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MEDICAL DEVICES

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Guideline for the CE marking of blood based *in vitro* diagnostic medical devices for vCJD based on detection of abnormal PrP

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GUIDELINES RELATING TO THE APPLICATION OF:

Decision 2002/364/EC on common technical specifications for *in vitro* diagnostic medical devices

Foreword

This guideline is not legally binding. This guideline has been produced further to the addition of vCJ D blood screening assay to Annex II List A of Directive 98/79/EC and the establishment of Common Technical Specifications for these assays.

It has been elaborated by an expert group including experts from Member States' Competent Authorities, National Public Bodies, Notified Bodies, Commission' services, as well as industry trade associations.

The guideline is limited to assays for abnormal PrP in human blood plasma.

Introduction

The appearance of cases of a new form of Creutzfeldt-Jakob Disease (CJD) in young adults followed the outbreak of bovine spongiform encephalopathy (BSE) in the United Kingdom. Compared with sporadic CJD, the new form of the disease, termed variant CJD (vCJD) has a characteristic pathology, including a different tissue distribution pattern, and clinical presentation. Up to July 2010 there had been 173 cases in the United Kingdom and of the order of 200 world-wide. The pathology and epidemiology of the disease are novel, and four apparent transmissions of infection by blood transfusion from donors later developing vCJD have been identified (resulting in three clinical and one asymptomatic disease courses in the recipients at the time of death). Infection is currently undetectable in the preclinical phase so that the effect of precautionary measures is difficult to judge and studies of prevalence are difficult to carry out. However, a number of *in vitro* diagnostics (IVD) manufacturers are working on the development of blood based assays for vCJD. The candidate assays are difficult to validate because of the small number of relevant cases and samples obtained from them and because of the nature of the disease and the infectious agent. This guideline gives some minimal criteria that such tests should meet and their scientific background.

Prion diseases including vCJD, BSE, scrapie, and others are associated with the accumulation of a normal host protein (PrP) in an abnormal isoform termed PrP^{res}, PrP^{ts} or PrP^{sc}. In this document, this will be referred to as abnormal PrP. Many assays in development are based on the detection of abnormal PrP in blood but other approaches might be followed. Current views are that the abnormal protein catalyses the conversion of the normal to the abnormal conformation and is considered as the infectious agent. However, the exact nature of the infectious agents is still uncertain and it is thus not possible to establish a clear correlation between the number of abnormal PrP molecules and infectivity. In particular, aggregates consisting of different numbers of molecules of the protein are believed to be different in their infectivity so that the mass of abnormal PrP alone is not an accurate measure of infectivity. The presence of abnormal PrP should therefore be considered only as a surrogate of infection rather than a direct measure of it. In addition in an infected individual and between infected individuals, these various forms distribute differently from tissue to tissue, both in quantitative and qualitative terms. Furthermore, the different aggregated forms are likely to vary in their sensitivity to conditions such as proteolysis or denaturing agents which are used in tests for the presence of abnormal PrP. As a consequence, the ratio of infectivity to mass of abnormal PrP may depend on the means used to assay it as well as the tissue examined.

Validated blood tests could be used for various objectives such as studies of prevalence, screening and/or monitoring the blood supply, assessing infection in potentially infected groups, monitoring the effectiveness of therapies or other areas of interest. They could provide a sound basis for policy development. However, it should be appreciated that these objectives differ in nature and the analytical performance needed from the designed test may thus differ

In Europe, blood screening markers (*e.g.* HIV, HBV, HCV) are listed in Annex II list A of Directive 98/79/EC (IVD Directive). The IVD Directive defines minimal requirements for assays to be placed on the European market (CE marking procedure). Blood screening assays undergo a more stringent assessment, including involvement of Notified Bodies and fulfilment of Common Technical Specifications (CTS). In principal, vCJD blood screening tests should fulfil minimal quality requirements equivalent to other IVDs qualified for blood screening. Recognizing that the current CTS cannot be adapted easily to vCJD assays, this guideline has been generated to identify basic quality requirements for vCJD assays.

This guideline defines the minimum desirable properties of a test for vCJD potentially suitable for blood screening. If other applications of the tests are proposed e.g. confirmatory, diagnostic, monitoring, these tests shall be assessed with similar rigour to screening tests and the evaluation of the performance of the tests shall be specifically adapted to the intended use.

The use of any test for blood screening is a matter for individual Member States, considering different aspects such as epidemiology, existing precautionary measures, features of the assays, specific needs.

Scope

This guideline is intended for manufacturers, Notified Bodies and Competent Authorities.

The guideline is limited to assays for abnormal PrP in human blood plasma. For assays based on other approaches be developed or using other blood components as the testing substrate other considerations will apply.

This guideline deals with test methods using plasma as test matrix and abnormal PrP as the analyte. The principle described could be adapted to other blood components.

The primary but not the sole routine application of such tests is assumed to be the screening of blood donations. As such, the validation criteria proposed in this guideline have been designed for this primary objective.

Sample preparation

The analyte may be aggregated and have amyloid properties. Handling of the sample may therefore affect the state of the analyte and its detection by a given assay in unpredictable ways. Consideration should be given to the possible effects of the specimen receptacles, and anticoagulant used. In particular the possible loss of prion protein through binding to the type of specimen receptacle that will be used if the test is implemented should be considered. Due to the nature of the analyte, particular attention should be paid to potential effects of pre-analytical procedures on its aggregation state (freezing/thawing, centrifugation...).

As the amount of abnormal protein present is likely to be small, a pre-concentration procedure may be required.

Sample selection and characterization

The specific characteristics of samples used to assess the performance characteristics of any assay can have a significant impact on the outcome of the assessment of performance. In the case of vCJD it has to be recognized that vCJD samples are poorly characterized. In particular, the commutability of animal samples and spiked human samples with actual patient samples is unknown. Therefore it is important to ensure that:

- The samples used should be traceable and available information on its characteristics and preparation be duly documented
- In the case of animal models, it should be clear whether the material was from an infected animal in a clinical or pre-clinical phase.

The final evaluation against the CTS requirements should be state of the art at the time of the evaluation. Samples from animals in the clinical phase can be used for the assessment of the sensitivity criteria.

Blinding of specimens

There has been previous experience of claims of successful detection of abnormal PrP in non-blinded tests and subsequent failure to correctly detect blinded samples. Therefore, it is important that a component of the evaluation should include panel of blinded samples.

Comparison with another blood based in vitro diagnostic medical device for vCJD

According to the current CTS GENERAL PRINCIPLE 3.1.4, "All performance evaluations shall be carried out in direct comparison with an established device with acceptable performance...". Clearly this will not be possible for the first test applying for CE marking.

Nevertheless, if more than one assay applies for CE marking, it would be desirable to evaluate them in a coordinated manner as it would allow better interpretation of performance data.

Analytical sensitivity

Analytical sensitivity may be expressed as the limit of detection, i.e. the smallest amount of the target marker, in this case abnormal PrP, that can be precisely detected.

For tests based on the detection of abnormal PrP the target can be an aggregated form of protein, where different tissues (brain, spleen or blood) are likely to have a different range of aggregated forms and different test procedures may detect different aggregated forms to different sensitivities. Analytical sensitivity established on one source may therefore be different from analytical sensitivity defined on another.

The form of abnormal PrP in brain homogenates might differ from that in spleen homogenates. The form in blood is likely to be different from both, but an assay that performs well on the different forms found in spleen and brain might also detect the form found in blood.

The precise level of infectivity in human blood is not known. Based on animal models it is estimated that there are approximately 1 to 10 infectious doses per ml whole blood. This is consistent with the transmission of infection by blood transfusion. Infected human brain has been titrated in animals and the titre is estimated at approximately 10^6 to 10^8 per gram of brain. This figure is uncertain because of the species barrier to infection which would make the titre lower in the experimental animals used for the titration than in humans by an indeterminate factor.

However, assuming that within a given species the infectivity in brain is in the same form as in blood an assay must be able to detect at least a 1 in 10^4 dilution of 10% homogenate of infected brain on one set of extreme assumptions (10 infectious units per ml of blood and 10^6 infectious units per gram of infected brain) and a 1 in 10^7 dilution of 10% homogenate of infected brain ¹ on the other (1 infectious unit per ml of blood and 10^8 infectious units per gram of infected brain). Based on the same reasoning, as spleen has about one hundred to one thousand fold less infectivity than brain the corresponding extreme figures for 10% homogenate of infected spleen are 1 in 10 and 1 in 10^5 .

In view of the difficulties in defining the meaning of analytical sensitivity for abnormal PrP, it is unreasonable to demand the highest sensitivity where a test has been proven to identify the marker in blood. The lower figures therefore seem the most suitable as a minimum acceptable requirement, i.e. the test must detect at least a 1 in 10^4 dilution of a 10% homogenate of vCJD infected human brain and a 1 in 10 dilution of a 10% homogenate of vCJD infected human spleen.

Working reagents of 10% homogenates of brains from patients infected with vCJD have been established by the World Health Organisation (WHO) following an international collaborative study and are available from the National Institute of Biological Standards (NIBSC) in the United Kingdom. NIBSC also holds and distributes preparations of 10% homogenates of spleens from patients infected with vCJD. The spleen preparations have no WHO status but have been calibrated in studies at NIBSC against the WHO working reagents.

¹ Reference material WHO number: NHBYO/003

Analytical specificity

Analytical specificity means the ability of the method to determine solely the target marker, in this case abnormal PrP.

As prion diseases are amyloidoses and neurological, other similar diseases may interfere with the tests designed for detection of the vCJD marker. At least 100 blood (plasma) samples should be tested from the diseases selected and justified by the manufacturer based on the principles of the test and could include the following:

- patients with other diseases involving aggregated misfolded proteins including conditions with systemic amyloidosis
- patients with neurological diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease
- patients with vascular dementia
- rheumatoid factor positive individuals
- multiparous women
- patients with diabetes.

The 100 samples should not be constituted from only one category, as wide a distribution as possible should be tested.

It is possible that a satisfactory blood test for vCJD will also detect other forms of CJD. If there is less abnormal PrP in blood in the other forms of the disease the test may discriminate between vCJD and other forms. It is believed that there is greater involvement of peripheral, non neurological tissues in the pathogenesis of vCJD compared to other prion diseases. At present there is no epidemiological evidence for the presence of infectivity in blood of patients with other forms of CJD but the study design should reflect this theoretical possibility. At least 20 samples from such patients should be tested. Analytical specificity should be known. If other forms of CJD are detected, it would be preferable that the test can distinguish vCJD from these other forms.

Diagnostic sensitivity

Diagnostic sensitivity is defined as the probability that the device gives a positive result in the presence of the target marker, in this case abnormal PrP. The objective of testing is to detect infected but asymptomatic individuals. For blood the aim is to prevent infected donations from being transfused and it is possible that an infected donor may not yet

have infectious material in the blood. The diagnostic sensitivity must be demonstrated but the number of relevant samples is currently so small that the statistical significance will be low.

Considering the rarity of human presumed infected vCJD samples, it would be preferable to demonstrate the sensitivity of the test using animal samples (e.g. sheep, mouse, hamster, non human primate and finally human samples).

Samples from animal models such as scrapie of sheep or laboratory models including hamsters, mice or transgenic mice may be more readily available but the assays will be designed to work optimally on human samples, and this is likely to affect both sensitivity and specificity. In such cases, a lower sensitivity than in human samples is acceptable when justified. Since the specificity might be affected, the panels from animal models should also include negative controls.

It may be necessary to replace components of a vCJD assay (e.g. monoclonal antibodies) for the optimal adaption of the assay to a certain animal model. At the current stage of knowledge justified modifications should be accepted for providing evidence for the proof of principle.

It is likely but not proven that all humans and most animals in the clinical stage do have infectivity in the blood. Samples of blood or plasma from humans or animal models (e.g. non-human primates) that are known to be infectious (for example because they have transmitted infection experimentally or through transfusion) are very rare, but a satisfactory assay should detect such materials at a high rate using as many samples as reasonably possible and available.

- Specimen from appropriate animal models:

As many specimen as reasonably possible and available, and at least 10 specimens, shall be tested. The diagnostic sensitivity should be at least 90%.

- Specimen from humans with known clinical vCJD:

- Where there are ten or more specimen from humans with known clinical vCJD the diagnostic sensitivity should be at least 90%.

- Where there are six to nine specimens from humans with known clinical vCJD there should be no more than one false negative result and all available specimens shall be tested. This only applies in case where 10 specimens from humans with known clinical vCJD are not available

Once sensitivity at the clinical end stage has been demonstrated and suspected pre-clinical patients are identified via population screening, further studies must be undertaken to attempt to establish the time in the incubation period when the assay gives a positive signal. There are very few samples from humans known to be infected but asymptomatic at the time of sampling. No scientifically sound requirement can be formulated to specify when during the incubation period the assay should give a positive result but studies should be undertaken to show that the assay can detect asymptomatic infection to some unspecified degree. From animal models including transfusion experiments in sheep, blood may become infectious at about one third through the incubation period. The incubation period for vCJD in humans is not known except for the cases of transmission of infection through blood, where the incubation periods were approximately six to ten years from transfusion from a donor developing vCJD up to three

years after the donation was given. The level of infectivity in the blood at this stage is not known, nor how it compares to the end stage. Further more as the route of infection may influence incubation period this can provide only a very approximate guide.

It is possible to prepare samples from animal models such as experimentally infected sheep or primates, where the pathogenesis and incubation periods will be different from humans. It is known that blood from infective animals become infectious before clinical onset. Therefore studies in animal models should indicate that the assay gives a positive signal at least some time before clinical onset.

Diagnostic specificity

Diagnostic specificity is defined as the probability that the device gives a negative result in the absence of the target marker, in this case abnormal PrP. The consequences of a false positive result due to insufficient diagnostic specificity will be severe. It is desirable for any confirmatory test to be based on a different principle. A high specificity for both primary and confirmatory tests is essential.

A satisfactory assay will achieve a diagnostic specificity greater than or equal to 99.5%. For the future assays, and in the absence of a true confirmatory assay, a higher level of specificity is desirable. This implies testing of at least of 5000 samples, as it is defined in the CTS for other blood screening markers. It may be preferable to test samples from areas with no known cases of vCJD or BSE. In practice the assay may detect cases of classical CJD which would arise even in such geographical regions, but it is statistically unlikely that in an area of low vCJD prevalence a positive donor for either vCJD or classical CJD will be found in this number of samples.