20th Annual Meeting of the TSE EURL

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# Diagnosis of CWD in Europe: evaluation of the performances of rapid tests and confirmatory western blot methods

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# Chronic Wasting Disease

1967 in Colorado (*Odocoileus hemionus*)

Highly widespread in North America and Canada

2016 in Norway (reindeer and moose)


2018 in Finland and Sweden (moose)

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# Chronic Wasting Disease


**Screening of cervids by diagnostic rapid tests approved by the EC Regulation**

## Regulation EU 2017/1972

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# OIE Terrestrial Manual - chapter 1.1.6. (OIE, 2018)


Screening tests should be validated for the species in which they will be used

There are three rapid tests that are commercially available and approved for the diagnosis of TSE in cattle and small ruminants:

- TeSeE™ SAP Combi Kit (Bio-Rad)
- TeSeE™ Sheep/Goat, (Bio-Rad)
- HerdChek BSE-Scrapie Antigen (Ag) test (IDEXX)

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# CRITICAL ISSUE

**Data not exhaustive for rapid tests in cervids affected with CWD**

paucity of positive reference samples from European CWD samples

**Novel Type of Chronic Wasting Disease Detected in Moose (*Alces alces*), Norway**

Laura Pirisinu, Linh Tran, Barbara Chiappini, Ilaria Vanni, Michele A. Di Bari, Gabriele Vaccari, Turid Vikøren, Knut Ivar Madslien, Jørn Våge, Terry Spraker, Gordon Mitchell, Aru Balachandran, Thierry Baron, Cristina Casalone, Christer M. Rolandsen, Knut H. Røed, Umberto Agrimi, Romolo Nonno, Sylvie L. Benestad

Tranulis *et al. Acta Veterinaria Scandinavica* (2021) 63:48
https://doi.org/10.1186/s13028-021-00606-x

Acta Veterinaria Scandinavica

REVIEW Open Access

**Chronic wasting disease in Europe: new strains on the horizon**

Michael Andreas Tranulis[1*], Dolores Gavier-Widén[2,3], Jørn Våge[4], Maria Nöremark[2], Sirkka-Liisa Korpenfelt[5], Maria Hautaniemi[5], Laura Pirisinu[6], Romolo Nonno[6] and Sylvie Lafond Benestad[4]

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**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 Mazza, Linh Tran, Daniela Loprevite, Maria C. Cavarretta, Daniela Meloni, Luana Dell'Atti, Jørn Våge, Knut Madslien, Tram T. Vuong, Elena Bozzetta , Sylvie L. Benestad

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# AIMS

Evaluate the diagnostic performance of three of the so-called 'rapid' tests, commercially available and approved for TSE diagnosis in cattle and small ruminants, to detect CWD strains circulating in Europe.

Compare the performance of the three screening tests with that of two different Western Blot confirmatory methods

**Sensitivity and specificity**

**Analytical sensitivity**

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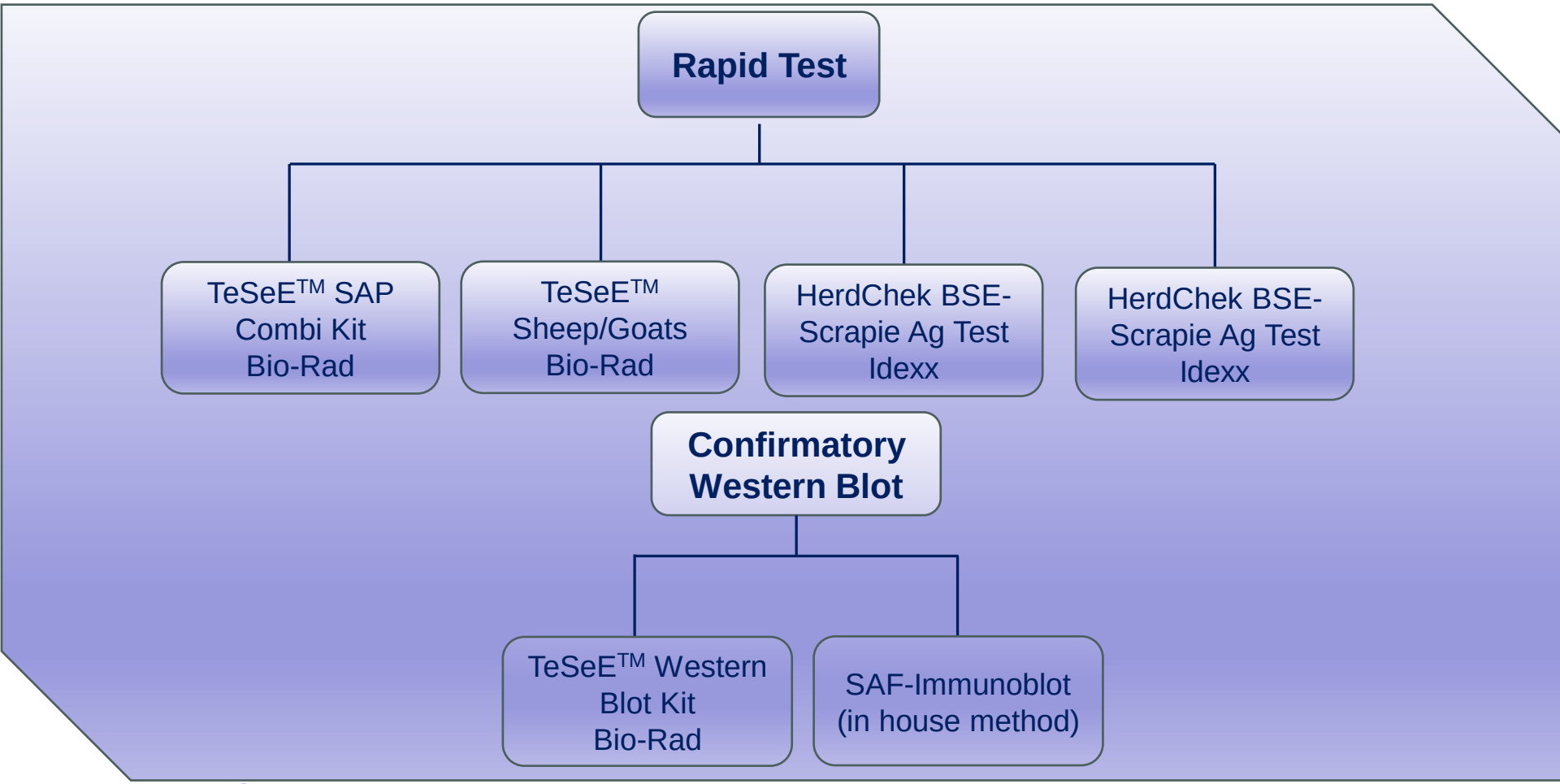
# Materials and methods (1/8)

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# Materials and methods (2/8)

CWD+

CWD -

| Species | CWD status | ID Number | Code | Prp genotype | Sex | Area | Set 1 | Set 2 |
|---|---|---|---|---|---|---|---|---|
| Moose | positive | 16-P138 | Moose A | $KK_{109}$ | Female | Selbu | X | X |
| | positive | 16-P153 | Moose B | $KK_{109}$ | Female | Selbu | | X |
| | positive | 17-CD11399 | Moose C | $KK_{109}$ | Female | Lierne | X | X |
| | positive | 19-CD24854 | Moose D | $QQ_{109}$ | Female | Sigdal | | X |
| | positive | 20-CD3380 | Moose E | $KK_{109}$ | Female | Steinkjer | | X |
| Reindeer | positive | 17-CD2788 | Reindeer A | A/C | Male | Nordfjella | X | |
| | positive | 17-CD20830 | Reindeer B | C/C | Male | Nordfjella | X | |
| | positive | 17-CD20831 lymph node | Reindeer B lymph node | C/C | Male | Nordfjella | X | |
| Moose | negative | 20-CD4385 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 20-CD4384 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 20-CD4380 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 20-CD4379 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 20-CD38 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 20-CD97 | //// | n.a. | n.a. | n.a. | | X |
| | negative | 18-80-55 | //// | n.a. | n.a. | n.a. | X | |
| | negative | 18-80-58 | //// | n.a. | n.a. | n.a. | X | |
| | negative | 18-04-V179 | //// | n.a. | n.a. | n.a. | X | |
| Reindeer | negative | 18-80-43 | //// | n.a. | n.a. | n.a. | X | |
| | negative | 18-80-57 | //// | n.a. | n.a. | n.a. | X | |
| | negative | 18-80-80 | //// | n.a. | n.a. | n.a. | X | |
| | negative | 18-80-78 | //// | n.a. | n.a. | n.a. | X | |

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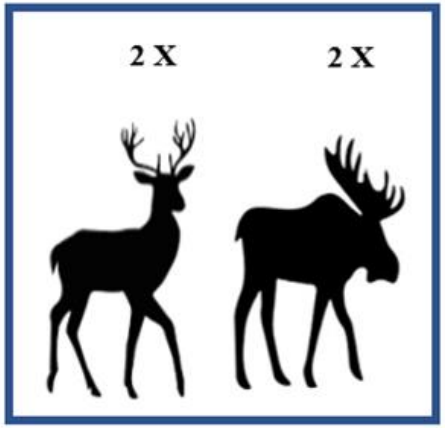
# Materials and methods (3/8)

Brain material from two moose, two reindeer and a retropharyngeal lymph node from one of the two reindeer were collected

Nervous tissue from each animal was thoroughly chopped and mixed well until the tissue appeared homogeneous

Each sample was diluted in negative brain material. Dilutions series from 1:2 to 1:128 were prepared. Each sample was analysed in duplicates




**SET 1: Homogenate samples prepared at NVI**

**Analyses at NVI**

- HerdChek CWD Antigen Test, IDEXX
- HerdChek BSE/Scrapie Antigen Test, IDEXX
- TeSeE™ SAP Combi Kit, Bio-Rad
- TeSeE™ Sheep/Goat, Bio-Rad

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# Materials and methods (4/8)


**SET 2: Homogenate samples prepared at IRL**

| Analyses at NVI | Analyses at IRL |
|---|---|
| • HerdChek BSE/Scrapie Antigen Test, IDEXX | • HerdChek BSE/Scrapie Antigen Test, IDEXX |
| • TeSeE™ Sheep/Goat, Bio-Rad | • TeSeE™ SAP, Bio-Rad |
| • TeSeE™ Western Blot, Bio-Rad | • Confirmatory SAF- Immunoblot |

Brain from five Norwegian CWD moose was prepared at the Italian TSE Reference Laboratory (IRL)

Brain samples were subjected to a pre-homogenisation protocol. A 50% w/v homogenate was made from CWD brain tissues in distilled water

Each sample was diluted in negative brain material. Dilutions series from 1:2 to 1:128 were prepared

The resulting homogenate was aliquoted into pre-labelled cryotubes distributed into either Bio-Rad or IDEXX grinding tubes

All dilutions of each moose sample were analysed in parallel at the NVI and the IRL

Each sample was analysed in duplicate or triplicate with the three screening tests and two WB methods

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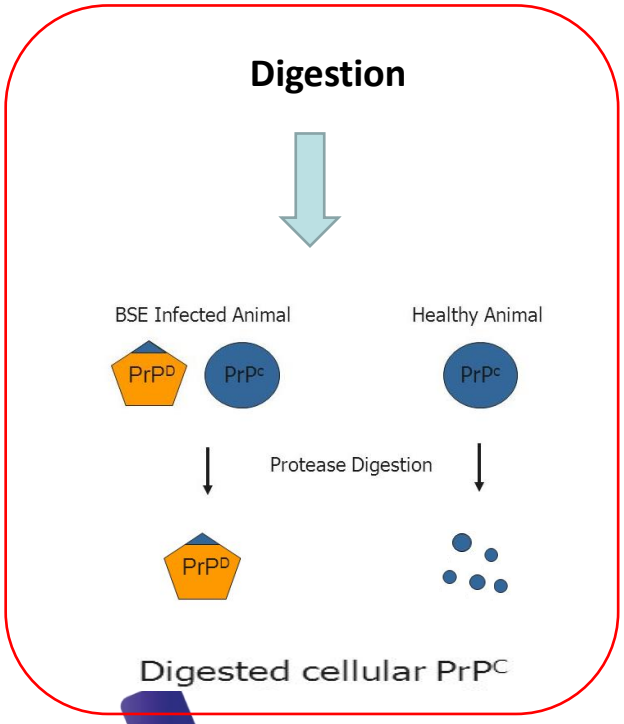
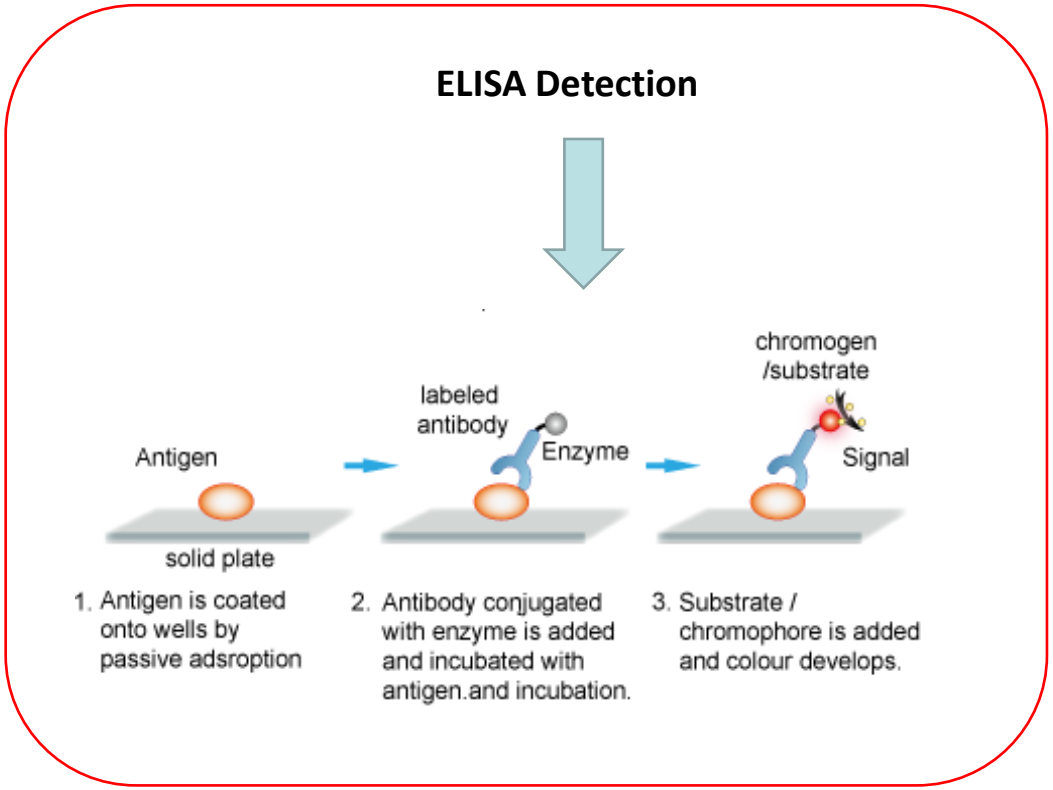
# Materials and methods (5/8)

## TeSeE™ SAP Combi Kit

## TeSeE™ Sheep and Goat

Enzyme immunoassay (sandwich ELISA) using 2 monoclonal antibodies for the detection of $PrP^{Sc}$

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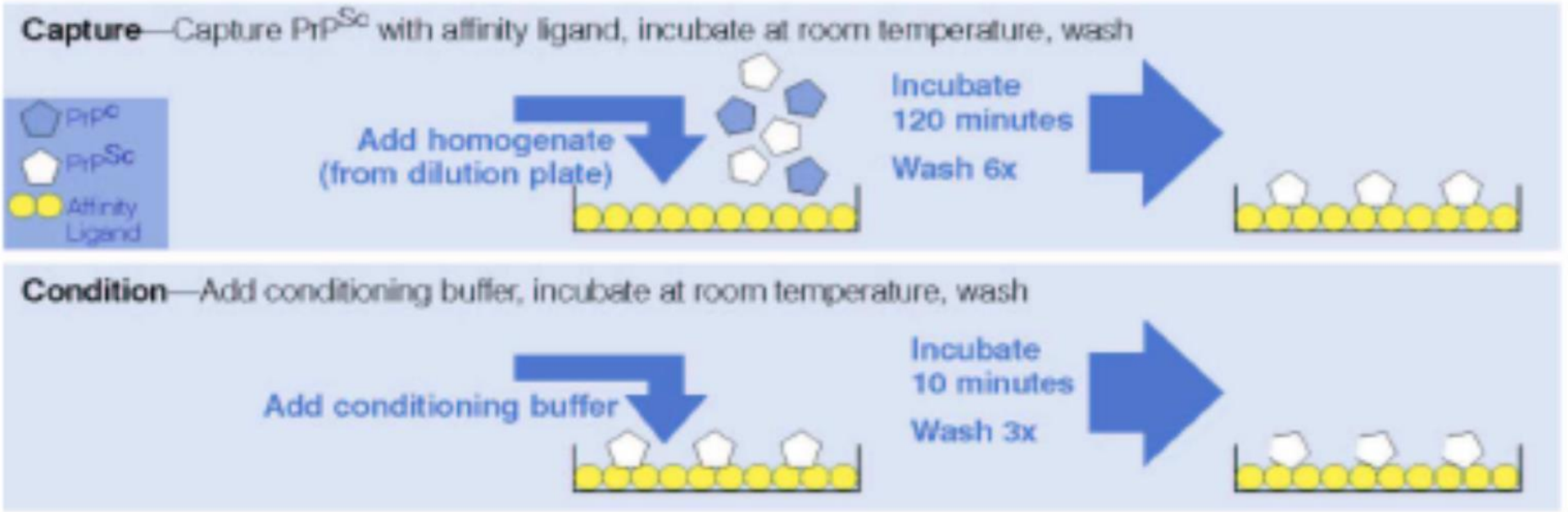
Short protocol

Ultra short protocol

# Materials and methods (6/8)

## Idexx HerdChek BSE-Scrapie Antigen Kit




This test does not involve any digestion with PK but uses a particular ligand that can capture $PrP^{Sc}$ by a specific conformational recognition of $PrP^{Sc}$ aggregates

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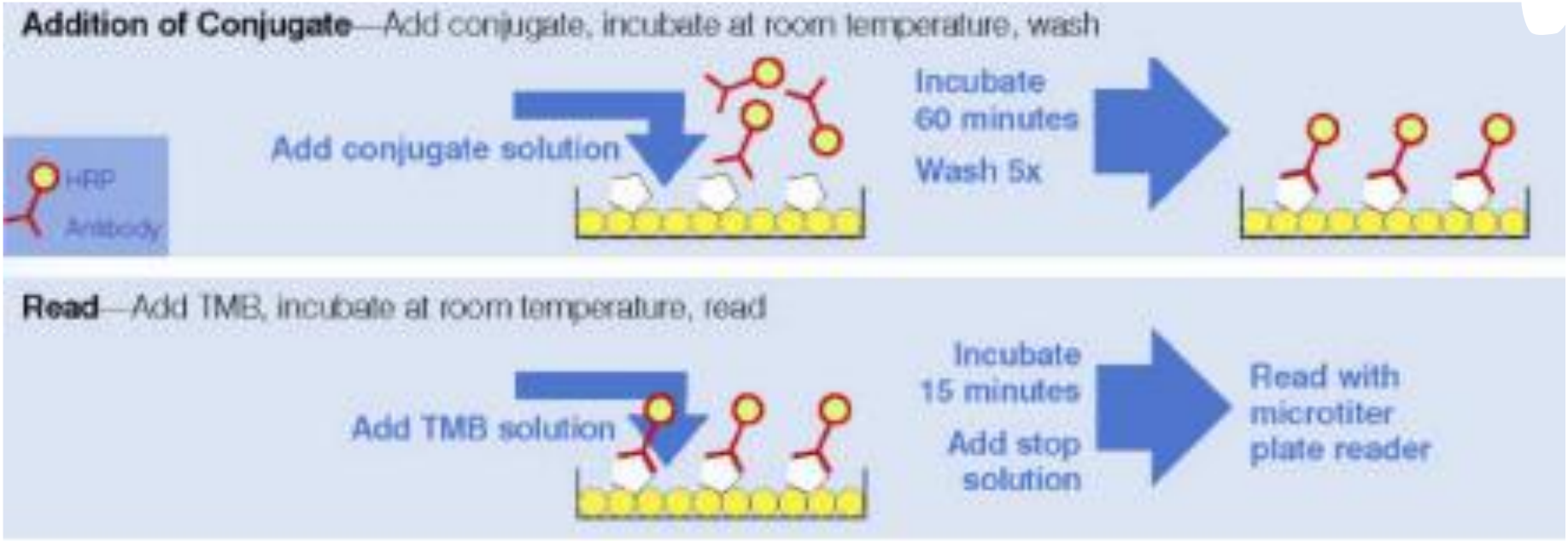
# Materials and methods (7/8)

## Idexx HerdChek CWD Antigen Kit




Abnormal PrP is detected using the kit conjugated anti-PrP antibody, Conjugate concentrate (CC) both for bovine and for small ruminants

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# Materials and methods (6/8)

## Idexx HerdChek CWD Antigen Kit

**HerdChek CWD Antigen Kit** ≠ **HerdChek BSE-Scrapie Antigen Kit**

- longer incubation times
- different cut-off value


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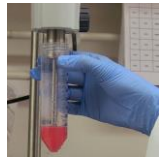
# Materials and methods (8/8)

## Confirmatory Western Blot



**TeSeE™ Western Blot**
**Bio-Rad**

**SAF- Immunoblot**

The test was carried out according to the manufacturer's instructions with slight modifications.

It is an in-house method based on pK digestion and centrifugation steps to concentrate the pathological prion protein. Immunodetection performed by 5 mAbs: 6H4, Sha31, 9A2, SAF84 and L42.

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## SET 1 - NVI

The O.D. represents the mean of duplicates values obtained from each dilution sample. The values above the cut-off indicate positive sample and those below the cut-off indicate negative sample.

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| ID sample | Dilution | HerdCheck CWD | HerdCheckBSE/Scrapie Ag Test with Bovine conjugate, ultra short protocol | HerdCheckBSE/Scrapie Ag Test with Bovine conjugate, short protocol | HerdCheckBSE/Scrapie Ag Test with small ruminants conjugate | TeSeE™ SAP | TeSeE™ Sheep & Goat |
|---|---|---|---|---|---|---|---|
| Moose A | 1 = 2 | 3, 45 | 3,5 | 3,5 | 3,382 | 0,069 | 2,788 |
| | 1 = 4 | 3,445 | 3,274 | 3,387 | 3,095 | 0,06 | 2,143 |
| | 1 = 8 | 3,247 | 1,659 | 3,18 | 2,675 | 0,057 | 1,159 |
| | 1 = 16 | 2,958 | 1,022 | 2,482 | 1,749 | 0,025 | 0,808 |
| | 1 = 32 | 2,027 | 0,569 | 1,364 | 1,072 | 0,012 | 0,442 |
| | 1 = 64 | 1,148 | 0,431 | 0,967 | 0,62 | 0,012 | 0,182 |
| | 1 = 128 | 1,037 | 0,305 | 0,773 | 0,556 | 0,014 | 0,077 |
| Moose C | 1 = 2 | 3,5 | 3,5 | 3,5 | 3,392 | 0,04 | 2,783 |
| | 1 = 4 | 3,453 | 3,269 | 3,5 | 3,219 | 0,03 | 2,283 |
| | 1 = 8 | 3,366 | 1,85 | 3,27 | 2,632 | 0,02 | 1,695 |
| | 1 = 16 | 2,94 | 1,186 | 2,699 | 1,801 | 0,016 | 0,77 |
| | 1 = 32 | 2,1418 | 0,595 | 2,071 | 1,324 | 0,012 | 0,702 |
| | 1 = 64 | 1,711 | 0,404 | 1,402 | 0,936 | 0,015 | 0,093 |
| | 1 = 128 | 0,899 | 0,187 | 0,815 | 0,483 | 0,009 | 0,043 |
| Reindeer A | 1 = 2 | 1,749 | 0,262 | 1,655 | 1,267 | 0,019 | 0,181 |
| | 1 = 4 | 0,936 | 0,106 | 0,841 | 0,506 | 0,016 | 0,115 |
| | 1 = 8 | 0,487 | 0,059 | 0,456 | 0,318 | 0,014 | 0,05 |
| | 1 = 16 | 0,259 | 0,039 | 0,245 | 0,199 | 0,011 | 0,038 |
| | 1 = 32 | 0,148 | 0,038 | 0,131 | 0,118 | 0,011 | 0,03 |
| | 1 = 64 | 0,089 | 0,04 | 0,08 | 0,069 | 0,008 | 0,03 |
| | 1 = 128 | 0,062 | 0,035 | 0,059 | 0,054 | 0,009 | 0,015 |
| Reindeer B | 1 = 2 | 3,11 | 2,837 | 3,102 | 2,343 | 1,97 | 2,793 |
| | 1 = 4 | 2,536 | 1,825 | 1,743 | 1,383 | 1,081 | 2,651 |
| | 1 = 8 | 1,422 | 0,619 | 0,963 | 0,791 | 0,514 | 1,136 |
| | 1 = 16 | 0,786 | 0,312 | 0,512 | 0,409 | 0,225 | 0,573 |
| | 1 = 32 | 0,399 | 0,193 | 0,285 | 0,24 | 0,098 | 0,57 |
| | 1 = 64 | 0,234 | 0,106 | 0,172 | 0,15 | 0,047 | 0,265 |
| | 1 = 128 | 0,156 | 0,069 | 0,107 | 0,109 | 0,028 | 0,137 |
| Reindeer B lymph node | 1 = 2 | 3,162 | 3,5 | 3,26 | 2,603 | 2,724 | 3,202 |
| | 1 = 4 | 2,473 | 3,427 | 2,369 | 1,238 | 1,876 | 2,389 |
| | 1 = 8 | 1,398 | 2,263 | 1,521 | 0,945 | 1,035 | 2,368 |
| | 1 = 16 | 0,769 | 1,185 | 0,658 | 0,474 | 0,495 | 0,965 |
| | 1 = 32 | 0,323 | 0,703 | 0,355 | 0,243 | 0,271 | 0,263 |
| | 1 = 64 | 0,219 | 0,397 | 0,207 | 0,149 | 0,159 | 0,568 |
| | 1 = 128 | 0,12 | 0,21 | 0,105 | 0,095 | 0,099 | 0,248 |
| Cut-Off | | 0,175 | 0,149 | 0,149 | 0,149 | 0,228 | 0,148 |


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RESULTS (2/4)

## SET21 – NVI & IRL

The O.D. represents the mean of duplicates values obtained from each dilution sample. The values above the cut-off indicate positive sample and those below the cut-off indicate negative sample.

| ID sample | Dilution | HerdCheckBSE/Scrapie Antigen Test with Bovine conjugate, short protocol | | TeSeE™ Sheep & Goat | TeSeE™ SAP | TeSeE™ Western Blot | SAF–Immunoblot | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NVI–Optical density | IRL–Optical density | Optical density | Optical density | Sha31 | Sha31 | 6H4 | 9A2 | L42 | SAF84 |
| Moose A | undiluted | 3,382 | 3,317 | 0,176 | 0,017 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 2 | 3,505 | 3,314 | 0,158 | 0,014 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 4 | 3,5 | 3,307 | 0,072 | 0,015 | Pos | Pos | Pos | Neg | Neg | Pos |
| | 1 = 8 | 2,947 | 3,097 | 0,04 | 0,012 | Pos | Pos | Neg | Neg | Neg | Pos |
| | 1 = 16 | 2,164 | 2,6 | 0,047 | 0,013 | Pos | Pos | Neg | Neg | Neg | Pos |
| | 1 = 32 | 1,555 | 2,068 | 0,027 | 0,012 | Pos | Pos | Neg | Neg | Neg | Pos |
| | 1 = 64 | 1,011 | 1,039 | 0,021 | 0,012 | Pos | Pos | Neg | Neg | Neg | Pos |
| | 1 = 128 | 0,711 | 0,694 | 0,024 | 0,025 | Pos | Pos | Neg | Neg | Neg | Neg |
| Moose B | undiluted | 3,418 | 3,395 | 0,08 | 0,022 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 2 | 3,478 | 3,374 | 0,101 | 0,012 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 4 | 3,152 | 3,287 | 0,031 | 0,014 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 8 | 2,28 | 2,878 | 0,025 | 0,014 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 16 | 1,555 | 1,919 | 0,014 | 0,012 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 32 | 0,815 | 1,527 | 0,027 | 0,01 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 64 | 0,45 | 0,666 | 0,012 | 0,011 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 128 | 0,277 | 0,354 | 0,013 | 0,012 | Pos | Pos | Neg | Neg | Neg | Neg |
| Moose C | undiluted | 3,372 | 3,24 | 0,027 | 0,461 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 2 | 3,509 | 3,274 | 0,051 | 0,151 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 4 | 3,5 | 3,263 | 0,061 | 0.024 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 8 | 2,957 | 3,067 | 0,025 | 0,018 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 16 | 2,291 | 2,571 | 0,032 | 0,013 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 32 | 1,481 | 1,901 | 0,026 | 0,018 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 64 | 0,994 | 1,292 | 0,019 | 0,036 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 128 | 0,638 | 0,794 | 0,016 | 0,016 | Pos | Pos | Neg | Neg | Neg | Neg |
| Moose D | undiluted | 3,372 | 3,192 | 2.17 | 0,501 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 2 | 3,5 | 3,198 | 1,987 | 0,446 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 4 | 3,5 | 3,185 | 0,651 | 0,336 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 8 | 3,432 | 3,408 | 1,624 | 0,202 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 16 | 2,906 | 3,366 | 0.543 | 0,329 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 32 | 2,139 | 2,878 | 0,672 | 0,129 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 64 | 1,386 | 1,725 | 0,201 | 0,107 | Pos | Pos | Pos | Pos | Pos | Pos |
| | 1 = 128 | 0,949 | 1,076 | 0,118 | 0,053 | Pos | Pos | Pos | Pos | Neg | Neg |
| Moose E | undiluted | 0,627 | 0,332 | 0,02 | 0,012 | Pos | Pos | Neg | Pos | Neg | Pos |
| | 1 = 2 | 0,253 | 0,3 | 0,021 | 0,012 | Pos | Pos | Neg | Pos | Neg | Pos |
| | 1 = 4 | 0,137 | 0,196 | 0,013 | 0,011 | Pos | Pos | Neg | Neg | Neg | Neg |
| | 1 = 8 | 0,071 | 0,118 | 0,011 | 0,01 | Pos | Neg | Neg | Neg | Neg | Neg |
| | 1 = 16 | 0,044 | 0,079 | 0,011 | 0,011 | Neg | Neg | Neg | Neg | Neg | Neg |
| | 1 = 32 | 0,028 | 0,047 | 0,014 | 0,008 | Neg | Neg | Neg | Neg | Neg | Neg |
| | 1 = 64 | 0,029 | 0,042 | 0,017 | 0,009 | Neg | Neg | Neg | Neg | Neg | Neg |
| | 1 = 128 | 0,021 | 0,063 | 0,017 | 0,008 | Neg | Neg | Neg | Neg | Neg | Neg |
| Cut-Off | | 0,168 | 0,191 | 0,151 | 0,116 | ////// | ////// | ////// | ////// | ////// | ////// |

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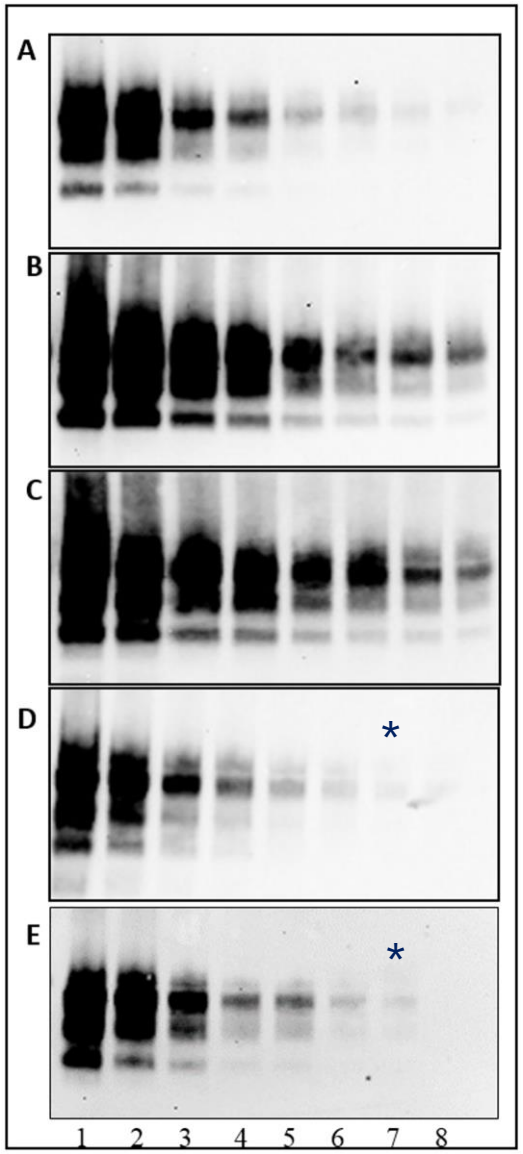
RESULTS (3/4)

## Moose D

Lane 1 = undiluted;
lane 2 = dilution 1:2;
lane 3 = dilution 1:4;
lane 4 = dilution 1:8;
lane 5 = dilution 1:16;
lane 6 = dilution 1:32;
lane 7 = dilution 1:64;
lane 8 = dilution 1:128.

## SAF-IMMUNOBLOT




6H4

Sha31

9A2

SAF84

L42

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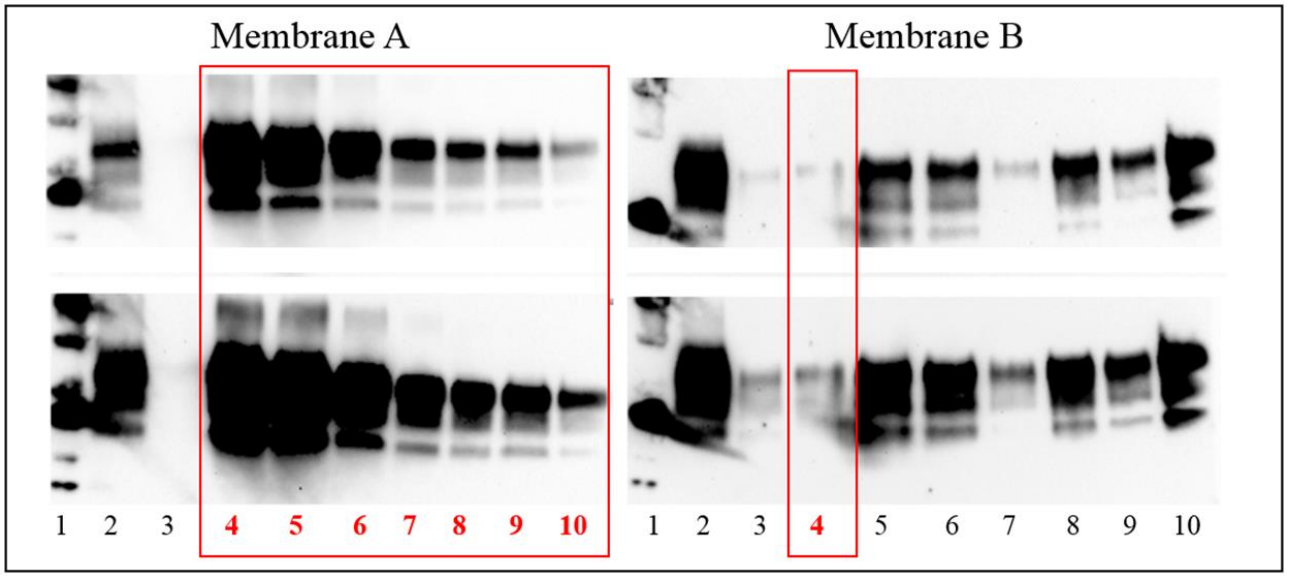
RESULTS (4/4)

# TeSeE™ WESTERN BLOT




Moose D

**Moose A, B, C**

**Membrane A.** Lane 1 = molecular weight; lane 2 = positive classical scrapie; 3 = negative moose control; lane 4 to 10: Moose D; lane 4 = undiluted; lane 5 = dilution 1:2; lane 6 = dilution 1:4; lane 7 = dilution 1:8; lane 8 = dilution 1:16; lane 9 = dilution 1:32; lane 10 = dilution 1:64.

**Membrane B.** Lane 1 = molecular weight; 2 = positive classical scrapie; lane 3 = Moose E undiluted; lane 4 = Moose D dilution 1:128; lane 5 to 7: Moose A; lane 5 = undiluted; lane 6 = dilution 1:2; lane 7 = dilution 1:4; lane 8 to 9: Moose B; lane 8 = undiluted; lane 9 = dilution 1:2; lane 10 = Moose C undiluted.

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## CONCLUSIONS

All rapid tests are able to identify the different strains of CWD circulating in the Nordic countries but 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.

The analytical sensitivity of both western blot methods was similar or higher to that of rapid tests, validating their ability to confirm CWD cases identified by the screening

Critical issue

Limitations related to the small number of animal samples, due to the lack of tissue especially from reindeer, do not allow to draw exhaustive diagnostic

**This study represents the first direct comparison between different diagnostic methods on European CWD cases. Despite the small number of samples, it is conceivable that the rapid and confirmatory diagnostic systems applied in Northern Europe for the CWD surveillance in cervid populations are reliable tools.**

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# many thanks for your attention!!!

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