**TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES**

**ADVISORY COMMITTEE MEETING**

**19 September 2006**

**Gaithersburg, MD**

**Topic II**

**Potential screening assays to detect blood and plasma donors infected with agents of transmissible spongiform encephalopathies (TSE agents or prions)**

**Possible FDA review criteria and related issues**

**Issue: FDA seeks the advice of the TSEAC regarding potential approaches and issues to consider when validating candidate screening tests for vCJD and other TSE infections in donors of blood, plasma and human cells, tissues and cellular and tissue-based products.**

## Background

TSE infectivity has not been reliably detected by bioassays of human blood, and epidemiological studies have failed to link most forms of Creutzfeldt-Jakob disease (CJD) to prior transfusions with labile blood components or treatment with plasma derivatives. However, variant CJD (vCJD) is a disease with clinical and histopathological characteristics that differ substantially from those of other forms of CJD; therefore, special concerns about the potential for transmission of vCJD by transfusions of blood from donors incubating vCJD emerged soon after the first published report of the disease in 1996 (1). In 1999, following advice sought from TSEAC, FDA recommended deferral of blood and plasma donors who had traveled or lived for six months or more in the UK from the presumed start of the BSE outbreak in the UK in 1980 until the end of 1996 when the UK had fully implemented a range of measures to protect animal feed and human food from contamination with the infectious agent causing BSE. In January 2002, FDA recommended enhancing the vCJD geographical donor deferral policy (2) by reducing the time that an otherwise suitable blood donor might have spent in the UK from six to three months, deferring donors who had spent longer periods of time in other European countries listed by the USDA as either having had BSE or having a significant risk of BSE, and adding certain other precautionary measures, such as deferring any donor with a history of blood transfusion in the UK after 1979. Taken together, these steps were estimated to have excluded donors representing slightly more than 90% of the potential BSE/vCJD risk and deferred a projected 7% of otherwise suitable donors. Since 2002, TSEAC has several times reviewed FDA vCJD/CJD blood donor deferral policies, most recently advising FDA to recommend deferral of blood donors transfused in France (2, 3), as reflected in a current published draft guidance (2).

The occurrence of three vCJD infections—two clinically apparent and one pre-clinical or sub-clinical—in a very small group of individuals receiving transfusions of red blood cell concentrates from three separate donors who later developed vCJD indicates that one blood component transmitted vCJD efficiently and indirectly suggests that other components might also pose a risk. (The three case histories and UK transfusion surveillance program were recently

summarized by R. Will (4)). No studies assaying TSE infectivity in blood of persons incubating vCJD or ill with vCJD have been reported, so we cannot estimate either the number of human intravenous infectious units or the time during which infectivity might be present in donor blood before the onset of vCJD, though implicated blood donations reported from the UK were drawn between 18 months and 42 months before donors showed clinical signs of vCJD. If the blood of humans with vCJD is like blood of TSE-infected animals, infectivity may first appear half way through the incubation period or earlier and rise in amount before onset of clinical illness, but even during overt illness concentrations of infectivity in blood are probably less than 100 intravenous infectious units per ml.

In concert with the geographical deferral policy described above, two additional strategies might be employed to reduce further the potential risk of exposing transfusion recipients (and persons treated with derivatives of human plasma) to vCJD agent: (i) eliminate the infectious agent during processing of blood components (discussed at the last meeting of TSEAC (3)) and during manufacture of plasma derivatives (see Issue I Summary), and (ii) identify and defer from donation asymptomatic vCJD-infected donors, using a sensitive, reliable and practical screening test.

An ideal vCJD donor screening test should detect low levels of TSE infectivity in the form circulating in human blood. Unfortunately, the physical form of TSE agent in blood is not known and might differ from that of TSE agent in the infected brain tissue usually studied. Several potential TSE blood-based screening tests have been described recently (4), all based on the detection in blood of the misfolded prion protein found in brain and sometimes other tissues of humans and animals with TSEs. (Consultants to the WHO recently recommended designating collectively all abnormally folded forms of prion protein associated with TSEs as $PrP^{TSE}$—a term essentially the same as the more common designations $PrP^{Sc}$ and $PrP^{res}$ (5).) FDA discussion in advance of the desirable performance characteristics of tests to detect $PrP^{TSE}$ in blood should be helpful for test developers, the public interest, and facilitation of test development and evaluation.

All but the earliest stages of TSE test development will almost certainly require the study of various materials containing TSE agents of animal origin and human origin. FDA believes it important to distinguish clearly between those biological materials used for method development, that is, for preliminary analytical studies to investigate the basic performance characteristics of a candidate method, from human-derived materials suitable for evaluating a test's probable clinical performance.

## Discussion

**The following discussion outlines a potential approach to validate screening tests for donors infected with vCJD and other TSEs.**

### Initial Analytical Studies

A test developer would have designed and conducted appropriate analytical studies demonstrating the basic performance characteristics of a candidate donor screening test. A sponsor would have successfully discriminated between samples of human blood spiked with

suspensions of solid tissues obtained from TSE-infected animals and blood spiked with similar tissue suspensions from matched uninfected control animals. Spiked TSE infectivity might be in the form of homogenates of solid tissue from animals (probably rodents) experimentally infected with scrapie agent or, possibly, with agents derived from cattle with BSE or from humans with vCJD or other human TSE; control samples would be prepared in a similar fashion from the same tissues of uninfected animals. Preliminary studies would have demonstrated that the TSE spiking materials selected generally contained both infectivity and $PrP^{TSE}$ and that the control spikes did not; retained spiking materials would be assayed after use to confirm that they actually contained both infectivity and $PrP^{TSE}$.

Following successful discrimination of animal-derived TSE-agent-spiked blood from control-spiked blood, similar studies might be performed with normal human blood spiked with suspensions of human TSE-infected and matched control tissues. The limits of detection and intrinsic variability of the candidate method should be determined by repeatedly spiking with serial dilutions of infected tissue suspensions. All studies, except for the earliest efforts to develop and optimize the method, would be performed and interpreted blindly using replicate coded specimens. The materials used for preliminary analytical studies would include brain tissue suspensions, because the highest concentrations of both infectivity and $PrP^{TSE}$ are found in brain, but might also include suspensions of other tissues. (UK authorities have proposed distributing suspensions of TSE-infected human spleen tissues as potentially more similar to endogenously infected blood than are brain suspensions.)

After successfully and repeatedly identifying blood spiked with animal-derived and human-derived TSE agents, the candidate method would be applied to endogenously infected animal blood and matched uninfected blood samples. In the current absence of well-characterized TSE-infected primate blood, blood of rodents infected with scrapie, BSE, vCJD or other TSE agent would be considered acceptable. Blood of sheep experimentally infected with BSE agent or scrapie agent might be studied as well. If endogenously infected blood from non-human primates eventually becomes available, successful identification of that material would be especially informative because of the antigenic similarities between humans and non-human primates. While it would be desirable to demonstrate that the candidate method identified endogenously infected blood from more than one animal model, FDA acknowledges the logistical difficulties involved in the study of TSE models other than rodents and is unlikely to require studies of blood from larger animals. The FDA would not expect the sponsor to select for study more than two animal TSE models with endogenously infected blood.

The most relevant preliminary studies might be conducted using blood obtained both during overt illness and the latter part of the incubation period. Infectivity of test blood samples would be confirmed by bioassays using a number of known susceptible animals sufficient to detect the expected levels. The detection limit of a candidate blood screening test might be compared with that of an infectivity assay by diluting endogenously animal infected blood in uninfected blood and then assaying by both methods. Just as for preliminary spiking studies, operators blinded to the identity of replicate randomized samples would conduct studies with endogenously infected blood in controlled tests. Successful completion of studies with endogenously infected animal blood samples might be considered as presumptive proof of principle and would demonstrate analytical sensitivity of the proposed test method.

Should it later prove difficult for a test developer to obtain the substantial number appropriate human blood samples usually needed for conventional clinical test validation, it would be especially important that well designed and conducted $PrP^{TSE}$ spiking studies and studies with endogenously infected animal blood have demonstrated the methodology to be robust with a reproducible analytical sensitivity adequate to identify blood samples containing very small amounts of endogenous infectivity.

At the last meeting of TSEAC (6), although directed towards validating performance of devices intended to remove TSE infectivity from human blood, the committee suggested that FDA consider a validation design that might be relevant to TSE blood donor screening tests as well: TSEAC suggested the use of at least two TSE animal models and two strains of infectious TSE agent, at least one strain derived either from a cow with BSE or from a human with vCJD. (The strains might have been adapted to infect rodents.)

## Clinical Validation Studies

FDA acknowledges the logistical difficulties in obtaining blood specimens from persons with CJD and other TSEs, especially vCJD; however, just as for other tests to screen donors for evidence of infections potentially transmitted by blood, a test sponsor would design and attempt to conduct appropriate clinical trials for a candidate test. Any test suitable for licensure would ordinarily have the demonstrated ability to discriminate blood samples from persons with vCJD (and from persons with other TSEs, if detection of those infections is to be claimed) from samples from persons with other neurological diseases and samples from age-matched individuals not having a neurological disease—all diagnoses to be later confirmed at autopsy. The blood tests would be performed with coded, replicate, randomized samples in multiple runs using a pedigreed panel of blood samples, including samples from persons with confirmed vCJD as well as other human TSEs. To that end, FDA seeks to assemble or obtain access to collections of TSE biological and control reference materials including brain, spleen and blood samples from humans and animals.

Recognizing the difficulty in obtaining blood from living patients with vCJD and other TSEs in amounts adequate for test development, FDA will consider the use of cadaveric blood samples for validation studies. If cadaveric blood samples are used, FDA would recommend that the sponsor attempt to investigate possible differences between ante-mortem and post-mortem blood in test performance, including false-positive and false-negative results, signal-cutoff ratios, endpoint dilutions, and coefficients of variation. As for all donor screening tests, the possible effects of potentially interfering substances in blood, including hemolysis and elevated levels of lipids, should also be evaluated.

FDA recognizes that any validated blood donor screening test for TSE would have additional applications both as a diagnostic test and for ante-mortem testing of human tissue donors. However, the suitability of post-mortem (cadaveric) tissue donors might be evaluated more reliably by post-mortem testing of brain and lymphoid tissues—materials that usually contain more infectivity than does blood and in which $PrP^{TSE}$ would be more readily detected.

**Sensitivity.** Consistent with its policies for other infectious agents transmissible by blood, FDA does not anticipate that a TSE screening test would replace current donor deferral recommendations but would view donor testing as providing additional safety. As such, a test with only modest clinical sensitivity might still be acceptable. The number of TSE blood samples

tested to investigate clinical sensitivity would presumably not be very large—perhaps fewer than 100 but more than 20 blood samples from persons with sporadic or familial CJD patients (ideally including some from patients with vCJD), to be controlled by at least equal numbers of samples from subjects with other neurological diseases and no neurological disease. Although it would be desirable for any screening test to identify infected persons during the incubation periods of TSEs, perhaps using blood samples drawn from populations at increased risk for TSE (for example, from asymptomatic members of families with TSE-associated mutations subsequently followed clinically), it might be very difficult or impossible for a sponsor to obtain such samples and so FDA might not require them.

FDA also acknowledges that, because epidemiological studies of recipients of blood components from donors subsequently found to have forms of CJD other than vCJD failed to identify any transfusion-transmitted cases (Page P and Dodd R, presentation to TSEAC 14 Oct 2004) it is possible that only in persons incubating vCJD does infectivity reach a level in blood sufficient to transmit infection. While detection of persons with other forms of CJD by a candidate blood test would provide compelling evidence of the test's probable utility in screening donors incubating vCJD as well, failure to identify persons with other human TSEs would not rule out the possible value of a test to screen for vCJD. In spite of the great difficulty anticipated in obtaining blood samples from persons with vCJD (increasingly difficult as new cases decline in UK), a complete clinical evaluation of method performance would ordinarily require testing such samples.

**Specificity.** As for other candidate donor screening tests, specificity of a TSE test might be determined by testing a large number of samples (usually at least 10,000) from healthy suitable donors presumed to be at very low risk for TSE. A target specificity of 99.5% or greater would be sought on practical grounds for implementation in the blood and plasma donor setting. Should a test have a lower specificity, its suitability for donor screening would be determined based on evaluation of its probable overall risk (predicted adverse consequences of repeatedly reactive results that are false positives) versus benefit (interdiction of blood components and plasma derivative infected with TSE agents).

**Confirmation.** For any disease of low prevalence, the positive predictive value of a test, even of high specificity, is generally low (i.e., most reactive results will be false positives). False-positive results are particularly problematic for TSE screening tests, because incubation periods of TSEs are very long, intervention during the incubation period is not available, the prognosis after onset of clinical disease is dire, and treatments have been ineffective; for those reasons, notification of donors with unconfirmed repeat reactive screening test results would almost certainly have adverse consequences, and notifications of substantial numbers of such persons would probably not be acceptable. Thus, a reliable confirmatory test—either based on the detection of abnormal PrP or some other marker shown to correlate with infectivity—would probably be warranted for every donor whose blood was repeatedly reactive on initial screening test. The ideal confirmatory test might be a sensitive bioassay for infectivity, however, bioassays for blood donors are currently unfeasible, although development of transgenic mice highly susceptible to the agents of vCJD and other human TSEs and methods for rapid identification of infected mice may eventually provide such assays. The FDA might consider, as a possible confirmatory assay, a supplemental test for abnormal PrP based on a principle somewhat different from that of the original screening test but at least as sensitive, for example, a test detecting $PrP^{TSE}$ using an antibody directed against a PrP epitope different from that reacting with the antibody used in the screening test.

**Counseling of deferred donors.** The predictable adverse consequences of informing deferred donors whose screening tests were repeatedly reactive—with or without reactive confirmatory assays—must be carefully considered in advance.

## Questions for the Committee

1) **Please comment on pre-clinical analytical studies needed to evaluate candidate donor screening tests for vCJD and other TSEs.**
2) **Please comment on clinical studies needed to evaluate candidate donor screening tests for vCJD and other TSEs.**
3) **Please discuss the relative merits of technical options that might be feasible to confirm screening test results for vCJD and other TSEs, e.g., bioassays, alternate immunoassays, prion protein amplification, etc.**

## References


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