

[Vet Rec. 2003 Aug 16;153\(7\):202-8. doi: 10.1136/vr.153.7.202.](#)

Cases of scrapie with unusual features in Norway and designation of a new type, Nor98

[S L Benestad ¹, P Sarradin, B Thu, J Schönheit, M A Tranulis, B Bratberg](#)



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Raid tests approved for active surveillance

REGULATION (EU) N. 110/2017
January 23 2017
Annex X

BSE		
RAPID TEST	METHOD	PRODUCER
HerdChek BSE-SCRAPIE Antigen Kit	EIA	Idexx
HerdChek BSE Antigen Kit	EIA	Idexx
PrioWESTERN BSE kit	Western blotting	Thermo Fisher
Prionics-Check LIA	ELISA	Prionics
PrioSTRIP BSE kit	Laterl flow immunoassay	Thermo Fisher
TeSeE SAP	Sandwich immunoassay	Bio-Rad
Beta Prion BSE EIA Test Kit	Sandwich immunoassay	Roboscreen

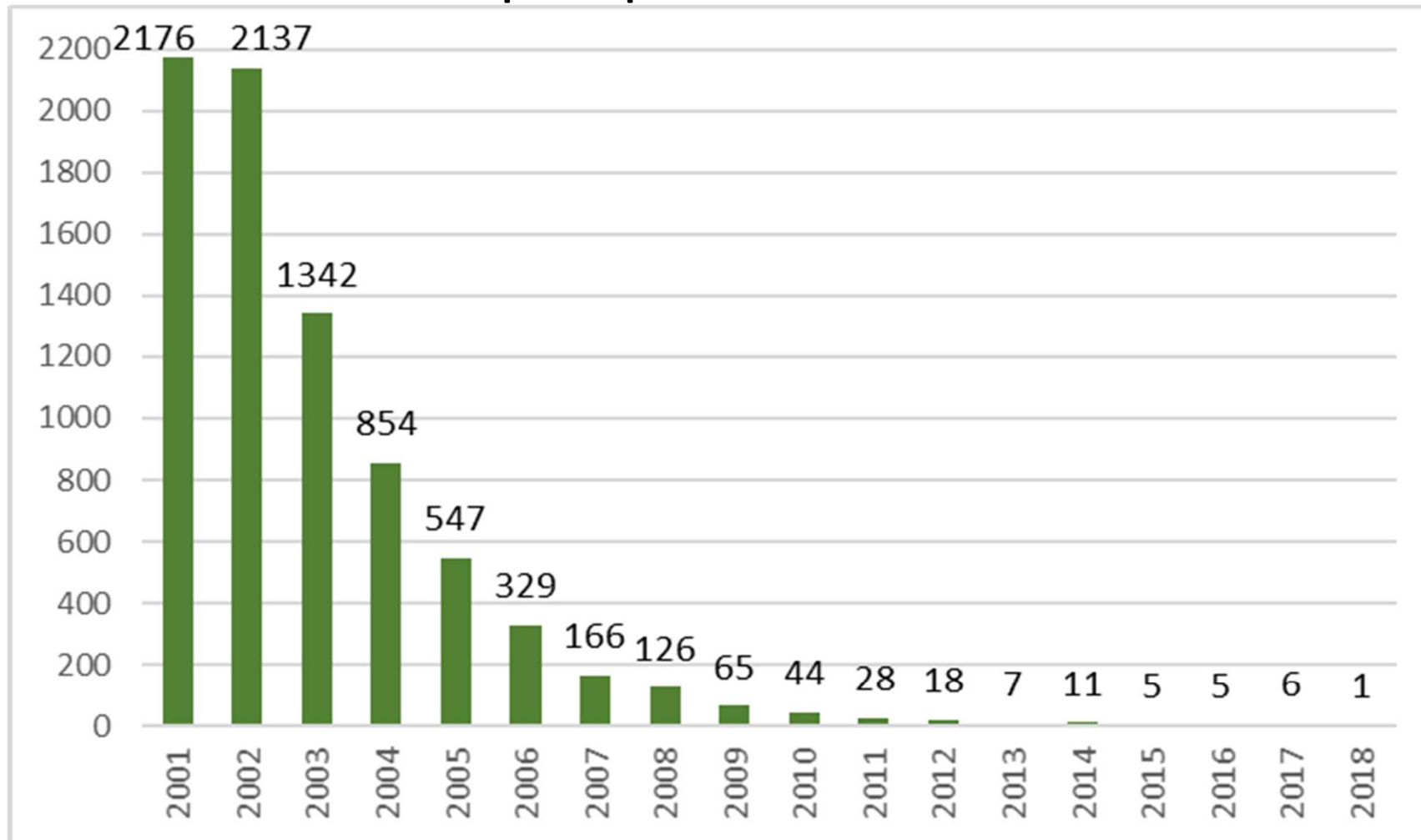
SCRAPIE		
RAPID TEST	METHOD	PRODUCER
HerdChek BSE-SCRAPIE Antigen Kit	EIA	Idexx
TeSeE SAP	Sandwich immunoassay	Bio-Rad
TeSeE Sheep/Goat	Sandwich immunoassay	Bio-Rad



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European epidemic curve



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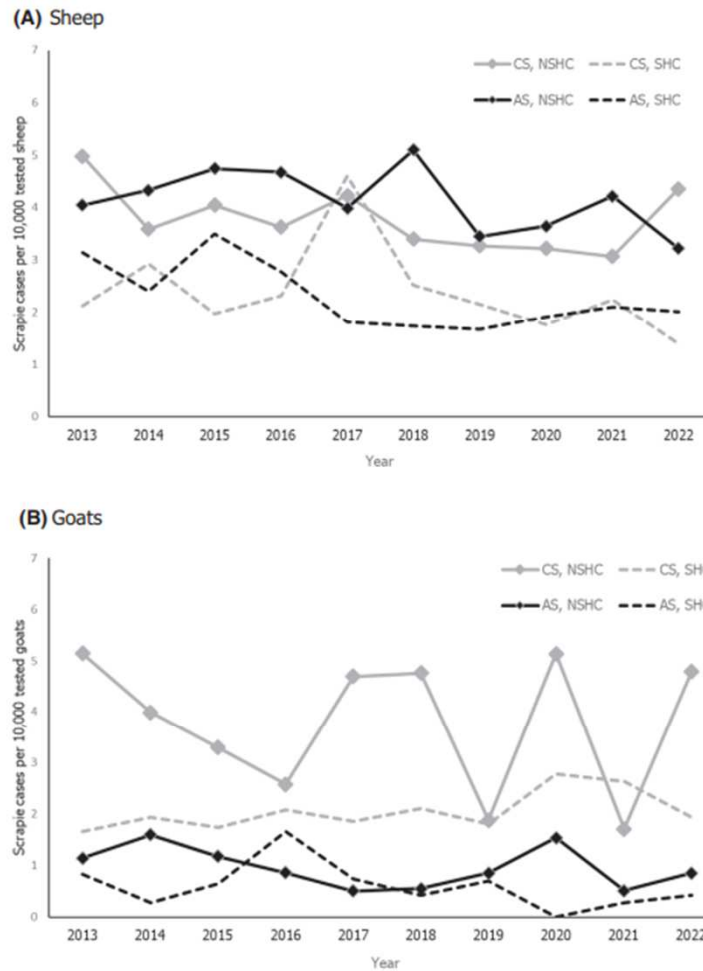


FIGURE 3 Number of scrapie (index) cases per 10,000 tests in the EU27 and the United Kingdom until 2020 and the EU27 and XI for the period 2021–2022 in (A) sheep and (B) goats in non-TSE-infected flocks/herds, reported by case type and target group in the period 2013–2022. This figure is restricted to active surveillance data, i.e. testing performed in NSHC and SHC target groups from non-infected flocks/herds or not previously known as infected. AS, atypical scrapie; CS, classical scrapie; NSHC, animals not slaughtered for human consumption; SHC, animals slaughtered for human consumption.



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RAPID TESTING IN ITALY FROM 2001 TO



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ACTIVE SURVEILLANCE IN EU

From all categories (including healthy slaughtered) > 24 months

....to risk categories > 48 months (NEGLIGIBLE RISK)



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12 PUBLIC Labs:

IDEXX HerdCheck Bovine
Spongiform Encephalopathy
(BSE) – scrapie Antigen Test Kit

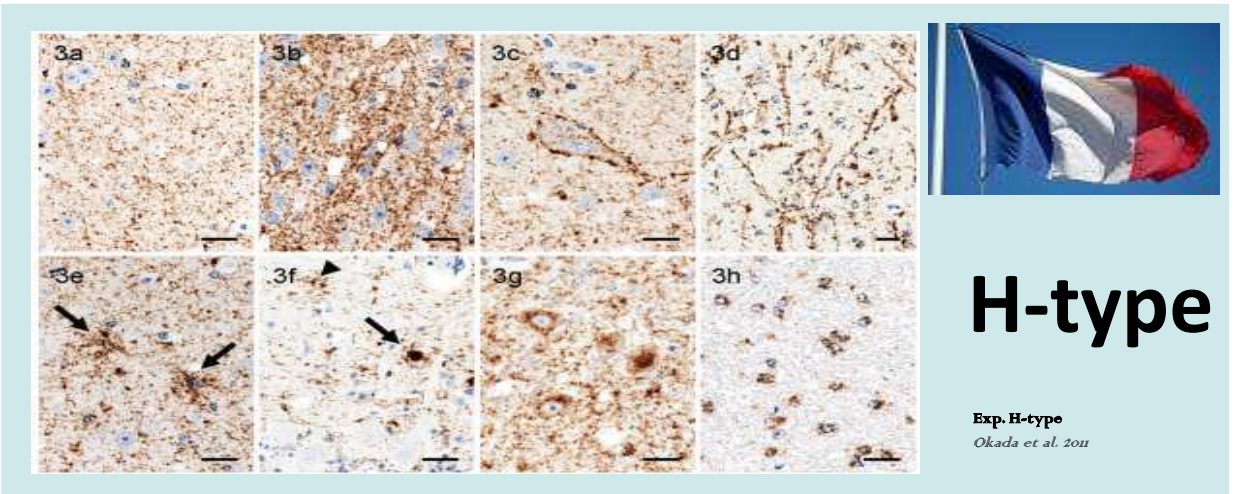
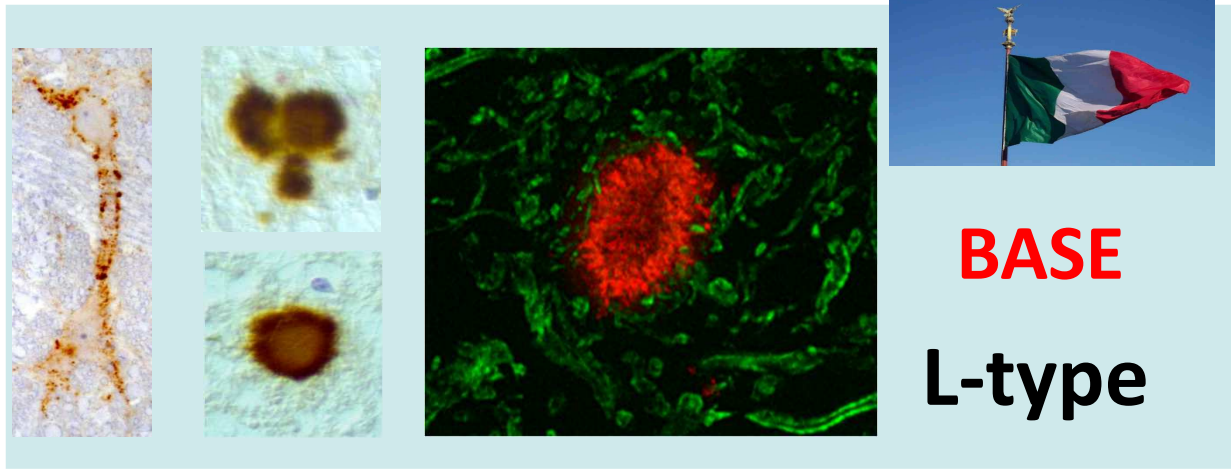
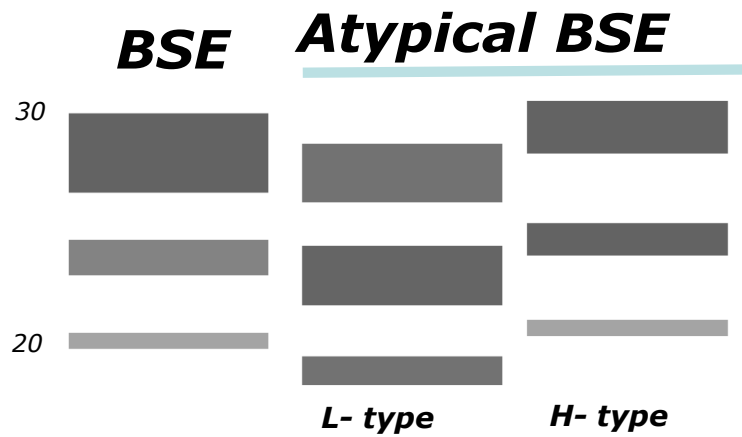


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FROM 2004: ATYPICAL BSE



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EU-Approved Rapid Tests for Bovine Spongiform Encephalopathy Detect Atypical Forms: A Study for Their Sensitivities

Daniela Meloni¹, Aart Davidse², Jan P. M. Langeveld², Katia Varello¹, Cristina Casalone¹, Cristiano Corona¹, Anne Balkema-Buschmann³, Martin H. Groschup³, Francesco Ingravalle¹, Elena Bozzetta^{1*}

¹ Centro di Referenza Nazionale per le Encefalopatie Animali, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, ² Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands, ³ Friedrich-Loeffler Institut, Federal Research Institute for Animal Health, Insel Riems, Germany



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Results

- Ranking the assays:
 - The sensitivity of *IDEXX® HerdCheck BSE-scrapie short protocol* test for all BSE types was higher than the other tests
 - Similar sensitivity for *IDEXX® HerdCheck BSE-scrapie ultra short protocol*, *Prionics® - Check WESTERN*, and *AJ Roboscreen® BetaPrion*
 - In decreasing order: *Roche® PrionScreen*, *Bio-Rad® TeSeE™ SAP* and *Prionics® - Check PrioSTRIP*



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Benestad *et al. Vet Res* (2016) 47:88
DOI 10.1186/s13567-016-0375-4



VETERINARY RESEARCH

SHORT REPORT

Open Access



First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer

Sylvie L. Benestad^{1*}, Gordon Mitchell², Marion Simmons³, Bjørnar Ytrehus⁴ and Turid Vikøren¹

Rapid tests and CWD

PLOS ONE

RESEARCH ARTICLE

Are rapid tests and confirmatory western blot used for cattle and small ruminants TSEs reliable tools for the diagnosis of Chronic Wasting Disease in Europe?

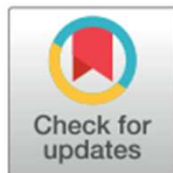
Maria Mazza¹*, Linh Tran², Daniela Loprevite¹, Maria C. Cavarretta¹, Daniela Meloni¹, Luana Dell'Atti¹, Jørn Våge², Knut Madslie², Tram T. Vuong², Elena Bozzetta¹‡, Sylvie L. Benestad²‡

1 European Reference Laboratory for Transmissible Spongiform Encephalopathies - Italian Reference Laboratory for Transmissible Spongiform Encephalopathies, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, **2** World Organisation for Animal Health (WOAH, founded as OIE) - Reference Laboratory for Chronic Wasting Disease, Norwegian Veterinary Institute, Oslo, Norway

☯ These authors contributed equally to this work.

‡ EB and SLB also contributed equally to this work.

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Rapid tests and CWD

(IDEXX) and HerdChek CWD Ag test (IDEXX). The second set of samples was tested in parallel in two different laboratories using the three commercial rapid tests: TeSeE™ SAP Combi Kit (Bio-Rad), TeSeE™ Sheep/Goats (Bio-Rad) and HerdChek BSE-Scrapie Ag test (IDEXX) and two confirmatory western blot methods, the one commercially available, TeSeE™ Western Blot (Bio-Rad) and one Scrapie Associated Fibrils (SAF) Immunoblot, developed at the Italian TSE Reference Laboratory (IRL).

The results of the present study showed that the most used rapid diagnostic tests are also able to identify the different strains of CWD circulating in the Nordic countries, although with different analytical sensitivity. HerdChek BSE-Scrapie Ag test and HerdChek CWD Ag test resulted the most sensitive and robust, especially in the moose samples, while both western blot methods adopted in these diagnostic investigations were able to confirm the presence of PrP^{Sc} in all samples positive by ELISA.



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WHAT ABOUT THE FUTURE OF RAPID TESTING?

- Strong pressure to dismiss active surveillance however...
- ...rapid testing can act as an early warning system in case of BSE reintroduction
- No new rapid test will be plausibly set up and validated, while few rapid tests are still available
- Perspectives for an *in vivo* test?



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Thank you!



Turin by night



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