

Detection of prions in body fluids – Diagnosis of CJD

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I. Priontype® Immuno-PCR

At present, there are no methods available that allow the routine diagnostic detection of the pathogenic isoform of the prion protein (PrP^{Sc}) in body fluids; this is due to the extremely low concentrations of PrP^{Sc} expected in e.g. blood. Therefore, a substantial increase in sensitivity of prion detection systems is necessary. On the basis of a classical ELISA we established such a highly sensitive test system by using a new detection technique, the so called Immuno-PCR (Imperacer™, Chimera BioTec). The Immuno-PCR combines the specific antigen-antibody reaction with the high signal amplification rate in PCR. Using Immuno-PCR, sensitivity of the corresponding classical ELISA was increased 10.000-fold, resulting in a detection limit of 10pg/mL recombinant prion protein (recPrP). In spiking experiments using recPrP in body fluids it was shown that 10pg/mL recPrP are clearly detectable even when spiked in serum or cerebrospinal fluid.

II. Sensitivity

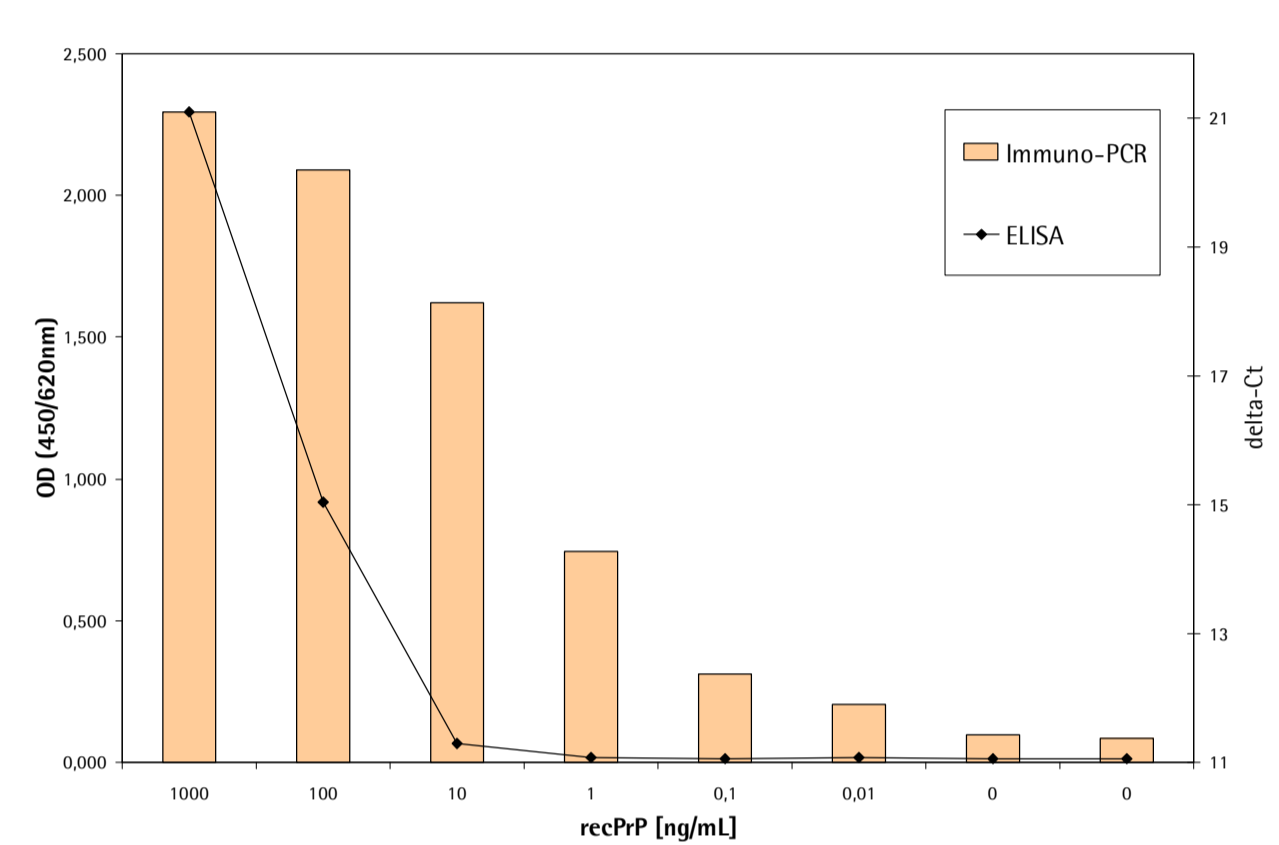


Fig. 2: Detection of recPrP spiked in PK-treated serum by conventional ELISA and Priontype® Immuno-PCR

- Spiking experiments were performed in order to estimate the sensitivity of the newly developed detection system.
- RecPrP was diluted in proteinase K (PK)-treated body fluids.
- 10pg/mL recPrP are clearly detectable even when spiked in serum (fig. 2) or cerebrospinal fluid (not shown).

III. Sample preparation

- PrP is masked in body fluids by abundant proteins (fig. 3, C).
- Before analysis of PrP abundant proteins must be removed.
- We optimized PK treatment of body fluids to unmask PrP.
- Fig. 3, A shows that PrP^{Sc} is stable under the preparation conditions established.

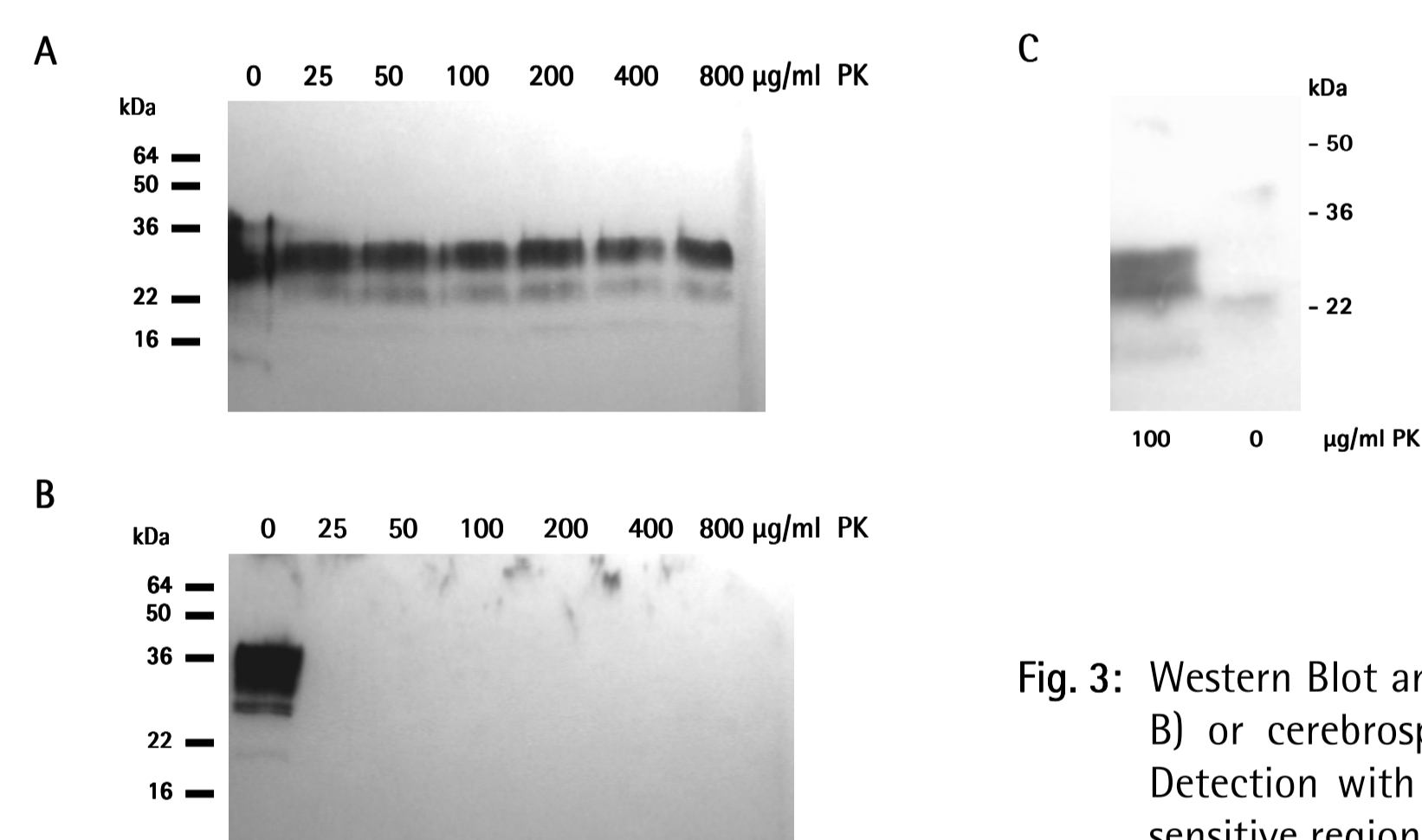


Fig. 3: Western Blot analysis. BSE positive brain homogenate diluted in buffer (A, B) or cerebrospinal fluid (C) treated with different PK concentrations. Detection with mAb having their epitope in the PK-stable (A, C) or PK-sensitive region of PrP (B).

IV. Validation 1

- To verify the suitability of the detection system a panel of spiked plasma samples was analyzed.
- These samples were kindly provided by the CJD Resource Centre of the NIBSC (UK).
- All samples spiked with normal brain or spleen and plasma samples alone were tested negative. This means that there were no false-positive samples (n=22).
- Plasma samples spiked with infected (nvCJD) spleen in a dilution up to 1:10 were detectable.
- In addition, all plasma samples spiked with infected (nvCJD) brain in a dilution up to 1:1000 were tested positive.
- This means that 3nL of infected brain were detectable in a Priontype® Immuno-PCR test.

Tab. 1: Results obtained for spiked plasma samples (CJD Resource Centre, NIBSC) using Priontype® Immuno-PCR

Spike	Dilution	Result
nvCJD brain	< 1:1000	100% positive
	> 1:1000	100% negative
nvCJD spleen	< 1:1000	33% positive
	> 1:1000	100% negative
Normal brain		100% negative
Normal spleen		100% negative
None		100% negative

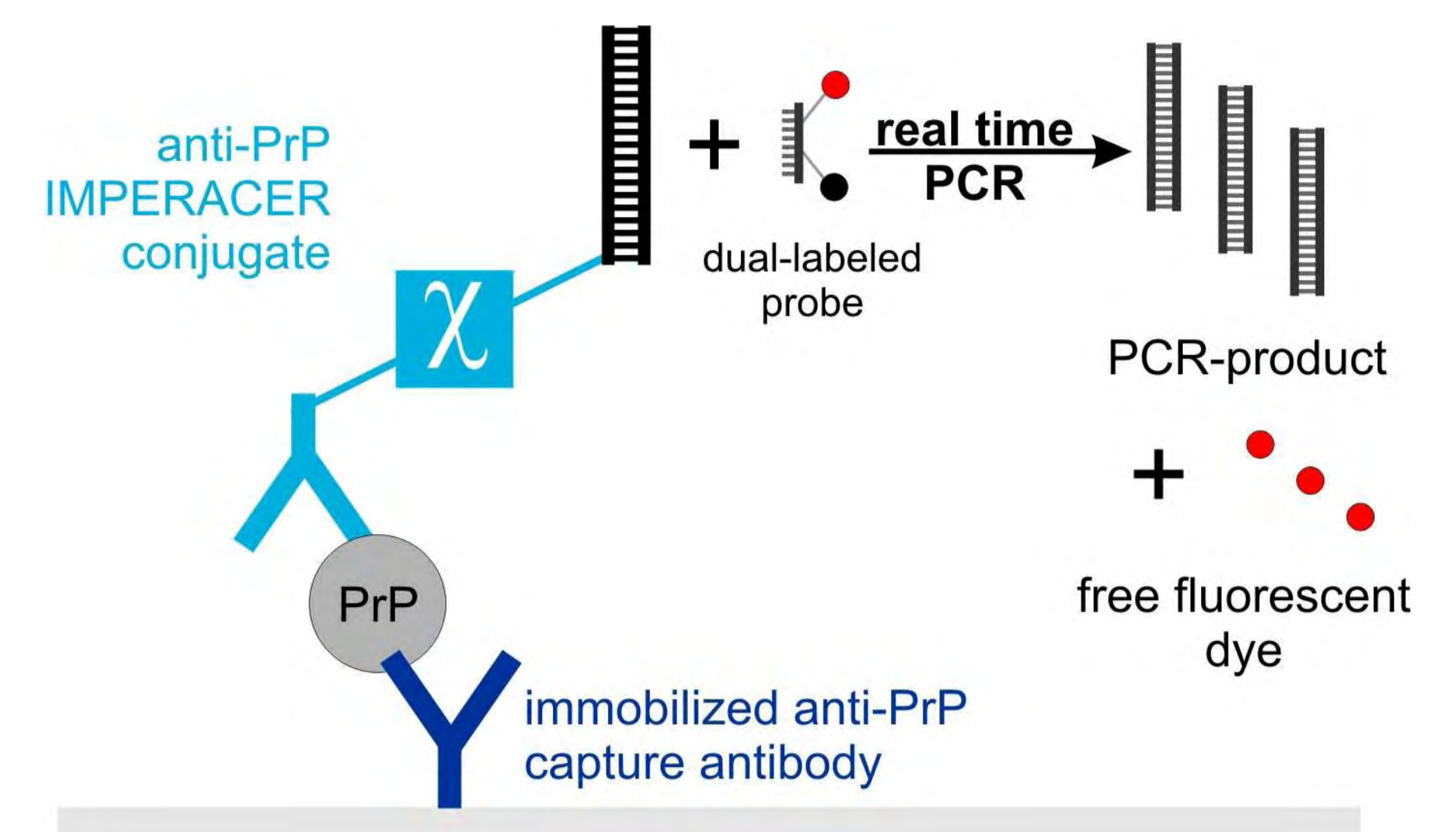


Fig. 1: Assay design of the Priontype® Immuno-PCR

V. Validation 2

Fig. 4: Analysis of CJD-negative plasma samples using the Priontype® Immuno-PCR

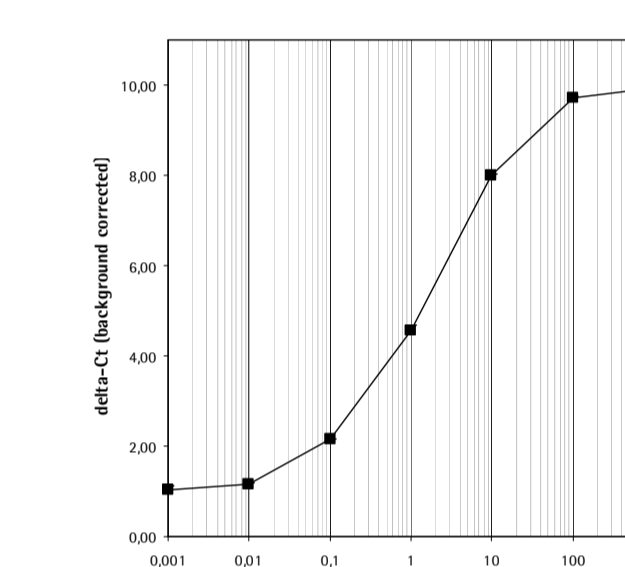
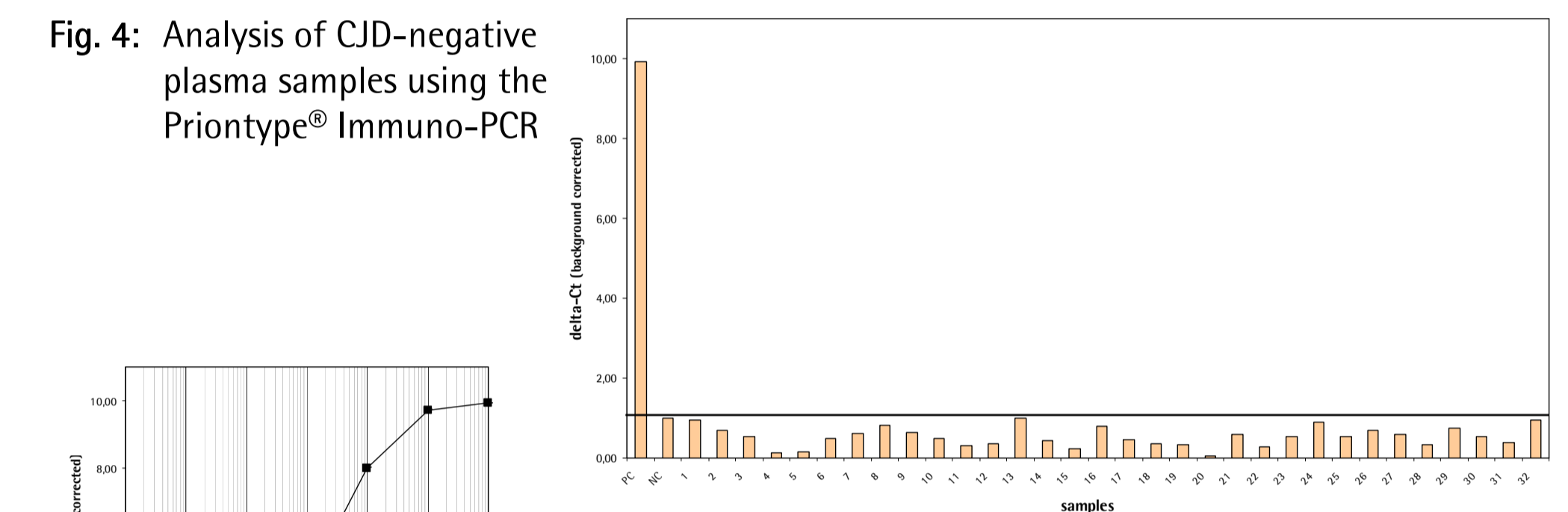


Fig. 5: Calibration curve for recPrP analyzed by Priontype® Immuno-PCR

- Plasma samples from healthy donors and AD patients were analyzed to demonstrate the specificity of the detection system.
- No false positive results were obtained.

Tab. 2: Results obtained for PrP^{Sc}-negative samples using Priontype® Immuno-PCR

	Positive	Negative	Total
Alzheimer's disease patients	0	28	28
healthy donors	0	347	347

VI. Immunoprecipitation

- Sensitivity of the detection system can be further improved by enrichment of PrP.
- We performed immunoprecipitation with mAbs coupled to magnetic beads.
- Using bovine brain tissue we demonstrate that immunoprecipitation is a valuable tool to improve the detection limit.
- A sample subjected to immunoprecipitation (+IP, fig. 6) shows a signal corresponding to 100ng of recPrP, an untreated sample (-IP, fig. 6) gives a signal corresponding to 1ng. This means a 100-fold improvement.

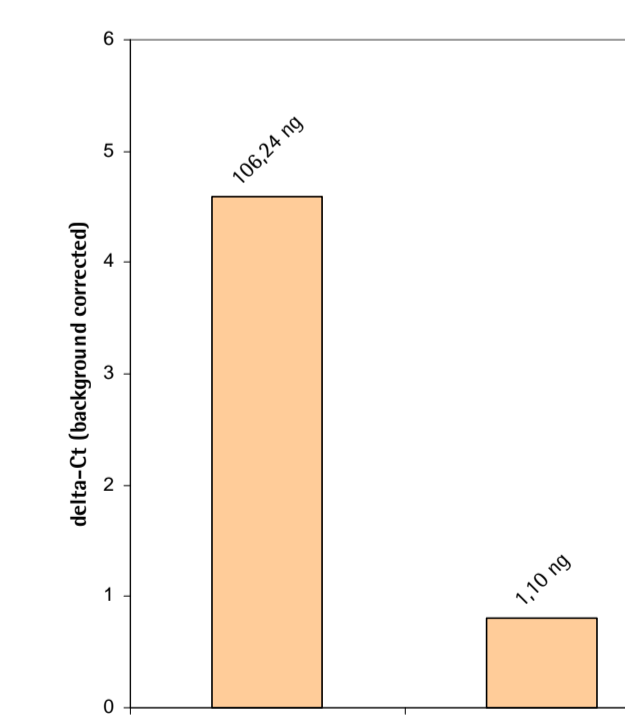


Fig. 6: Detection of samples subjected to immunoprecipitation (+IP) and untreated samples (-IP) using Priontype® Immuno-PCR. Amounts indicated above were calculated using a calibration curve for recPrP (not shown).

Conclusion

Immuno-PCR is a highly sensitive detection system for PrP^{Sc} in body fluids with a detection limit of 10pg/mL recPrP. We tested a panel of spiked plasma samples (CJD Resource Centre, NIBSC) and thereby show that as little as 3nL of infected brain are detectable in this system. We demonstrate 100% specificity by testing several hundred plasma samples from healthy donors and patients with other neurodegenerative disorders. For further improvement of the detection limit we introduced an enrichment step through immunoprecipitation of PrP. First experiments showed that immunoprecipitation allows a further increase of sensitivity. Taken together, this is an exciting approach towards the very sensitive, i.e. the very early diagnosis of PrP and towards a general screening possibility of all donors for transfusions and transplantations.