During active surveillance with a different type of test kit to be approved by the Swiss authorities in 1998. In 1999 it was officially acknowledged by the EU as the only test to achieve 100% sensitivity and 100% specificity without retesting. In the CRL Analytical sensitivity study in 2009 was concluded that Prionics®-Check WESTERN performed as the only test to achieve this sensitivity and specificity. Following EURL approval, details of the modification, shall be communicated to the Commission and to European Union National Reference Laboratories.

Test Principle

After Sample Collection and Registration, samples are analyzed with the Prionics®-Check WESTERN. The Prionics®-Check WESTERN follows a five step protocol, consisting of Homogenization, Protease digestion, Gel Electrophoresis, Blotting and Immunological Detection. One person can process 100 samples (duplicate assays) within 6-8 hours.

Samples are collected, registered, and a homogenate is prepared from a defined piece of brain tissue. Treatment with Proteinase K degrades PrP completely while PrPSc is reduced to the 27 - 30 kD fragment. The proteolytic reaction is stopped, and PrPSc is detected in the Prionics®-Check WESTERN assay.

Digested homogenates are subjected to gel electrophoresis and Western blotting. The blot membranes are incubated with a monoclonal antibody – with high affinity for PrP – for the detection of protease resistant PrPSc. The signal is visualized using the secondary antibody-alkaline phosphatase (AP) conjugate.

Various tissues of a prion-infected animal contain a pathologically altered, disease specific form of the prion protein, PrPSc. The altered prion protein is denominated PrPSc. The normal isomorph of PrP is termed PrP (the cellular form of PrP).

PrPSc differs from PrP in its protease resistance: Upon treatment with Proteinase K, PrPSc is degraded, while PrP is reduced from its original size of 32-35 kD to a smaller size of 27-30 kD. The remaining protease-resistant PrPSc fragment is referred to as PrP27-30.

The Prionics®-Check WESTERN achieves its high precision and reliability by monitoring three independent criteria: protease-resistance, glycosylation pattern and lower molecular weight of the protease-resistant PrPSc-fragment (27-30 kD) compared to normal, undigested PrP.

The unique properties of the buffer solutions used in Prionics®-Check WESTERN and the high affinity of the antibodies can be confirmed by the modification of the dilution with tissue homogenates combining the reliability of the Western blotting procedure with the speed needed for mass screening.

The Prionics®-Check WESTERN was the first BSE-test kit to be approved by the Swiss authorities in 1998. In 1999 it was officially acknowledged by the EU as the only test to achieve 100% sensitivity and 100% specificity without retesting. In the CRL Analytical sensitivity study in 2009 was concluded that Prionics®-Check WESTERN performed with a maximal 2 log10 inferiority range as compared to the most sensitive test system.

The validation data for this kit have been certified by the OIE, based on expert review, as fit for the following purposes:

1. To confirm diagnosis of suspect or clinical cases (includes confirmation of a positive screening test);
2. To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control, e.g. surveys, implementation of disease control measures) and to assist in the demonstration of the efficiency of control policies;
3. To confirm a non-negative test result obtained during active surveillance with a different type of test.

Producers of TSE rapid tests must have a quality assurance system in place, agreed by the European Union Reference Laboratory (EURL), which ensures that test performance does not change. Sampling tools and modifications to the rapid test or to the test protocol (including sampling) may only be made following advance notification to the EURL and will only be granted provided that the EURL finds that the modification does not reduce the sensitivity, specificity or reliability of the rapid test. Following EURL approval, details of the modification, shall be communicated to the Commission and to European Union National Reference Laboratories.

The validation data for this kit have been certified by the OIE, based on expert review, as fit for the following purposes:

1. To confirm diagnosis of suspect or clinical cases (includes confirmation of a positive screening test);
2. To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control, e.g. surveys, implementation of disease control measures) and to assist in the demonstration of the efficiency of control policies;
3. To confirm a non-negative test result obtained during active surveillance with a different type of test.

Prionics®-Check WESTERN

Test for in vitro detection of TSE-related PrPSc

Within the European Union, this test is approved as rapid test for the BSE testing program on cattle which is set up in accordance with Regulation (EC) No 999/2001

Kit for 100 samples (duplicate analyses)
©Prionics AG
Version 12.0_e

Component 1
Homogenisation Buffer Concentrate (5x)
(5x concentrate, dilute before use). One bottle containing 200 ml of 5x concentrated Homogenization Buffer. Prepare 1x homogenisation working solution by mixing 1 part Homogenisation Buffer (5x) with 4 parts purified water.

Component 2
Digestion Buffer (1x) (Ready-to-use)
One vial containing 4 ml of Digestion Buffer. Cap color code: yellow

Component 3
Proteinase K (Ready-to-use)
One vial containing 4 ml of Proteinase K. Cap color code: white

Component 4
Digestion-Stop (1x) (Ready-to-use)
One vial containing 4 ml of Proteinase K blocker to stop proteolytic activity of the Proteinase K. Cap color code: red

Component 5
Control Sample (Ready-to-use)
One vial containing 200 µl functional control (normal PrPSc) and molecular weight markers (97/68/45/30/20/14 kD) in PAGE Sample Buffer. Mix before use, e.g. by flicking the tube.

Component 6
PAGE Sample Buffer (1x) (Ready-to-use)
One vial containing 25 ml of Sample Buffer for SDS PAGE Gel Electrophoresis (PAGE). (Contains 3-mercaptoethanol. Opened vials release a bad smell. However, even if 100 vials are opened simultaneously in a normal airtight room, air concentrations do not reach the Workplace Environmental Exposure Level of 0.65 mg/m3 defined by the American Industrial Hygiene Association.)

Component 7
PVDF Blocking Buffer Concentrate (5x)
(5x concentrate, dilute before use). One bottle containing 100 ml of concentrated Blocking Buffer to block unspecific binding sites. Dilute 100 ml of Blocking Buffer with purified water to a final volume of 0.5 liter.

Component 8
1. Antibody 6H4
One vial containing 30 µl of monoclonal antibody to PrP (mouse anti-PrP IgG1). Working dilution: 1:5000 (In case fluid sticks to wall or lid, the tube can be centrifuged).

Component 9
2. Antibody-AP
One vial containing 30 µl of goat anti-mouse IgG-AP, an antibody to mouse IgG that is conjugated to alkaline phosphatase. Working dilution: 1:5000 (In case fluid sticks to wall or lid, the tube can be centrifuged).

Component 10
Luminescence Buffer Concentrate (10x)
(10x concentrate, dilute before use). One bottle containing 27 ml of Luminescence Buffer concentrate. Dilute with purified water to 270 ml before use.
Additional Kit Contents:
- Package insert
- Labels for working solutions

Additional Material Required

- The highlighted items have been validated for the use with the PrioCLIP® Check WESTERN. The use of different devices is in the responsibility of the user.
- Please see also our list "PrioCLIP® Check WESTERN additional material and devices" for more information (contact your local distributor or info@prionics.com).

General:
- Laboratory equipment according to national safety regulations
  - Purified water: at least equivalent to Grade 3 water as defined by ISO 3696:1987 (E)
  - Single channel pipette (1 - 10 µl)
  - Single channel pipette (10 - 100 µl)
  - Single channel pipette (100 - 1000 µl)
  - Single channel pipette (1 - 5 ml)
  - Multichannel pipette (0.5 - 10 µl)
  - Multichannel pipette (10 - 100 µl)
  - Pipette tips (as recommended by pipette manufacturer)
  - Solution reservoirs
  - Incubation trays
  - 15 ml conical tubes
  - 50 ml conical tubes

Homogenization:
- Cutting tool and forceps
- Balance
- Dispenser for homogenization working solution
- 1.2 ml 96-deep well plate (used as sample Master Plate)
- PrioGENIZER™ homogenization device with six racks and one tray (Prionics AG, Product No: 10010)
- or FASTH/MediFASTH or FASTH 2 homogenization device (Consul AR S.A., Product No: 80300)
  - or Omnistem homogenization device (Omni International Inc., Product No: TH220P) and Omni tips (Omni International Inc., Product No: 32750)

Protease Digestion:
- 96-well microplates (0.2 ml wells; used as Digestion Plate)
- Sealing film
- Microplate incubator (reaching at least 100 °C)

Gel Electrophoresis:
- 12% NuPAGE Gels (17 slots) (Invitrogen™, Product No: NP0349BOX)
- NuPAGE MOPS/SDS Running Buffer (Invitrogen™, 500 ml: Product No: NP0001; 5 l: Product No: NP0001-02)
- NuPAGE Antioxidant (Invitrogen™, Product No: NP0005)

Blotting:
- PVDF membrane, Immobilon-P 0.45 µm (Millipore, Product No: IPVH 00010)
- Methanol (approx. 98%)
- Transfer Buffer (10x): 30.28 g Tris base/144.13 g Glycine/ add purified water to 1000 ml.

Immunological Detection:
- Tris-Buffered-Saline (TBS, pH 7.4): 8 g NaCl/0.2 g KCl/3 g Tris base. Add purified water to 1000 ml, adjust pH to 7.4 with HC1.
- Tris-Buffered-Saline with Tween (TBST): TBS with 0.05% (v/v) Tween-20
- Ponceau S (20x): 0.5% (w/v) Ponceau S/5% (v/v) acetic acid. Dilute with TBST to 1x for use
- CDP-Star concentrate (+ Alkaline Phosphatase Substrate) (Applied Biosystems, 12.5 mM; Cat No: M90509) or Roche Diagnostics GmbH, (25 mM, Cat No. 1759051) or CDP-Star, ready-to-use (Roche Diagnostics; Cat No. 2041677)
- X-Ray films

Test Procedure

Precautions
- National guidelines for working with prions must be strictly followed (see also section “Safety Regulations and R&S Statements” Appendix V). The PrioCLIP® Check WESTERN must be performed in laboratories suited for this purpose.
- Persons performing the test have to be trained generally in working with prions and specifically in performing the PrioCLIP® Check WESTERN.
- Samples should be considered as potentially infectious and all items which were in contact with the samples as potentially contaminated.

Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix V).

Notes
- To achieve optimal results with the PrioCLIP® Check WESTERN, the following aspects must be considered:
  - The Test Procedure protocol must be strictly followed.
  - Pipette tips have to be changed for every pipetting step.
  - The use of either pipette filter tips or separate pipettes for the different pipetting steps is strongly recommended. In addition, the accuracy of pipettes should be calibrated regularly.
  - Separate solution reservoirs must be used for each reagent.
  - Kit components must not be used after their expiry date or if changes in their appearance are observed.
  - Kit components of different kit lot numbers must not be used together.
  - Non-disposable cutting tools and forceps must be decontaminated according to guidelines enforced by national authorities.
  - When the PrioGENIZER™ is used for homogenization, only program P0 PRIONICS TSE must be used for homogenization of brain tissue.

Sampling and Homogenization

- Take 0.45 - 0.70 g nervous tissue from the preferred area of the left or the right side of the brainstem with e.g. a scalpel.

Sampling and laboratory testing must follow the regulations (EC) No 999/2001. Chapter C, which refers in terms of collection of samples to the latest edition of the "Manual Standards for Diagnostic Test and Vaccines of the International Office of Epizootic Diseases (OIE)" stating: "The preferred sample for immunosassay should be at, or as close to the obex as possible, but no further than 1.5 cm anterior to the obex." The picture below shows the sampling area within box 4.

Medulla oblongata
- The tissue sample is an approx. 8 cm long piece of brainstem/cervical spinal cord.

For a detailed sampling protocol contact info@prionics.com

Note: after sample collection, a complete hemi-section of the brain stem with an intact obex region must remain available for confirmatory testing

Homogenization:

Preparatory Steps
- Dilute 5x Homogenization Buffer (Component 1) with purified water to prepare homogenization working solution (Appendix I).

Homogenization
- Transfer sample to a homogenization container and determine weight on balance (0.45 - 0.70 g).
- Add ten volumes of homogenization working solution (w/v; e.g. 5 ml to 0.50 g brain tissue) and homogenize sample using the PrioGENIZER™ (Program P0 PRIONICS TSE), the FASTH/MediFASTH/FASTH 2 (45 sec ± 5 sec, 20’000 ± 1’000 rpm) or the Omnistem (60 ± 10 sec at maximum speed) homogenization device.
- Store two 1 ml samples per homogenate in a 96-well sample Master Plate. (From now on, each step will be done with two samples per original homogenate).
- PrioCLIP® and Prypcon homogenization containers of samples tested "TSE negative" may be washed for reuse (see PrioCLIP®/Prypcon Wash Protocol, Appendix IV).

Protease Digestion

Following amounts are for 48 samples
(See Appendix II for volumes needed for samples numbers other than 48)

Preparatory Steps
- Set the temperature of the microplate incubator to 48±1°C approx. 1 hour prior to use.
- Add 10 µl of Digestion buffer (Component 2) to each well of the Digestion Plate.

Protease Digestion
- Transfer 100 µl (mix first by pipetting up and down at least three times) of each homogenate from the Master Plate to the corresponding well of the Digestion Plate with a multichannel pipette. Afterwards, the Master Plate may be covered and stored at -20°C to -80°C for up to 12 months.
- Add 10 µl of Proteinase K (Component 3) to each well of the Digestation Plate and mix by pipetting up and down at least three times.
- Cover the Digestion Plate with a Sealing Film. Digest for 40±1 min at 48±1°C.
- Stop the reaction by adding 10 µl Digestion Stop (Component 4). Mix by pipetting up and down at least three times.

Gel Electrophoresis

Preparatory Steps
- Mount 17-slot 12% NuPAGE gels: Carefully remove the comb and white plastic foil at the bottom of the gel.
- Heat Control Sample (Component 5) to 65±3°C for 2 - 5 min.
- Set the temperature of the microplate incubator to 68±3°C approx. 1 hour prior to use.

Gel Electrophoresis
- Add 100 µl of PAGE Sample Buffer (Component 6) to the digested homogenate in the Digestion Plate and mix by pipetting up and down at least three times.
- Boil samples at 98±1°C for 5 min ± 30 s.
- The Digestion Plate may be covered with a Sealing Film and stored at 20°C to -50°C for up to 5 days.
- Previously prepared samples are heated to 65±3°C for 2 - 5 min before loading.

Sample loading:
- Load 10 µl of the Control Sample in the first lane.
- Load 10 µl of the heated samples per lane.
- Fill up inner and outer chamber with 1x NuPAGE SDS-MOPS Running Buffer and add 500 µl NuPAGE Antioxidant to the inner chamber only.
Blocking:
- Place membrane on Whatman Paper moistened with 1x Transfer Buffer or purified water.
- Open plastic frame of NuPAGE gel. Remove the top part of the gel containing the slots and the bottom part below the dye front. Place the gel on the membrane (avoid air-bubbles). Up to 6 gels can be placed on one membrane of the above size (see Appendix III).
- Overlay gels with moistened Whatman Paper, place sponge on top.
- Close transfer cassette and place in transfer unit. Proteins are negatively charged and move towards the positive (red) pole of the transfer unit. Make sure that the cassette is inserted with the PVDF membrane towards the positive pole and the gels towards the negative pole.
- Transfer at 150 V for 60±2 min at 5±3°C with continuous cooling.
- Remove the membrane and stain bound proteins with 1x Ponceau S. Label the position of the size markers. Destaining is performed with TBST until the red color has disappeared (approx. 2 x 1 min).

IMMUNOLOGICAL DETECTION

Blocking:
- Incubate the membrane in a plastic incubation tray with 50±2 ml of 1x PVDF Blocking Buffer (Component 7; see Appendix I for the dilution table) for 3±5 min at 22±3°C on a rocking platform with gentle agitation.

1st Antibody:
- Dilute 10 µl of 1. Antibody 6H4 (Component 8) in 50±2 ml of TBST (1:5000 dilution), add to membrane and incubate for 60±15 min at 22±3°C (or alternatively for 12-18 h at 5±3°C) with gentle agitation on a rocking platform.

2nd Antibody:
- Dilute 10 µl of 2. Antibody-AP (Component 9) in 50±2 ml of TBST (1:5000 dilution). Incubate for 30±1 min at 22±3°C with gentle agitation.

Transfer:
- Equilibrate membrane for 5 - 10 min in 50±2 ml 1x Luminescence Buffer (Component 10; see Appendix I for the dilution table).
- Dilute 100 µl CDP-Star (12.5 mM; 50x) or 50 µl (25 mM; 100x) in 5 ml 1x Luminescence Buffer.

General Remarks

Notice
This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

Liability
Prionics AG warrants its products will meet their applicable published specification when used in accordance with their applicable instructions and within the declared product life time. Prionics AG makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of Prionics AG products appearing in Prionics AG published catalogues and product literature may not be altered without express written agreement signed by an officer of Prionics AG. Representation, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Prionics AG’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Prionics AG promptly of any such breach. If after exercising reasonable efforts, Prionics AG is unable to repair or replace the product or part, then Prionics AG shall refund to the customer all monies paid for such applicable product or part.

Prionics AG shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by any customer from the use of its products.

Prionics AG is an ISO 9001:2000 certified company.

Appendix I

Tables for preparation of working solutions

Homogenization working solution
Mix indicated volumes of purified pure water and 5x Homogenization Buffer (Component 1) to obtain the desired volume of homogenization working solution:
- Shelf life of homogenization working solution: 1 week at 5±3°C

<table>
<thead>
<tr>
<th>Vol. of homogenization solution</th>
<th>Volume of Homogenization Buffer (5x) (Component 1)</th>
<th>Volume of purified water</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ml</td>
<td>50 ml</td>
<td>200 ml</td>
</tr>
<tr>
<td>500 ml</td>
<td>100 ml</td>
<td>400 ml</td>
</tr>
<tr>
<td>1000 ml</td>
<td>200 ml</td>
<td>800 ml</td>
</tr>
</tbody>
</table>

PVDF Blocking Buffer
Mix indicated volumes of purified water and 10x PVDF Blocking Buffer (Component 7) to obtain the desired volume of PVDF Blocking Buffer (1x):

<table>
<thead>
<tr>
<th>Vol. of PVDF Blocking Buffer (1x)</th>
<th>Vol. of PVDF Blocking Buffer (5x) (Component 7)</th>
<th>Vol. of purified water</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ml</td>
<td>100 ml</td>
<td>400 ml</td>
</tr>
</tbody>
</table>

Luminescence Buffer
Mix indicated volumes of purified water and 10x Luminescence Buffer (Component 6) to obtain the desired volume of Luminescence Buffer (1x):

<table>
<thead>
<tr>
<th>Vol. of Luminescence Buffer (1x)</th>
<th>Vol. of Luminescence Buffer (10x) (Component 6)</th>
<th>Vol. of purified water</th>
</tr>
</thead>
<tbody>
<tr>
<td>270 ml</td>
<td>27 ml</td>
<td>243 ml</td>
</tr>
</tbody>
</table>

NuPage SDS-MOPS Running Buffer 1x
Mix indicated volumes of purified water and 10x NuPage SDS-MOPS Running Buffer (1x) to obtain the desired volume of NuPage SDS-MOPS Running Buffer (1x):

<table>
<thead>
<tr>
<th>Vol. of NuPage SDS-MOPS Running Buffer (1x)</th>
<th>Vol. of purified water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ml</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

Transfer Buffer

<table>
<thead>
<tr>
<th>Vol. of Transfer Buffer (1x)</th>
<th>Vol. of Transfer Buffer (10x) (Component 1)</th>
<th>Vol. of purified water</th>
<th>Vol. of methanol (98%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 ml</td>
<td>200 ml</td>
<td>1800 ml</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

TBST

<table>
<thead>
<tr>
<th>Vol. of TBST (1x)</th>
<th>Vol. of TBS (20x)</th>
<th>Vol. of purified water</th>
<th>50% Tween 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ml</td>
<td>50 ml</td>
<td>950 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Ponceau S

<table>
<thead>
<tr>
<th>Vol. of Ponceau S (1x)</th>
<th>Vol. of Ponceau S (10x) (Component 2)</th>
<th>Vol. of TBST (1x) (Component 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ml</td>
<td>50 ml</td>
<td>950 ml</td>
</tr>
</tbody>
</table>
Preparatory Steps
- Fill two vessels with sufficient amounts of de-ionized water (at least 25 l) in order to allow complete submersion of the PrioCLIP™/Pryqpon during the washing steps.

Draining
- Empty containers with homogenates tested “TSE negative” into an autoclavable, heat-resistant bottle or a disposable canister/flask.
- Containers whose contents have been identified “initial reactive” must not be re-used and have to be disposed of according to the national safety guidelines.

Washing
- Immerse the empty PrioCLIP™/Pryqpon in a vessel with de-ionized water, rinse thoroughly.
- Inspect the homogenization containers visually for possible damage and tissue contamination during transfer from vessel one to vessel two. Discard any damaged or contaminated PrioCLIP™/Pryqpon homogenization containers.
- Submerge containers and incubate at least 30 min at 22±3°C.

Drying
- Take the PrioCLIP™/Pryqpon out of the vessel, shake out remaining water and let them dry completely at 22±3°C.
- Alternatively, PrioCLIP™/Pryqpon can be dried in a fluid or tissue.
- Visualy check PrioCLIP™/Pryqpon. Discard damaged or contaminated PrioCLIP™/Pryqpon.
- Now PrioCLIP™/Pryqpon are ready for re-use.

Waste disposal
- Homogenates and washing solutions have to be disposed of according to national safety guidelines.

Appendix II

Volumes needed for different numbers of samples

<table>
<thead>
<tr>
<th>No. of gels</th>
<th>Tray size (cm)</th>
<th>Membrane size</th>
<th>TBST</th>
<th>1. Antibody</th>
<th>Luminescence Buffer</th>
<th>CDP-Star</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Large (22.4 x 15.6)</td>
<td>13 x 17 cm</td>
<td>50 ml</td>
<td>10 l</td>
<td>12.5 ml</td>
<td>25 ml</td>
</tr>
<tr>
<td>4</td>
<td>Large (22.4 x 15.6)</td>
<td>8 x 17 cm</td>
<td>50 ml</td>
<td>10 l</td>
<td>100 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>3</td>
<td>Medium (15 x 11.4)</td>
<td>13 x 8.5 cm</td>
<td>25 ml</td>
<td>5 l</td>
<td>5 ml</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Medium (15 x 11.4)</td>
<td>9 x 8.5 cm</td>
<td>25 ml</td>
<td>5 l</td>
<td>60 ml</td>
<td>30 ml</td>
</tr>
<tr>
<td>1</td>
<td>Small (5.5 x 9.5)</td>
<td>4.5 x 8.5 cm</td>
<td>10 ml</td>
<td>2 l</td>
<td>3 ml</td>
<td>40 ml</td>
</tr>
</tbody>
</table>

Appendix III

Scheme for placement of gels on blot membrane

Recommended scheme for placement of gels on blotting membrane. For a detailed PrioCLIP™/Pryqpon wash protocol (including pictures) can be requested at info@prionics.com.

Appendix IV

PrioCLIP™/Pryqpon Wash Protocol

General instructions
- Sample traceability: PrioCLIP™/Pryqpon homogenization containers must be labeled with sample number – e.g. a water-proof pen or labels – to guarantee the sample traceability. Labeling of the containers can only be removed after release of results.
- PrioCLIP™/Pryqpon usage traceability: Homogenization containers should not be used more than 5 times. PrioCLIP™/Pryqpon have to be labeled with dashes or dots using a waterproof pen after each use.

Do not use hypochlorite-containing disinfectants for washing.

Appendix V

Safety Regulations and R&S Statements

Safety Regulations
1. National Safety Regulations must be strictly followed.
2. ACPD guidelines
   Laboratories MUST adhere to National Safety Regulations, but the following information – published by the Advisory Committee on Dangerous Pathogens (ACPD) – is available for guidance: “Transmissible spongiform encephalopathies: safe working and the prevention of infection”. Department of Health, London, UK (can be ordered at the Stationary Office, ISBN 0113221665, phone number +44 (20) 8773 9090). An update is available under www.advisorybodies.doh.gov.uk/acdp/fsguidance/index.htm

R&S Statements

Component 1
Homogenization Buffer (5x)

Hazard Code: This product is not classified according to EU regulations.

Component 2
Digestion Buffer (1x)

Hazard Code: R22 Harmful if swallowed.
R36/37 Irritating to eyes and skin.
S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S36/37 Wear suitable protective clothing and gloves.

Appendix VI

OIE certification: Summary of validation studies

Analytical characteristics

Analytical sensitivity
- Dilution series were tested in the course of the European Union validation study. Of 20 positive homogenates tested at a 10−1 dilution, 15 scored positive, two doubtful and three negative. At the 10−2 dilution three samples were scored doubtful and the remainder negative. Two samples were also scored doubtful at 10−3 and a single sample at 10−4 dilution. [Positive samples were supplied by the Central Veterinary Laboratory (CVL), Weybridge: brainstem and spinal cord samples were selected from bovines showing clinical signs of BSE with confirmation by histological examination.]

Analytical specificity
- There has been not been specifically examined. However some field studies using fallen stock samples (animals suffering from disorders other than BSE, e.g. encephalitis, brain oedema, rabies, listeriosis) have consistently shown that Prionics AG-Check WESTERN ERN kit has a good analytical specificity [see Surveillance of BSE, D. Heim and al, Arch Virol, Suppl. 2000, (16):127-33].

Repeatability data
- Tests carried out over a period 2002 to 2007 show that the kit could detect a BSE positive sample at a 1/10 dilution.
Diagnostic characteristics

Threshold determination
This test does not give a quantitative reading. The response is qualitative and is based on the two criteria of the presence of the signal and its position. This allows easy discrimination between true positives and (potentially) false positives due to undigested normal proteins.

Diagnostic sensitivity (DSn) and specificity (DSp)
Tests performed at the Swiss BSE reference laboratory (Neurocenter/University of Bern) in February 2003 on 38 positive samples (18 homogenates & 20 tissue samples) from the UK/190 negative samples from the Swiss BSE surveillance program (tissue samples from cattle older than 30 months that had tested negative by histology/imunohistochemistry).

<table>
<thead>
<tr>
<th></th>
<th>BSE positive by IHC</th>
<th>BSE negative by IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive by Prionics Check-WESTERN</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Test Negative by Prionics Check-WESTERN</td>
<td>0</td>
<td>190</td>
</tr>
</tbody>
</table>

Diagnostic Sensitivity: 100%, CI [90.7 – 100.0%]
Diagnostic Specificity: 100%, CI [96.1 – 100.0%]

Reproducibility
During the EU field evaluation of 2004, samples were tested using the Prionics AG-Check WESTERN. These results were compared to the results achieved with other tests under evaluation. The samples were divided into three categories: positive samples, negative samples and samples of poor quality.

- A total of 335 BSE positive samples were tested in three labs (VLA, Newcastle, UK - AnalytiCo Food BV, Heerenvenn, Holland and Laboratorio Central de Veterinaria, Algecete, Spain).
- A total of 24,534 BSE negative samples were tested in eight labs (AnalytiCo Food BV, Heerenvenn, Holland; Institut für Veterinärmedizin, Mödling, Austria; Instituto de Referência en Dierhouderij en Diergezondheid, Lelystad, Holland; Laboratorio EET, Leon, Spain; Labor Dr. Guenteer, Luzern, Switzerland; Instituto Zooprofilattico Sperimentale del Piemonte, Turin, Italy; Irish Equine Centre, Kil dane, Ireland; Arthur Biotec GmbH, Hamburg, Germany).

Canadian field study, 2003. As a follow up to the detection of the index case in May, 2003, 2036 birth and feed cohorts were tested by immunohistochemistry (IHC) and the Check WESTERN kit. This work was carried out by Canadian Food Inspection Agency laboratories at Lethbridge, Winnipeg, New pean and St-Hyacinthe.

<table>
<thead>
<tr>
<th></th>
<th>BSE positive by IHC</th>
<th>BSE negative by IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive by Prionics Check-WESTERN</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Test Negative by Prionics Check-WESTERN</td>
<td>0</td>
<td>2036</td>
</tr>
</tbody>
</table>

Diagnostic Sensitivity: 100%, CI [99.0 – 100.0%]

Evaluation of tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines for the European Commission, 1999. All samples were prepared by the EU Institute for Reference Materials and Measurements (IRMAM) at Geedi, Belgium and presented for testing in a coded ‘blind’ format to the different participants (Prionics AG-Check WESTERN kit was one of the four selected kits): A total of 300 positive samples (collected from cattle showing clinical signs of BSE and confirmed by histological examination - supplied by CVL Weybridge) and 1000 negative samples (collected in New Zealand from healthy adult cattle of at least 4 years of age and confirmed negative by histological examination).

<table>
<thead>
<tr>
<th></th>
<th>BSE positive by histology</th>
<th>BSE negative by histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive by Prionics Check-WESTERN</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>Test Negative by Prionics Check-WESTERN</td>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

Diagnostic Sensitivity: 100%, CI [98.8 – 100.0%]
Diagnostic Specificity: 100%, CI [99.6 – 100.0%]

Agreement between tests
The agreement of the Prionics AG-Check WESTERN with histological examination and IHC is high.

Applications
Prionics AG-Check WESTERN is currently in use in both reference and routine laboratories throughout the world.
Testing laboratories should participate in proficiency testing and laboratory training programmes organized by OIE Reference Laboratories.

References:
2. DOHERR MG, OESCH B, MOSER M, VANDEVEELDE M, HEIM D.; Targeted surveillance for bovine spongiform encephalopathy; Veterinary Record 1999 Dec 4; 145:672
5. HEIM, D AND WILESMITH, JW; Surveillance of BSE; Arch Virol. Suppl. 2000, (16):127-33