4. Discussion

Using IPCR, we have consistently detected recombinant hamster PrP^C at 1 fg/mL and detected 100 ag/mL (2600 molecules) in 50% of our experiments. Scrapie infected hamster brain homogenate is detected at the equivalent of 10–100 IU. As with PCR, IPCR incorporates an amplification process (signal amplification) that allows an exponential increase in sensitivity for detection of an analyte. The IPCR method for prion protein detection described here has been developed as a semi-quantitative analytical method that exhibits a greater sensitivity of detection than any test for prion protein, which is presently available. Although the IPCR method is not yet reproducible to the standards required for clinical use, there is little doubt that it has the potential with continued refinement to become the most sensitive analytical method for the detection of pathogenic protein and could be translated for use in appropriate laboratories.

4.1. Units of infectivity required for diagnostic detection

For the diagnosis of prion-infected animals, the units of infectivity (e.g., LD_{50}, IU) as well as the detection limits necessary to obtain valid diagnoses must be well defined. Different literature sources may cite slightly different values for equivalent PrP^{Sc} concentrations to LD_{50} and these sources are often used to estimate the number of LD_{50}, which must be detected for pre-clinical diagnosis of BSE and human infection. The number of PrP^{Sc} molecules which define an estimate of LD_{50} from different sources usually do not exceed a 2-log range. Prusiner et al. (1982) estimated 1 LD_{50} to be equivalent to 10^5 molecules or 5 fg PrP^{Sc}. Brown et al. (2000) estimated that 1 LD_{50} is equivalent to 10 IU, which is approximately equal to 100 fg/mL PrP^{Sc}. The limit of sensitivity of the Western blot is 1000–3000 IU/mL (Lee et al., 2000, 2001; Brown et al., 2000; Brown, 2001; MacGregor, 2001), or approximately 10–30 ng/mL PrP^{Sc}. MacGregor (2001) estimated the sensitivity of detection of the Western blot and most serology assays (1000 IU) is at least 100-fold less than that required for detection of abnormal prion in blood of pre-clinical infected animals.

4.2. Other test formats

Other tests described in the literature which report greater sensitivity of detection (<100 pg/mL) than the Western blot include: (1) a quantitative sandwich ELISA using time-resolved dissociation-enhanced fluorescence technology (DELFIA, EG&G Wallac, Turku, Finland) which showed a detection limit for PrP^C in plasma samples of approximately 50 pg/mL (1.4 pM) in vCJD (Völkel et al., 2001); (2) dual-color fluorescent confocal scanning (Bieschke et al., 2000) that detects PrP^{Sc} aggregates in CSF down to approximately 0.1 pg/mL (2.8 fM); (3) the conformation-dependent immunoassay (Safar et al., 1998) which showed a sensitivity of 100 pg/mL–1 ng/mL (2.8–28 pM); and (4) amplification of PrP^{Sc} in vitro by a cyclical process involving alternate phases of incubation and sonication of the sample (Saborio et al., 2001; Soto et al., 2002) where a 640-fold dilution of scrapie infected hamster brain homogenate generated 250 pg or 8.3×10^{-15} mol of dectectable PrP^{Sc} after 10 amplification cycles; PrP^{Sc} was detected even after >10,000-fold dilution under these conditions. All of these procedures appear promising and offer greater sensitivity than currently available methods, but (similar to IPCR) none have been standardized for routine use in laboratories.

4.3. Detection in blood

Although transmission of blood infectivity has been documented in either naturally or experimentally infected animals, and there are now two reports of transmission from vCJD blood donors to blood recipients (Llewelyn et al., 2004, Peden et al., 2004), the infectious agent has not been serologically detected in the blood of infected cattle or humans. Only Schmerr et al. (1999) reported the detection of PrP^{Sc} in sheep and elk blood before clinical symptoms of disease occur (3 months after exposure to PrP^{Sc}) with a sensitivity of detection of 0.5–13.5 pg/mL (MacGregor, 2001) using ICCE. However, Cervenakova et al. (2003a) were unable to distinguish between extracts from leucocytes from healthy and CJD-infected chimpanzees, or between healthy human donors and patients affected with various forms of CJD using ICCE. Inconsistencies in the detection of PrP^{Sc} in the blood of pre-clinical animals may be because of differences between the various animal and human model systems. There may be other reasons why PrP^{Sc} is undetectable in blood or pre-clinical specimens by present methodologies. For instance, PrP^{Sc} may be blocked from molecular interactions by other (ill-defined) protein chaperone molecules in the blood (Telling et al., 1995; DebBurman et al., 1997) or may take on a different conformation in the blood, which is unrecognizable by antibodies presently available and used in serological tests. However, it is highly probable that at least one of the reasons PrP^{Sc} has been undetectable in blood is because of a lack of sensitivity of currently available methods, which cannot efficiently reach sub-pg/mL levels of detection.

4.4. Caveats of the IPCR method

The IPCR standard curve for recombinant hamster PrP^C displayed a correlation coefficient of 0.961 (Fig. 3b), which is not as precise as an optimized PCR amplification of a DNA template alone. Unlike PCR, the IPCR protocol involves the primary step of antibody recognition of recombinant hamster PrP^C. This variable in the IPCR test may account for a loss of precision when compared to a standard curve generated by PCR of a DNA template alone. However, a semi-quantitative dose response represented by increasing Ct with decreasing recombinant hamster PrP^C molecule numbers or increasing dilutions of scrapie infected hamster brain homogenates was consistently observed.

One caveat of any test that uses PK digestion (and not an antibody specific to PrP^{Sc}) as the defining criterion for determination of scrapie infected versus normal tissues is that normal PrP^{C} may not be completely digested and will be detected by the non-PrP^{Sc} specific antibody. The fraction of PrP^{C} remaining in scrapie infected brain from terminally ill animals is estimated to be lower than 0.1% of the original amount of PrP^{C} (European Commission Directorate-General JRC, 2002, p. 69) and thus has not been a serious problem with less sensitive testing methods (e.g., the ELISA) where the cutoff range for negative (i.e., mean NSB + 3SD) will exclude 99% of the normal (uninfected) population. Frequently, five times the standard deviation is added to the mean of the NSB samples to insure 100% exclusion of samples, which may be designated as falsely positive (European Commission Directorate-General JRC, 2002, p. 29). However, it is likely that if dilutions of brain homogenates are not optimized for complete PK digestion, highly sensitive assay methods such as IPCR will detect residual PrP^{C} in the PK-digested sample resulting in a false positive result. It has been documented that the level of PrP^{Sc} in the brains of clinically ill animals exceeds that of PrP^{C} by 3–10-fold (Safar et al., 1998) and therefore, is not likely to pose a problem after optimized PK digestion. Various studies have shown that hamster PrP^{C} is expressed at levels of approximately 70 μg/gm in brain tissue (Pan et al., 1992; Groschup et al., 1997). Safar et al. (1998) have stated that the PrP^{C} concentration in eight different prion strains was <5 μg/mL. We have not found data in the literature quantifying the exact ratio of PrP^{C} to PrP^{Sc} in the same sample of scrapie infected hamster brain homogenate. The reason for this is that the presence of only PrP^{C} in each sample cannot be ascertained because all commercial anti-prion antibodies will detect both PrP^{C} and PrP^{Sc}. Thus, it is important when using a highly sensitive assay such as IPCR, to insure the removal of all PrP^{C} to avoid false positive results.

Because of the lack of exquisite sensitivity of the Western blot or ELISA in comparison to IPCR, there is no adequate experimental protocol to definitively show that PrP^{C} is completely digested in scrapie infected hamster brain homogenates when using highly sensitive assays (such as IPCR). Therefore, it was necessary to perform IPCR on several dilutions of PK-digested sample using a N-terminus versus a C-terminus specific antibody (e.g., 8B4 and 7A12) to define the presence of PrP^{Sc}. Only at the dilution where the N-terminus specific antibody is non-reactive and the C-terminus specific antibody is reactive should the sample be considered suitable for diagnostic interpretation when using an ultra-sensitive test method. This approach is necessary with IPCR when detection of PrP^{Sc} is not performed using a specific antibody.

Using IPCR, only a semi-quantitative dose response was observed when analyzing increasing dilutions of PK-digested scrapie infected hamster brain homogenates. Various reasons for this observation include the fact that the IPCR methodology is not fully standardized and many variables, which exist within the test format impact upon the final accuracy and reproducibility of the assay. These variables include the ratio of DNA reporter molecules to antigen, which may in turn be affected by several other variables (e.g., the number of biotins per antibody molecule, the number of biotins which bind to streptavidin, the number of biotins per DNA molecule, the number of biotinylated DNA reporter molecules which bind to the tetravalent streptavidins), as well as the exponential amplification of the PCR method which accentuates the upstream effects of any of the above variables. Another reason that a quantitative dose response is not always observed in the IPCR analysis is that the PrP^{Sc} molecule may be present as aggregates in solution and difficult to solubilize. If these aggregates are not adequately dispersed during the initial preparation of the sample, variations or sampling error between dilution series may result. In fact, when grouping our data from two adjacent dilutions of homogenates, more precise dose responses were often observed. Finally, other uncontrollable factors, such as very low target analyte concentrations, or pipettor coefficient of variations may account for imprecision (e.g., the Amplicor RT-PCR test for HIV-1 detection has CVs which range from 47 to 90% for samples which contain 25–50 viral RNA copies) (Amplicor HIV-1 Monitor Test, version 1.5; Roche Diagnostic Systems Inc., Branchburg, NJ).

It has been previously shown that one of the most common problems associated with the use of IPCR is the reduction of sensitivity due to high background (non-specific amplification) in the negative controls (McKie et al., 2002a, 2002b, 2002c). For example, if more than 33% of the negative controls react as false positives in a typical FDA approved ELISA test (e.g., Coulter HIV-1 p24 Antigen Assay, Coulter Corp., Miami, FL), then the test results are invalid. We enforced a more stringent criterion for the acceptance of false positive control reactions in this study; i.e., a run was not accepted if ≥25% (two out of eight) of the negative controls reacted as false positive. When higher levels of non-specific background fluorescence are present in the IPCR, the fluorescent threshold may need to be raised, thereby decreasing the sensitivity of detection. High (non-specific background) RFU levels displace the optimal setting of the BFT which, in turn, affects Ct values (Figs. 3a and 4b).

4.5. Our modifications to the IPCR method

There are relatively few publications describing the use of real-time IPCR (Sims et al., 2000; McKie et al., 2002b, 2002c; Adler et al., 2003). Others continue to make novel modifications to the standard IPCR method, which have shown dramatic improvements in the sensitivity and specificity of the method (Hendrickson et al., 1995; Niemeyer et al., 1998, 1999, 2003). We have made modifications to reported IPCR protocols in several ways to substantially reduce the occurrence of false positives (see Section 2). Our modified IPCR protocol significantly reduces false positives in the negative controls down to a range where 75–80% of normal controls

remain below the BFT. The specificity of the modified IPCR method approaches 100% because positive samples were defined only if above the threshold setting for the negative controls. It is likely that these technical modifications may compromise the sensitivity of the IPCR. However, detection down to 1 molecule/mL of PrP^{Sc} in a biological specimen may not be a prerequisite for diagnosis of pre-clinical prion infection. Presently, based on the IPCR standard curve and calculation by the regression equation, the limit of detection for scrapie infected hamster brain homogenate using the modified IPCR protocol is 1×10^4 PrP^{Sc} molecules/mL, or 330 PrP^{Sc} molecules per reaction (because 33 μL is used in the final PCR reaction). This number of PrP^{Sc} molecules is equal to 19 fg/mL.

Our study sought to confirm, and is the first to demonstrate, that ultra-low detection of PrP^{Sc} is possible with IPCR without the use of large sample volumes, sample concentration methods, or extensive sample processing. We have shown that with modifications to the IPCR method, non-specific background may be substantially reduced, if not completely eliminated, and IPCR can be used as a highly sensitive and specific method. However, further specificity studies must be performed to verify a lack of interference from other causes. A recent report describing the detection of PrP^{Sc} in BSE infected bovine brain extract by IPCR has shown the potential of IPCR to exceed the sensitivity of a corresponding ELISA method (Gofflot et al., 2004). Although this report supports the validity of IPCR as a test method for the detection of pathologic prion protein, the sensitivity was only 10 times that of their ELISA method, with a detection limit of only 750 pg/mL. In contrast, our modified IPCR method detected 100 ag/mL of recombinant hamster PrP^C and 70–700 fg/mL of scrapie infected hamster brain homogenate. This level of detection is one million times more sensitive than our ELISA, and 1000–10,000 times more sensitive than the method as described by Gofflot et al. (2004) for prion detection. We note that the IPCR procedure detailed by Gofflot et al. was not substantially different from the original procedure described by Sano et al. (1992). Our modifications to the IPCR method (Provisional Patent Application # 60/546,204) most likely contributed to the dramatic increase in the sensitivity of our method over that used by Gofflot et al. (2004).

5. Conclusions

We have shown that PrP^{Sc} can be detected by real-time IPCR in biological samples from PK-digested scrapie infected brain homogenates at concentrations which are 100,000 to 1,000,000 fold lower than those detected by Western blot and ELISA. We have also shown that our modified IPCR method possesses the potential to eliminate non-specific background in approximately 80% of assay runs, thereby increasing the sensitivity of the method to detect fg/mL to ag/mL levels. Future studies are planned to challenge our IPCR method to detect PrP^{Sc} in blood from infected animals and humans. Although not standardized to the level of performance required by a national certification agency, we believe that with further standardization, IPCR has the potential to be the most sensitive method for the detection of PrP^{Sc}, thereby offering the ability to further protect the blood supply, monitor animals and commercial products, and detect the presence of low levels of the pathogenic prion protein in asymptomatic animals and humans.

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References

Adler, M., Wacker, R., Niemeyer, C.M., 2003. A real-time immuno-PCR assay for routine ultrasensitive quantification of proteins. Biochem. Biophys. Res. Commun. 308, 240–250.

Barletta, J.M., Edelman, D.C., Constantine, N.T., 2004. Lowering the detection limits of HIV-1 viral load using real-time immuno-PCR for HIV- p24 antigen. Am. J. Clin. Pathol. 122, 20–27.

Bieschke, J., Giese, A., Schulz-Schaeffer, W., Zerr, I., Poser, S., Eigen, M., Kretzschmar, H., 2000. Ultrasensitive detection of pathological prion protein aggregates by dual-color scanning for intensely fluorescent targets. PNAS U.S.A. 97, 5468–5473.

Biffiger, K., Zwald, D., Kaufmann, L., Briner, A., Nayki, I., Purro, M., Bottcher, S., Struckmeyer, T., Schaller, O., Meyer, R., Fatzer, R., Zurbriggen, A., Stack, M., Moser, M., Oesch, B., Kubler, E., 2002. Validation of a luminescence immunoassay for the detection of PrP(Sc) in brain homogenate. J. Virol. Methods 101 (1–2), 79–84.

Brown, P., Cervenakova, L., McShane, L.M., Barber, P., Rubenstein, R., Drohan, W.N., 1999. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt–Jakob disease in humans. Transfusion 39, 1169–1178.

Brown, P., Cervenakova, L., Diringer, H., 2000. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt–Jakob disease. J. Lab. Clin. Med. 137, 5–13 (Review).

Brown, P., 2001. Transfusion medicine and spongiform encephalopathy. Transfusion 41, 433–436.

Bruce, M.E., Will, R.G., Ironside, J.W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., et al., 1997. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389, 498–501.

Bruce, M.E., McConnell, I., Will, R.G., Ironside, J.W., 2001. Detection of variant Creutzfeldt–Jakob disease infectivity in extraneural tissues. Lancet 358, 208–209.

Casaccia, P., Ladogana, A., Xi, Y.G., Pocchiari, M., 1989. Levels of infectivity in the blood throughout the incubation period of hamsters peripherally injected with scrapie. Arch. Virol. 108, 145–149.

Case, M.C., Burt, A.D., Hughes, J., Palmer, J.M., Collier, J.D., Bassendine, M.F., Yeaman, S.J., Hughes, M.A., Major, G.N., 1999. Enhanced ultrasensitive detection of structurally divere antigens using a single immuno-PCR assay protocol. J. Immunol. Methods 223, 93–106.

Cervenakova, L., Brown, P., Soukharev, S., Yakovleva, O., Diringer, H., Saenko, E.L., Drohan, W., 2003a. Failure of immunocompetitive capillary electrophoresis assay to detect disease-specific prion protein in buffy coat from humans and chimpanzees with Creutzfeldt–Jakob disease. Electrophoresis 24, 853–859.

Cervenakova, L., Yakovleva, O., McKenzie, C., Kolchinsky, S., McShane, L., Drohan, W.N., Brown, P., 2003b. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 43, 1687–1694.

Chang, T.C., Huang, S.H., 1997. A modified immuno-polymerase chain reaction for the detection of b-glucuronidase from *Escherichia coli*. J. Immunol. Methods 208, 35–42.

Collinge, J., 1999. Variant Creutzfeldt–Jakob disease. Lancet 354, 317–323.

DebBurman, S.K., Raymond, G.J., Caughey, B., Lindquist, S., 1997. Chaperone-supervised conversion of prion protein to its protease-resistant form. PNAS U.S.A. 94, 13938–13943.

Edelman, D.C., Barletta, J., 2003. Real-time PCR provides improved detection and titer determination of bacteriophage. Biotechniques 35 (2), 368–375.

European Commission Directorate-General JRC {Institute for Reference Materials and Measurements (IRMM)}, 2002. The evaluation of five rapid tests for the diagnosis of transmissible spongiform encephalopathy in bovines (2nd study). 27 March, p. 29.

European Commission (EC), 2003. Opinion of the Scientific Steering Committee on the field trial evaluation of two new rapid BSE post mortem tests. Health and Consumer Protection Directorate-General, EC, Brussels, Belgium.

Glatzel, M., Abela, E., Maissen, M., Aguzzi, A., 2003. Extraneural pathologic prion protein in sporadic Creutzfeldt–Jakob disease. New Engl. J. Med. 349, 1812–1820.

Gofflot, S., Moualij, B.E., Zorzi, D., Melen, L., Roels, S., Quatpers, D., Grassi, J., Vanopdenbosch, E., Heinen, E., Zorzi, W., 2004. Immuno-quantitative polymerase chain reaction for detection and quantitation of prion protein. J. Immunoassay Immunochem. 25, 241–258.

Grassi, J., Comoy, E., Simon, S., Creminon, C., Frobert, Y., Trapmann, S., Schimmel, H., Hawkins SAC, Moynagh, J., Deslys, J.P., Wells, G.A.H., 2001. Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue. Vet. Rec. 149, 577–582.

Groschup, M.H., Harmeyer, S., Pfaff, E., 1997. Antigenic features of prion proteins of sheep and of other mammalian species. J. Immunol. Methods 207, 89–101.

Hendrickson, E.R., Hatfield-Truby, T.M., Joerger, R.D., Majarian, W.R., Ebersole, R.C., 1995. High sensitivity multianalyte immunoassay using covalent DNA-lAbeled antibodies and polymerase chain reaction. Nucleic Acids Res. 23 (3), 522–529.

Hill, A.F., Zeidler, M., Ironside, J., Collinge, J., 1997. Diagnosis of new variant Creutzfeldt–Jakob disease by tonsil biopsy. Lancet 349, 99–100.

Hilton, D.A., Fathers, E., Edwards, P., Ironside, J.W., Zajicek, J., 1998. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt–Jakob disease. Lancet 352, 703–704.

Houston, F., Foster, J.D., Chong, A., Hunter, N., Bostock, C.J., 2000. Transmission of BSE by blood transfer in sheep. Lancet 356, 999–1000.

Hunter, N., Foster, J., Chong, A., et al., 2002. Transmission of prion diseases by blood transfusion. J. Gen. Virol. 83, 2897–2905.

Kang, S.C., Li, R., Wang, C., Pan, T., Liu, T., Rubenstein, R., Barnard, G., Wong, B.S., Sy, M.S., 2003. Guanidine hydrochloride extraction and detection of prion proteins in mouse and hamster prion diseases by ELISA. J. Pathol. 199 (4), 534–541.

Kascsak, R.J., Rubenstein, R., Merz, P.A., Tonna-DeMas, M., Fersko, R., Carp, R.I., Wisniewski, H.M., Diringer, H., 1987. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril protein. J. Virol. 61 (12), 3688–3693.

Lee, D.C., Stenland, C.J., Hartwell, R.C., Ford, E.K., Cai, K., Miller JLC, Gilligan, K.J., Rubenstein, R., Fournel, M., Petteway Jr., S.R., 2000. Monitoring plasma processing steps with a sensitive Western blot assay for the detection of the prion protein. J. Virol. Methods 84, 77–89.

Lee, D.C., Stenland, C.J., Miller JLC, Cai, K., Ford, E.K., Gilligan, K.J., Hartwell, R.C., Terry, J.C., Rubenstein, R., Fournel, M., Petteway Jr., S.R., 2001. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. Transfusion 41, 449–455.

Llewelyn, C.A., Hewitt, P.E., Knight RSG, Amar, K., Cousens, S., Mackenzie, J., Will, R.G., 2004. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. Lancet 363, 417–421.

MacGregor, I., 2001. Prion protein and developments in its detection. Transfus. Med. 11, 3–14.

McKie, A., Vyse, A., Maple, C., 2002a. Novel methods for the detection of microbial antibodies in oral fluid. Lancet Infect. Dis. 2, 18–24.

McKie, A., Samuel, D., Cohen, B., Saunders, N.A., 2002b. Development of quantitative immuno-PCR assay and its use to detect mumps-specific IgG in serum. J. Immunol. Methods 261, 167–175.

McKie, A., Samuel, D., Cohen, B., Saunders, N.A., 2002c. A quantitative immuno-PCR assay for the detection of mumps-specific IgG. J. Immunol. Methods 270, 135–141.

Moynaugh, J., Schimmel, H., 1999. Tests for BSE evaluated: bovine spongiform encephalopathy. Nature 400 (6740), 105.

Niemeyer, C.M., Burger, W., Hoedemakers, R.M., 1998. Hybridization characteristics of biomolecular adaptors, covalent DNA–streptavidin conjugates. Bioconjug. Chem. 9 (2), 168–175.

Niemeyer, C.M., Adler, M., Pignataro, B., Lenhert, S., Gao, S., Chi, L., Fuchs, H., Blohm, D., 1999. Self-assembly of DNA–streptavidin nanostructures and their use as reagents in immuno-PCR. Nucleic Acids Res. 27 (23), 4553–4561.

Niemeyer, C.M., Wacker, R., Adler, M., 2003. Combination of DNA-directed immobilization and immuno-PCR: very sensitive antigen detection by means of self-assembled DNA–protein conjugates. Nucleic Acids Res. 31 (16), e90.

Pan, K.M., Stahl, N., Prusiner, S.B., 1992. Purification and properties of the cellular prion protein from Syrian hamster brain. Protein Sci. 1, 1343–1352.

Peden, A.H., Head, M.W., Ritchie, D.L., Bell, J.E., Ironside, J.W., 2004. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 364, 527–529.

Prusiner, S.B., Bolton, D.C., Groth, D.F., Bowman, K.A., Cochran, S.P., McKinley, M.P., 1982. Further purification and characterization of scrapie prions. Biochemistry 21 (26), 6942–6950.

Prusiner, S.B., 1991. Molecular biology of prion diseases. Science 252 (5012), 1515–1522 (Review).

Saborio, G.P., Permanne, B., Soto, C., 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411, 810–813.

Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F.E., Prusiner, S.B., 1998. Eight prion strains have PrP^{Sc} molecules with different conformations. Nat. Med. 4, 1157–1165.

Safar, J.G., Scott, M., Monoghan, J., Deering, C., Didorenko, S., Vergara, J., Ball, H., Legname, G., Leclerc, E., Solforosi, L., Serban, H., Groth, D., Burton, D.R., Prusiner, S.B., Williamson, R.A., 2002. Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice. Nat. Biotechnol. 20 (11), 1147–1150.

Sano, T., Smith, C.L., Cantor, C.R., 1992. Immuno-PCR: very sensitive antigen detection by means of specific Ab–DNA conjugates. Science 258, 120–122.

Schmerr, M.J., Jenny, A.L., Bulgin, M.S., Miller, J.M., Hamir, A.N., Cutlip, R.C., Goodwin, K.R., 1999. Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J. Chromatogr. A 853, 207–214.

Scott, M.R., Will, R., Ironside, J., Nguyen H-OB, Tremblay, P., DeArmond, S.J., et al., 1999. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci. U.S.A. 96, 15137–15142.

Sims, P.W., Vasser, M., Wong, W.L., William, P.M., Meng, Y.G., 2000. Immunopolymerase chain reaction using real-time polymerase chain reaction for detection. Anal. Biochem. 281, 230–232.

Soto, C., Saborio, G.P., Anderes, L., 2002. Cyclic amplification of protein misfolding: application to prion-related disorders and beyond. Trends Neurosci. 25 (8), 390–394.

Taylor, D.M., Fernie, K., Reichl, H.E., Somerville, R.A., 2000. Infectivity in the blood of mice with a BSE-derived agent. J. Hosp. Infect. 46, 78–79.

Telling, G.C., Scott, M., Mastrianni, J., Gabizon, R., Torchia, M., Cohen Fe, DeArmond, S.J., Prusiner, S.B., 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83 (1), 79–90.

Völkel, D., Zimmermann, K., Zerr, I., Bodemer, M., Lindner, T., Turecek, P.L., Poser, S., Schwarz, H.P., 2001. Immunochemical determination of cellular prion protein in plasma from healthy subjects and patients with sporadic CJD or other neurologic diseases. Transfusion 41, 441–448.

Wadsworth, J.D.F., Joiner, S., Hill, A.F., et al., 2001. Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive immunoblotting assay. Lancet 358, 171–180.

Will, R.G., Ironside, J.W., Zeidler, M., et al., 1996. A new variant of Creutzfeldt–Jakob disease UK. Lancet 347, 921–925.