

# Real-time immuno-PCR: a new perspective for prion blood screening tests.

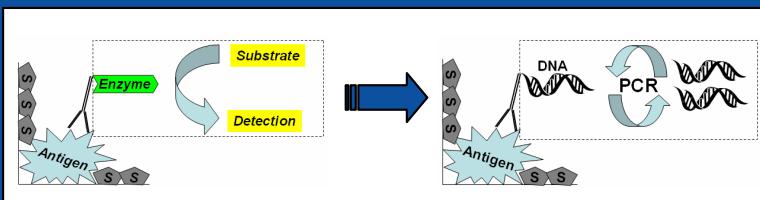
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Prion diseases such as Creutzfeldt-Jakob, scrapie and Bovine Spongiform Encephalopathy (BSE) are fatal neurodegenerative disorders characterized by behavioural and locomotor changes, cerebral amyloid plaques and spongiform degeneration of the brain<sup>1</sup>. In 1996, the first case of variant Creutzfeldt-Jakob disease (vCJD) was diagnosed in the United Kingdom, through the consumption of prion-contaminated food. Since, their number increased whereas cases of BSE were declining. Until the end of January 2007, 219 people were death from vCJD<sup>2</sup>. The potential for transmission of vCJD by blood transfusions was demonstrated experimentally<sup>3</sup> prior the apparition of apparent cases of human transmission<sup>4</sup>. From December 2004, 3 people are already death following a transfusion of contaminated blood. The classical techniques used to diagnose prion protein (Western blot assays and ELISA)<sup>5</sup> are not sensitive enough to detect the low levels of prion in the blood. In this study, we focussed on the detection of the prion protein in blood by immuno-quantitative-PCR (iqPCR) <sup>6-7</sup>.

## Principle

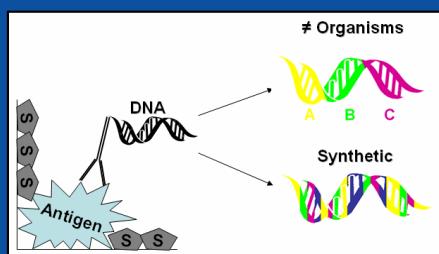
The iqPCR is based on the same technology than the ELISA, except that the detection system (based on an enzyme) is replaced by a DNA marker, allowing a signal amplification by PCR.



This ultra-sensitive technology combines the **MOLECULAR SPECIFICITY** of the antibodies and the **SENSITIVITY** of the PCR.

## Chimeric DNA

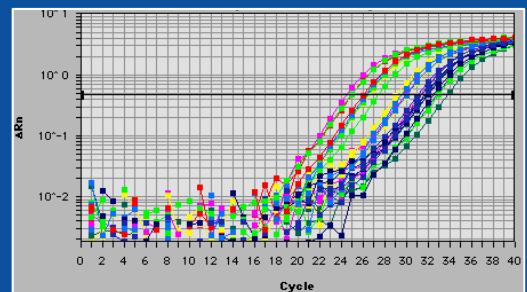
To reduce the risk of false positive due to an exogenous DNA contamination, we developed a chimeric DNA marker which does not exist in the matrix to analyze nor in nature.



Patent :  
EP1232283,  
2002-08-21.

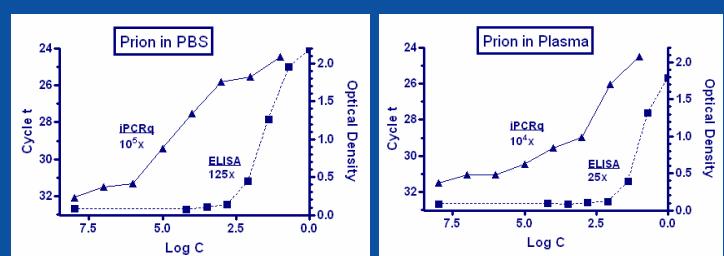
## Results

To illustrate the advantages of iqPCR, we have compared it with a conventional ELISA technique in experiments aimed to detecting the resistant form of prion protein in human plasma. The infectious prion protein was detected, in PBS and human plasma, using ELISA and iqPCR techniques.



### sensitivity improvement by iqPCR :

800 x      in PBS  
400 x      in human plasma



## Bibliography :

<sup>1</sup> Vana, K. et al. Cell. Mol. Neurobiol., 2007, 27(1), 107-128

<sup>2</sup> <http://www.cjd.ed.ac.uk/vcjdworld.htm>

<sup>3</sup> Hunter, N. et al. J. Gen. Virol., 2002, 83, 2897-2905.

<sup>4</sup> Llewelyn, C. A. et al. Lancet, 2004, 363, 417-421.

<sup>5</sup> Soto, C. Nat. Rev. Microbiol. 2004, 2, 809-819.

<sup>6</sup> Gofflot, S. et al. J. Immunoassay Immunochem. 2004, 25, 241-258.

<sup>7</sup> Gofflot, S. et al. Clin. Chem., 2005, 51(9):1605-11.

**The immuno-quantitative PCR could be used to detect a minute quantity of prion in blood spiked with infected Creutzfeldt-Jakob sample.**

**This technology could be a technique of choice for the development of a new blood screening test. This could allow the diagnosis of prion protein both at ante- and post-mortem stage.**