Conference
Vaccine Cell Substrates 2004

Diagnostics for TSE agents

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TSE Diagnostics
OVERVIEW

- Post mortem diagnostics
  - Histopathology (vacuolar changes)
  - Immunohistochemistry (PrP^{Sc})
  - EM/SAF (Electron Microscopy of Scrapie Associated Fibrils)
  - Bioassay
  - **Rapid tests (immunochemical detection of PrP^{Sc})**
- Ante mortem diagnostics
  - **PrP^{Sc} (conformation specific antibodies)**
  - Surrogate markers
    - Erythroid differentiation related factor (EDRF)
    - Fatty acid binding protein (FABP)
    - Cellular nucleic acids
    - Cystatin C

TSE Diagnostics
RAPID TESTS

1999 EU Approved

> **Prionics®-Check WESTERN**	**WB**
> Bio-Rad Platelia	ELISA
> Enfer BSE Test	ELISA

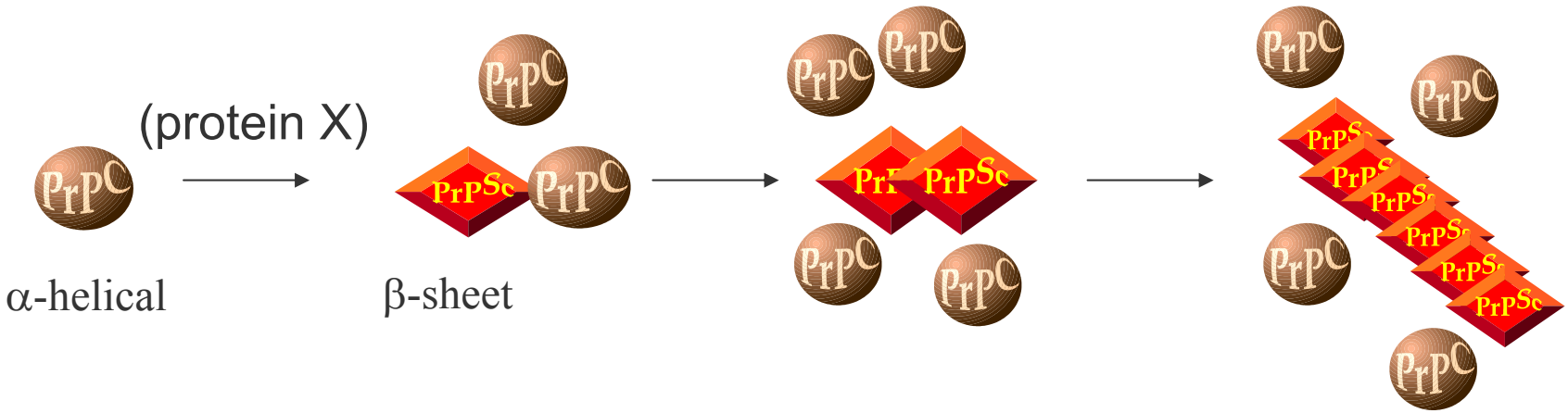
2003 EU Approved

> **Prionics®-Check LIA**	**ELISA**
> InPro CDI-5	ELISA

2004 EU Evaluation

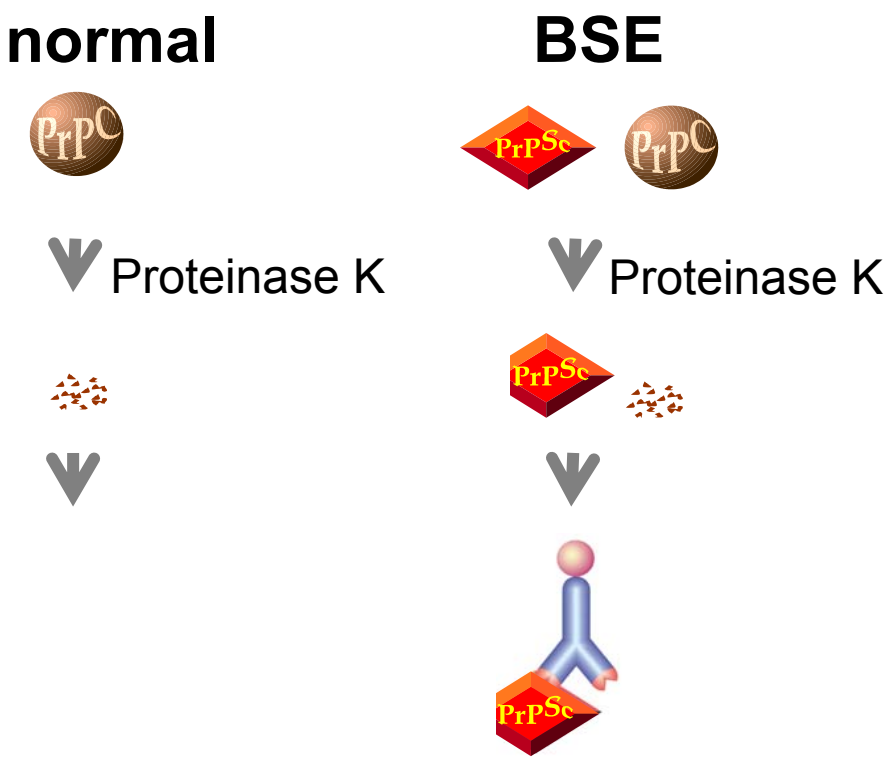
> **Prionics®-Check PrioSTRIP**	**Strip test**
> Several new tests	

Prion Biology
THE ‚PROTEIN ONLY‘ THEORY



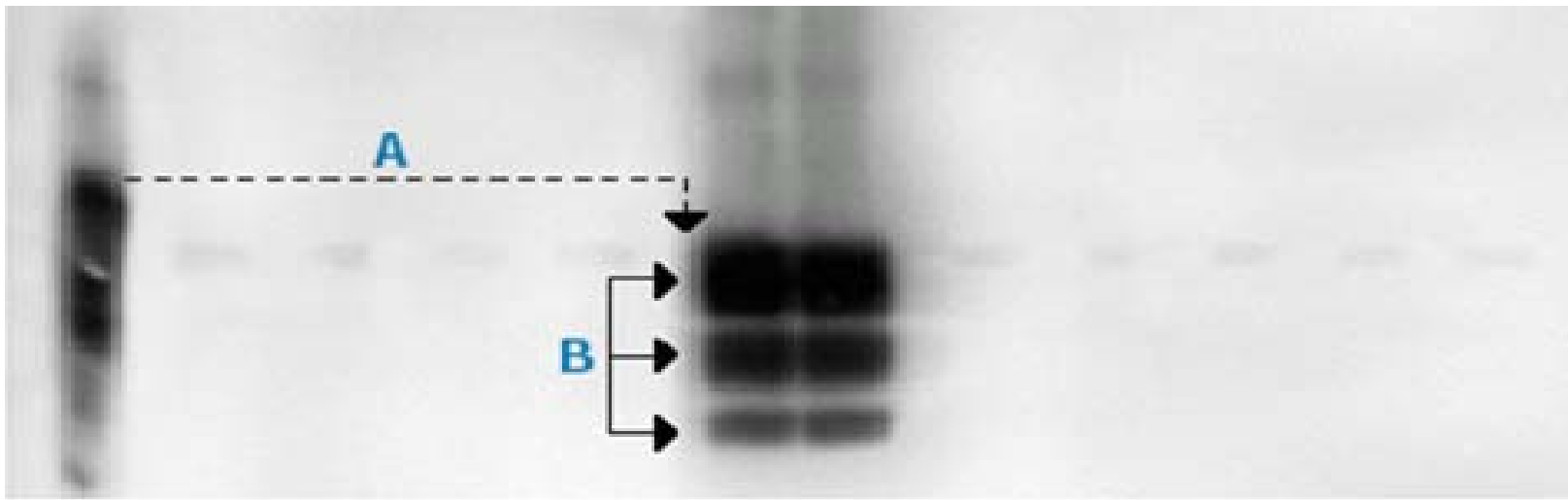
> Prusiner (1982) ‚Novel proteinacious particles causing scrapie‘
> Nobel price, 1997

TSE Diagnostics
DETECTION PRINCIPLE



Prionics®-Check WESTERN RESULT

Control BSE Normal

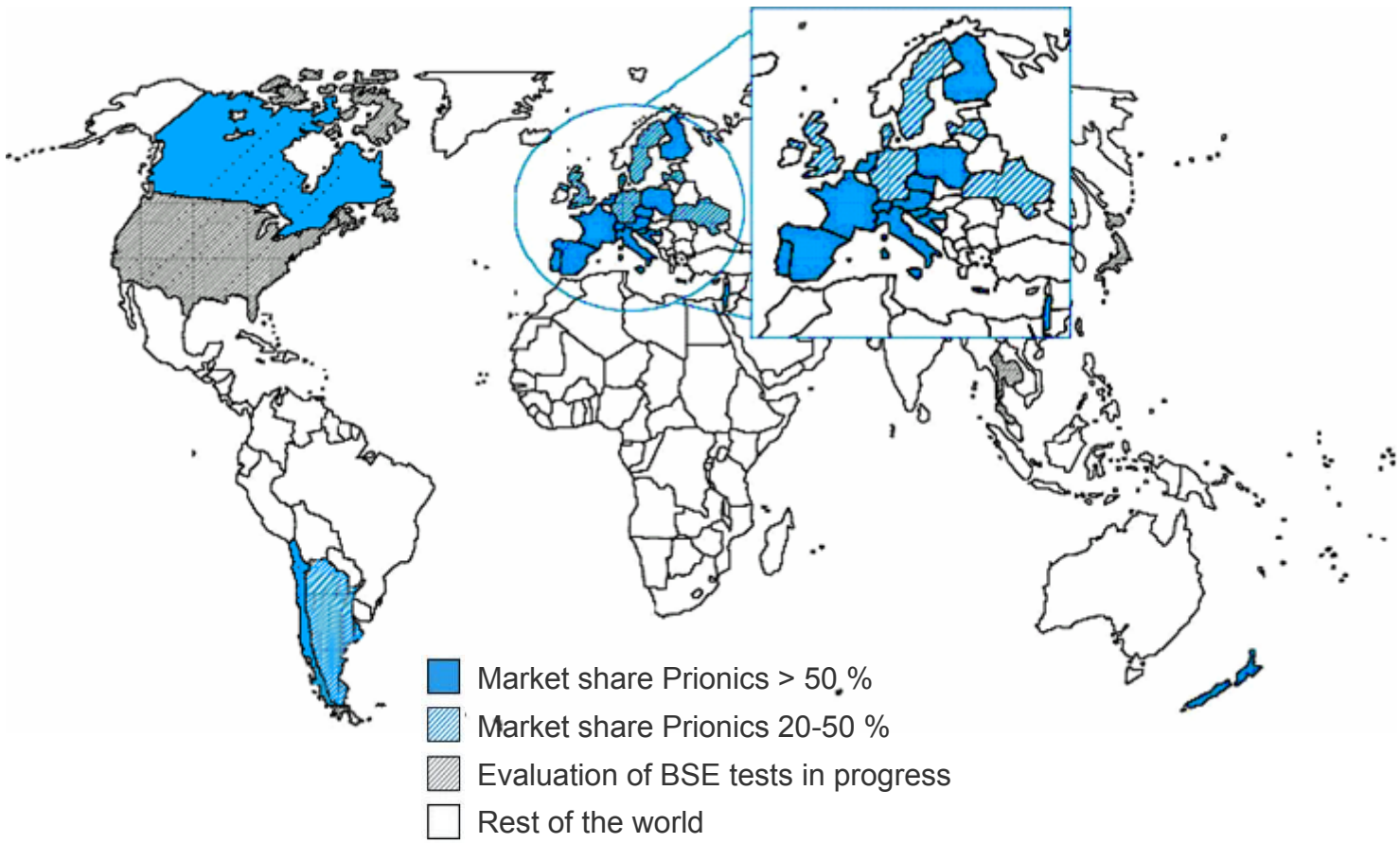


Immunological detection: Identification of PrP through presence of signal.

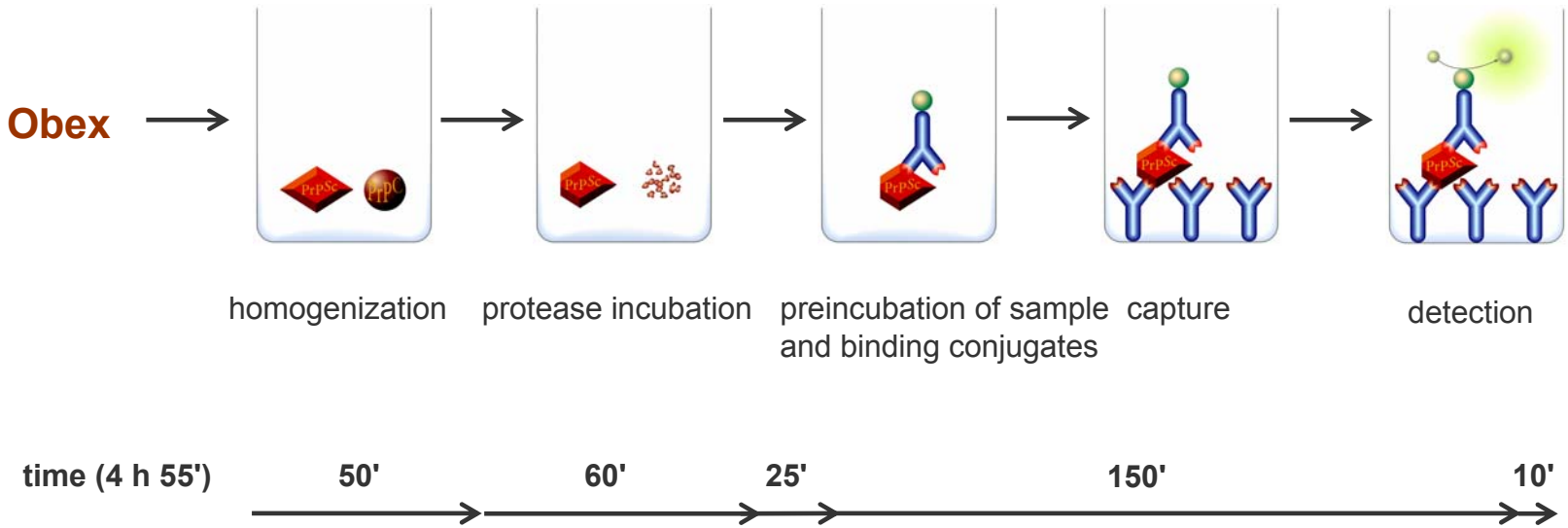
A Molecular weight: Absence of higher molecular bands serves as an internal control for complete digestion of PrP^{C}.

B Glycosylation Pattern: Characteristic band pattern confirms PrP^{SC}, rather than putative contaminants, as the origin of signal.

Prionics®-Check WESTERN
> 20 MIO TESTS: NO FALSE POSITIVES



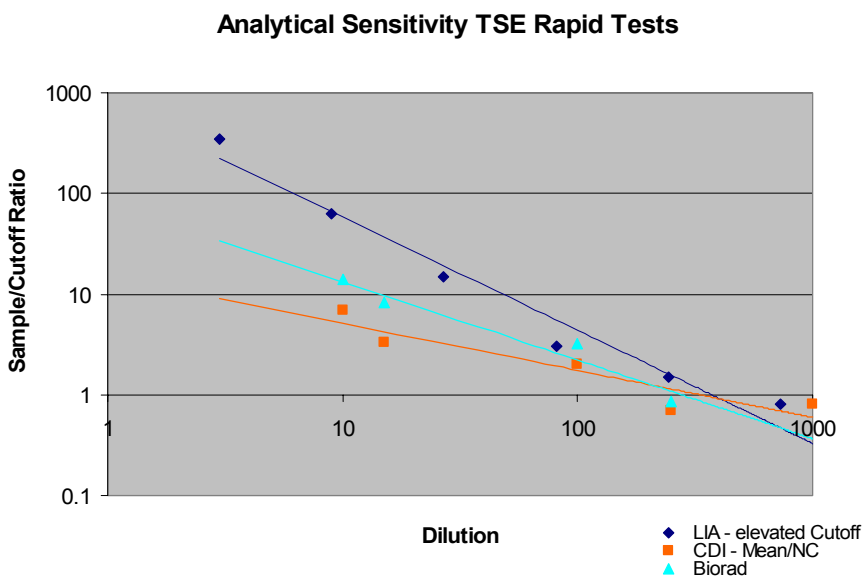
Prionics®-Check LIA
TEST PRINCIPLE



2002 EU Field Trial
ANALYTICAL SENSITIVITY

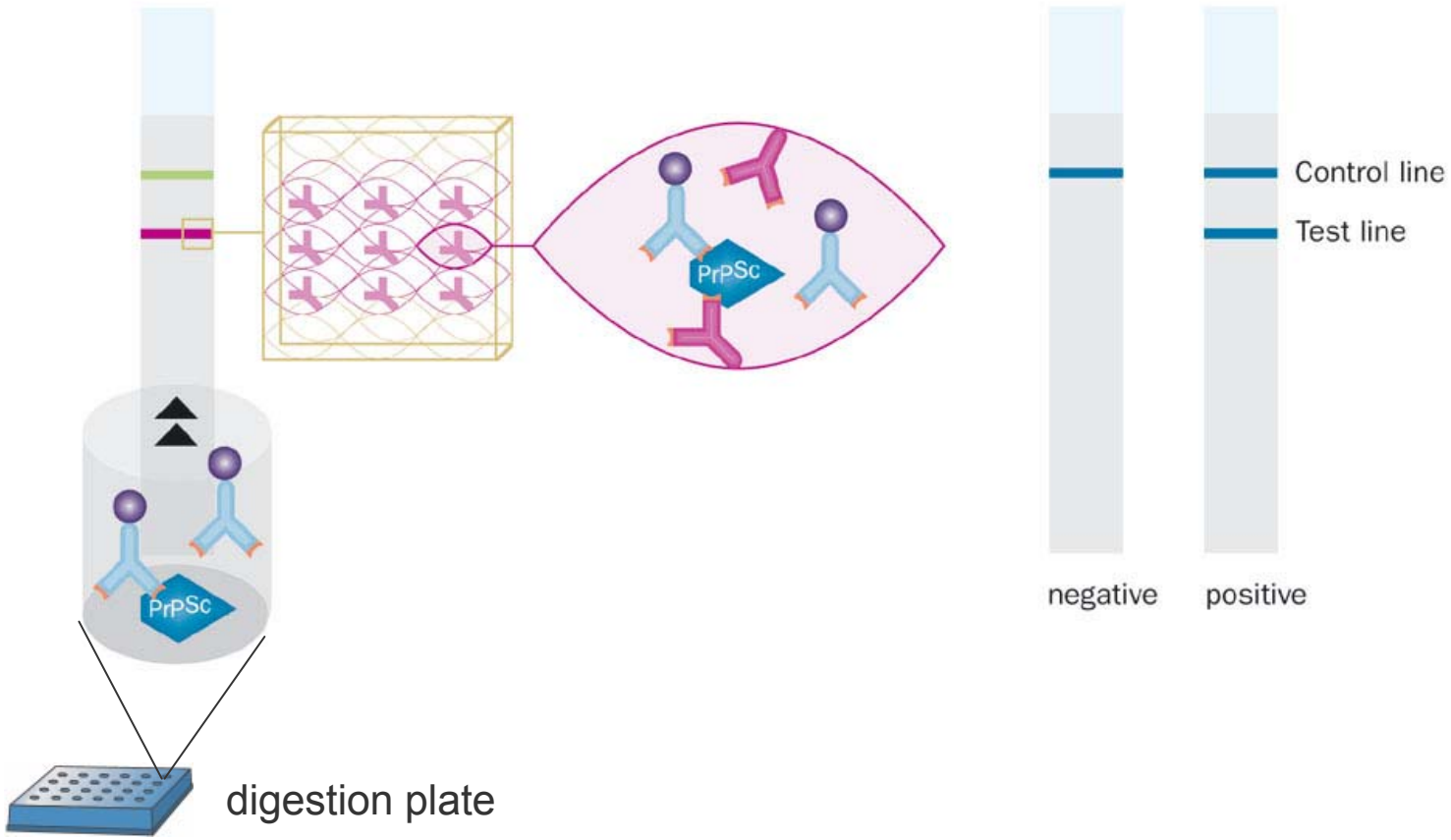
> Report at http://europa.eu.int/comm/food/fs/bse/bse42_en.pdf

> Analytical sensitivity



Data taken from European Comission Report „The Evaluation of Five rapid tests for the diagnosis of TSE“ March 2002

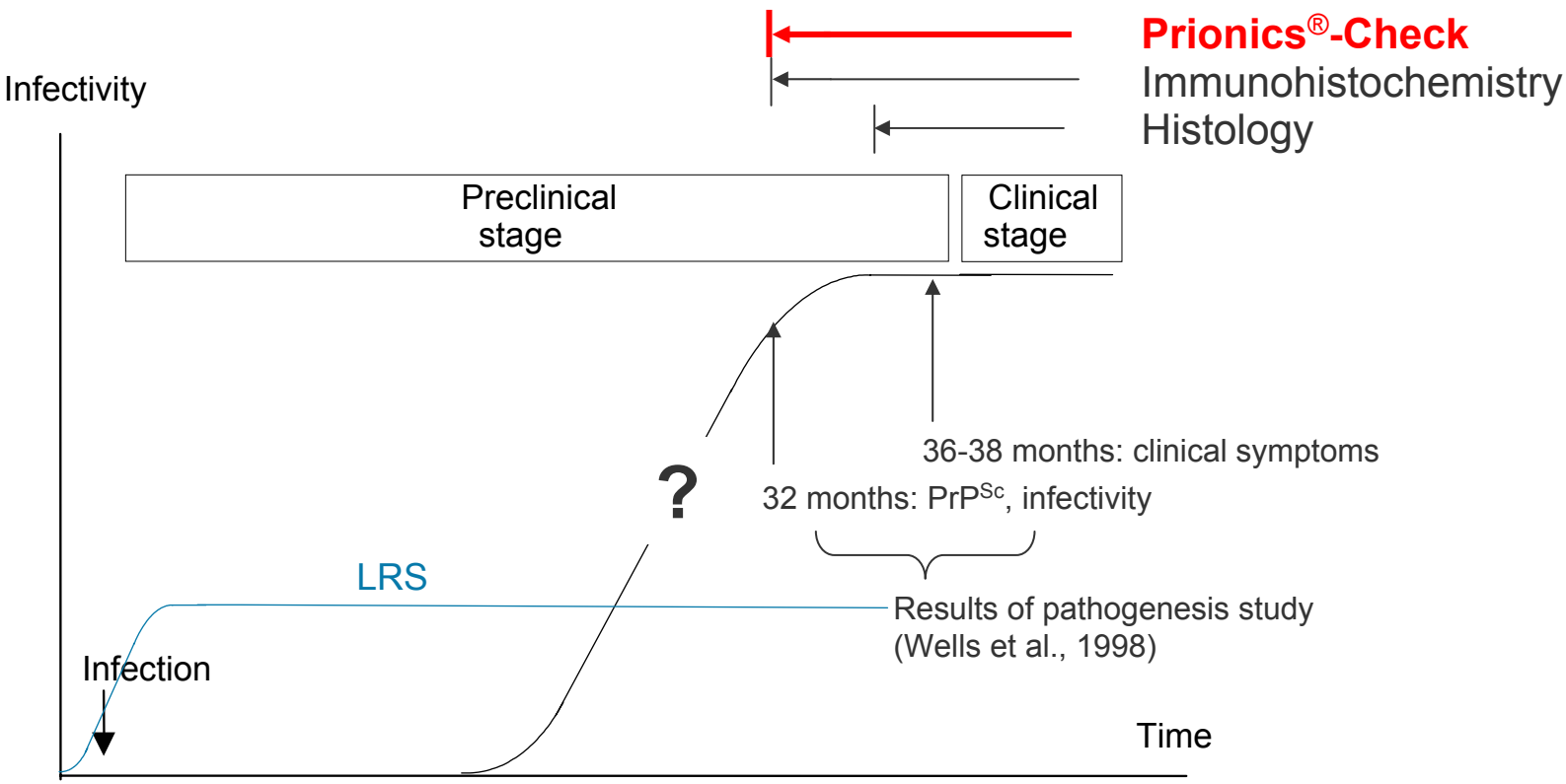
Prionics®-Check PrioSTRIP
DETECTION PRINCIPLE



Prionics®-Check PrioSTRIP
SUMMARY

- Sensitivity and specificity of 100%
  - More than 4500 negatives and 240 positives correctly identified
- EU field studies completed
- Validated in Switzerland
- High analytical sensitivity in the low picogram range
- Very rapid kinetics giving results within minutes
- No need for laborious sample preparation steps such as centrifugation or precipitation and no need for sophisticated laboratory equipment

Prionics®-Check Immunoassay
COMPARISON WITH REFERENCE METHODS



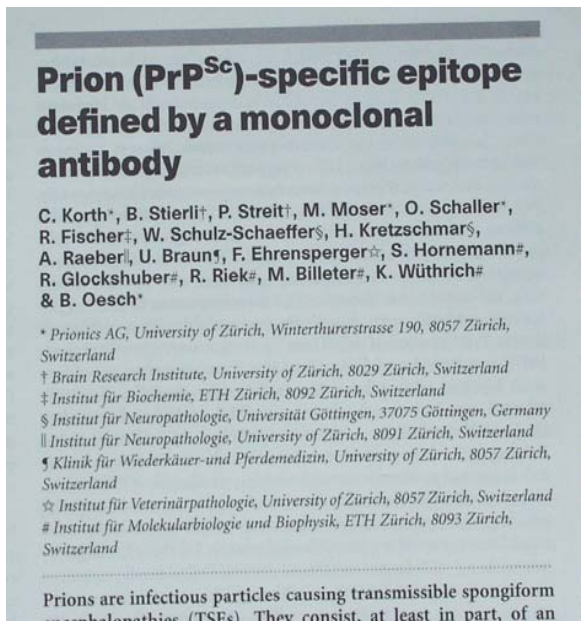
In vivo TSE Diagnostics
GOALS

- Detection of preclinical/subclinical TSE cases
- Assessment of TSE epidemics (vCJD, BSE, CWD, Scrapie)
- Safety of blood supply and blood-derived products
- Safety of pharmaceutical products produced from animal tissues

In vivo TSE DIAGNOSTICS
INFECTIFITY/ PrP^{Sc} IN BLOOD

- TSE infectivity was documented to be present in the blood of experimentally infected rodents and sheep models
- Blood and blood fractions of CJD patients was occasionally infectious to rodents
- First vCJD case likely to be caused by blood transfusion
- **The European CJD surveillance system**
  - More than 12500 specimens of tonsil and appendix screened
  - 3 showed PrP^{vCJD}
  - Potentially 3800 people could be carriers of vCJD in the UK

Detection of PrP^{Sc}
PrP^{Sc} SPECIFIC ANTIBODY 15B3



Prion (PrP^{Sc})-specific epitope defined by a monoclonal antibody

C. Korth*, B. Stierli†, P. Streit†, M. Moser*, O. Schaller*, R. Fischer‡, W. Schulz-Schaeffer§, H. Kretzschmar§, A. Raeber‖, U. Braun¶, F. Ehrensperger☆, S. Hornemann#, R. Glockshuber#, R. Riek#, M. Billeter#, K. Wüthrich# & B. Oesch*

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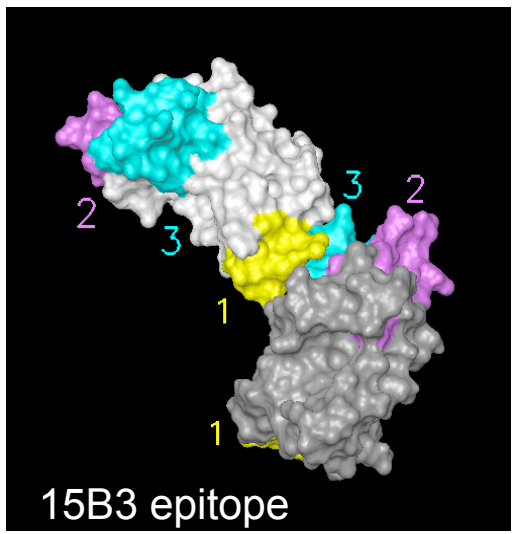
Institut für Molekularbiologie und Biophysik, ETH Zürich, 8093 Zürich, Switzerland

Prions are infectious particles causing transmissible spongiform

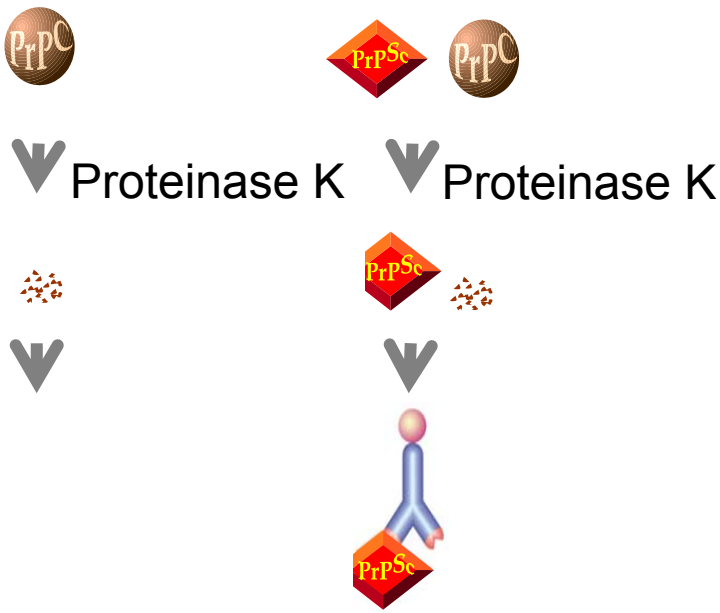


Nature 390 (6655) : 74-77, 1997

TSE Diagnostics
DETECTION PRINCIPLES

6H4 antibody

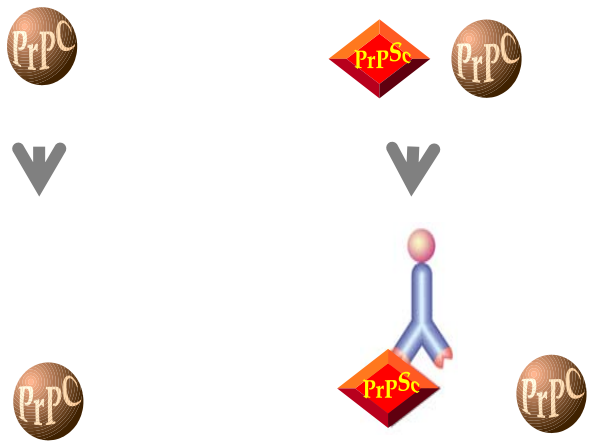
normal **BSE**



Prionics®-Check WESTERN
Prionics®-Check LIA
Prionics®-Check PrioSTRIP

15B3 antibody

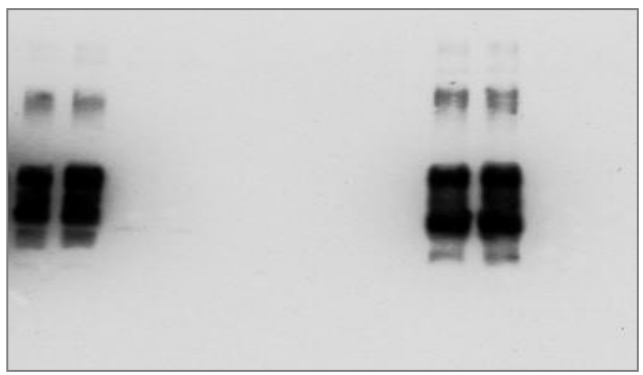
normal **BSE**



Immunoprecipitation
ELISA

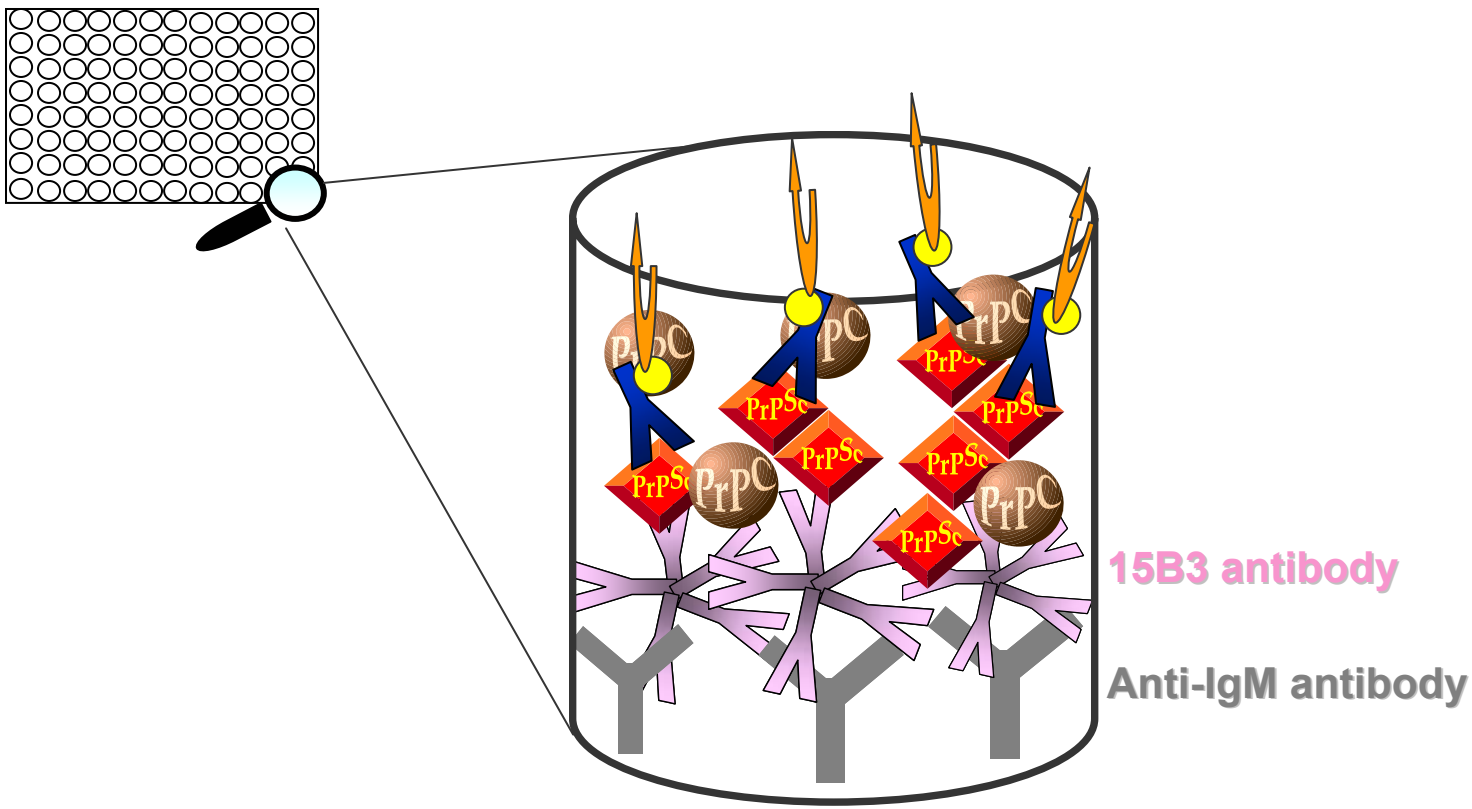
15B3 Immunoprecipitation
DETECTION OF PrP^{Sc} AND PrP^{BSE}

	Sheep				Cattle	
	Sc	N	Sc	N	BSE	N
15B3	+	+	-	-	+	+

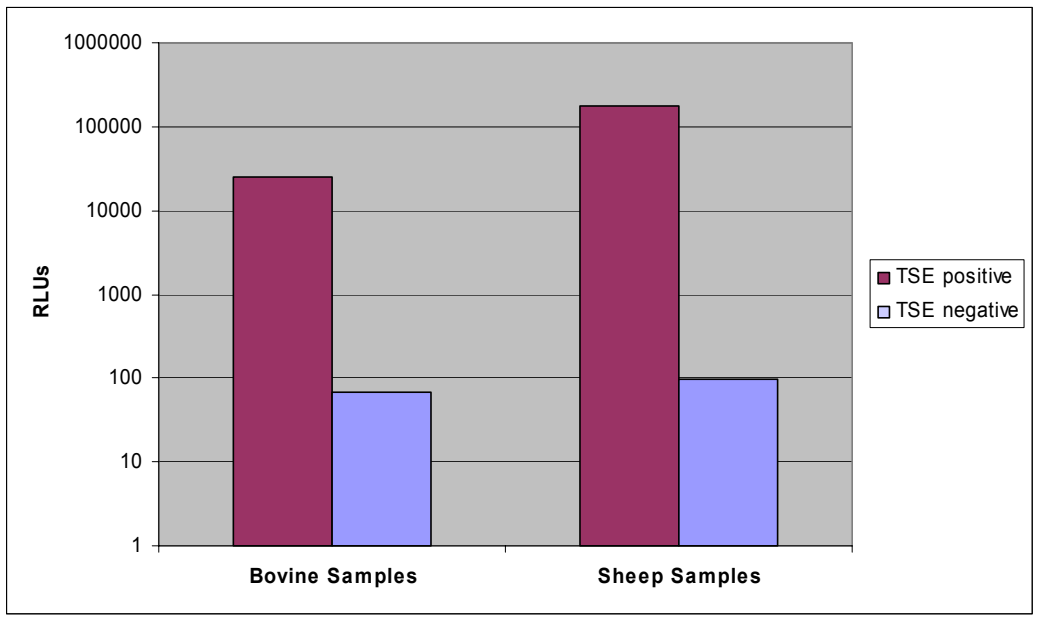


Monoclonal antibody 15B3 specifically immunoprecipitates PrP^{Sc} and PrP^{BSE} from brain of TSE infected animals

Detection of PrP^{Sc}
ELISA TEST PRINCIPLE



15B3 Immunodetection by ELISA
DETECTION OF PrP^{Sc} IN BRAIN



Monoclonal antibody 15B3 specifically detects PrP^{BSE} and PrP^{Sc} in a Sandwich ELISA

SUMMARY

- The current TSE diagnostics (Prionics®-Check WESTERN, LIA and PrioSTRIP) are well suited for postmortem detection of prions in cattle, sheep and deer
- Conformation-specific antibody 15B3 detects only the pathological isoform of PrP in cattle, humans, sheep, and deer
- Sensitive and specific detection of PrP^{Sc} without protease-digestion in combination with the presented ELISA platform opens the way for early diagnostic testing in peripheral tissues and body fluid