

CRL GUIDANCE DOCUMENT ON TSE-RELATED SAMPLING ISSUES

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Summary

The first stage of all TSE testing procedures is sampling. It is vital to obtain the correct sample, as PrP^d is not evenly distributed in brain tissue. If a sub-optimal sample is taken, which is either too small, not from the correct area or causes damage to tissue needed for confirmation; the whole testing programme is compromised.

This paper discusses common problems encountered when taking samples of brainstem for TSE testing and recommends approaches which can minimise the inherent risks.

Aim

To ensure that appropriate samples are taken for TSE rapid testing.

Introduction

The first stage of all the current TSE diagnostic or screening tests involves the sub-sampling of the central nervous system at the level of the brainstem, and the subsequent examination of the sampled tissue for the presence of disease-specific PrP using immunochemical methods.

PrP has proved to be the most consistent marker for TSE, being present in the CNS of all recognised clinically suspect TSE cases, and it has been shown experimentally that demonstrable accumulations of PrP arise in the CNS (and in a more variable way the lymphoreticular system) in advance of any clinical disease. It is thus a useful marker in pre-clinical animals, as well as in those presenting with overt disease.

The brain consists of multiple inter-related but anatomically and functionally distinct areas, and PrP accumulation shows distinct anatomically-specific tropisms which result in clear-cut patterns of PrP accumulation. These patterns are specific both in end-stage disease, and through the pathogenesis of each form of TSE.

Cattle

In BSE the pattern appears to be highly consistent, with early changes appearing first in certain nuclei in the brainstem at the level of the obex. Following experimental oral challenge with BSE, the earliest visible PrP accumulation is consistently seen (using immunohistochemistry (IHC)) in the nucleus of the solitary tract, with involvement of the adjacent dorsal motor nucleus of the vagus nerve (DNV) and the nucleus of the spinal tract of the trigeminal nerve (V) following soon after. The vestibular nuclei in the rostral medulla may also become involved at an early stage. The IHC patterns

observed in many 'early' field cases (detected through both active and passive surveillance) support the consistent early involvement of these areas in natural disease (See figure 4).

In advanced clinical cases, all the grey-matter areas within the brainstem become affected to some extent.

The current EU regulations require that large numbers of clinically normal cattle are tested each year as part of the Community-wide active surveillance programme. This has led to a large commercial market for 'rapid' immunochemical tests which work on fresh brain tissue, and allow the timely release of carcasses from the abattoirs.

These rapid tests generally require a specific weight of brainstem tissue, taken at the level of the obex, to be presented to the test. Initially this was achieved by taking a cross-sectional slice of brainstem with a scalpel. However, this approach gave rise to some health and safety concerns, and also required that each sample was weighed before use, which can be very time-consuming. This has led to the development of safer plastic sampling tools, many of which offer the additional advantage of collecting a measured volume of tissue, thereby dispensing with the need to weigh each sample before testing.

Another hypothetical drawback of the transverse slice approach (ie using a blade) was the possibility that a full cross-sectional sample, if taken from an animal with very restricted PrP distribution, might suffer a 'dilution effect' from the non-involved areas which would compromise the sensitivity of the test. This led to the development of increasingly popular sampling techniques in which a graduated syringe is used to take a longitudinal 'core' sample of brainstem, focussing on the target areas.

In addition to collecting a fresh tissue sample for rapid testing, it is a requirement that the remaining brainstem is left in a condition that is suitable for confirmatory testing, most usually by fixation and subsequent immunohistochemistry.

The requirement is that the sampling method:

- obtains the correct amount of sample
- from the correct anatomical area
- leaves appropriate residual tissue for confirmatory testing

CRL role in evaluation of sampling tools

The CRL has a role in evaluating and approving rapid test kits for use within the EU. Sampling tools are increasingly offered as part of a commercial 'package', and as such have to be evaluated as fit for purpose, in particular to ensure that any tissue disruption as a result of sampling does not compromise the suitability of material for confirmatory testing should it be required.

In principle these tools take a core of grey matter from the obex region of the brainstem thereby reducing any dilution effect of the peripheral white matter, and increasing the sensitivity of the test. If used correctly, and with a clear understanding of the three-dimensional anatomy of the TSE target areas these tools perform adequately, and can dispense with the need to assess the weight of each individual sample, with considerable savings in time. However, there are a number of potential drawbacks with this technique that users should be aware of, and conduct appropriate monitoring checks.

These drawbacks can be broadly divided into two categories.

1. Inadequately sampled brainstem delivered to the laboratory.

This can be a problem, especially when dealing with fallen stock, where material may be significantly autolysed before sampling takes place. In such cases, the obex region may be damaged or incomplete in some way (Figure 1) or the sample may be so distorted or autolysed that anatomical orientation is not possible (Figure 2).

In these cases it will not be possible to take an anatomically targeted sample. This cannot be avoided, but it should be recorded, to assist with interpretation of the resulting test results, and to feed back to the personnel collecting the samples that ***such poor quality samples should be avoided whenever possible.***

This problem affects both tissue slice and sampling tool samples.

Figure 1

a) Sampling damage
– obex incomplete



b) Autolysis – obex identifiable, but
tissue integrity compromised



Figure 2

Distortion of brainstem – obex not identifiable

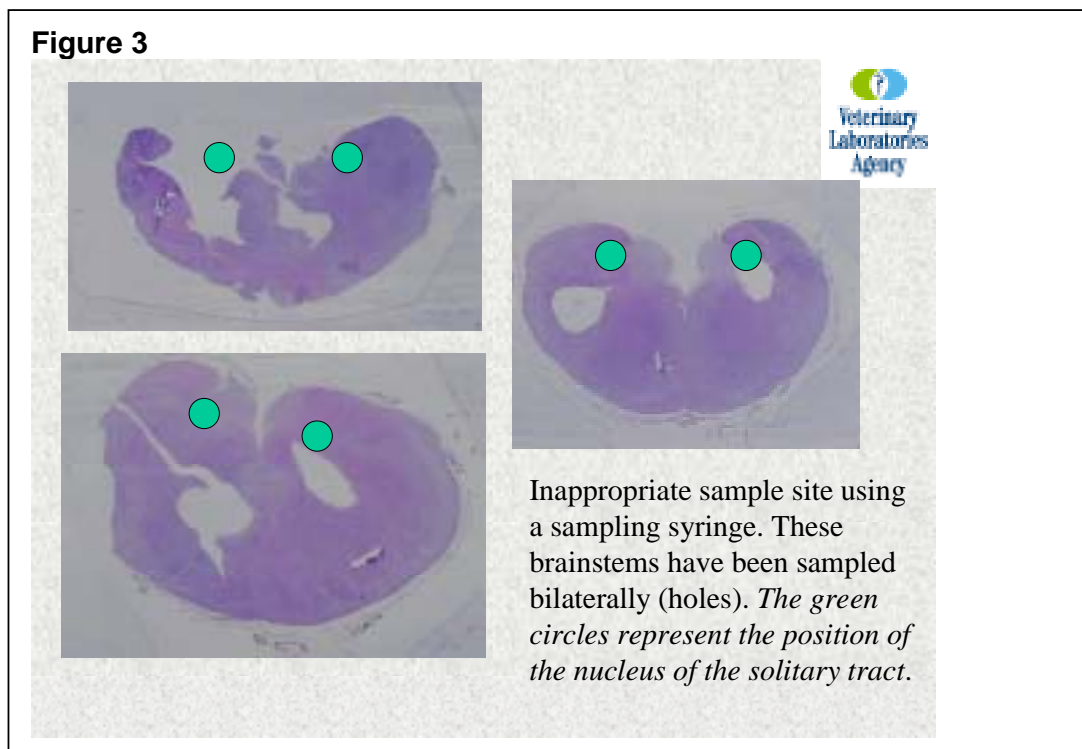


2. Inappropriately targeted sample in early/ pre-clinical disease

To avoid compromising the sensitivity of the test, the sampler must ensure that the relevant TSE target areas are adequately represented, in addition to the sample being of a consistent and appropriate weight for the test.

The nucleus of the solitary tract, which is the earliest site of PrP accumulation at the obex in pre-clinical disease in cattle, runs rostro-caudally throughout the caudal brainstem [1,2], but in its most caudal portion (caudal to the obex) it runs more medially, immediately adjacent to the spinal canal, and dorsal to the parasympathetic nucleus of the vagus nerve.

One potential danger with syringe-type 'core' extractor samples taken in their current position from variable distances caudal to the obex is that the resulting sample is too caudal to incorporate the nucleus of the solitary tract at the entry point, and may potentially miss the target areas more rostrally if the sampling tract veers laterally or ventrally (Figure 3).



This presents a potential sensitivity problem, especially in active surveillance cases where the PrP accumulation is likely to be very focal, and anatomically targeted to the nucleus of the solitary tract, the dorsal motor nucleus of the vagus nerve and the nucleus of the spinal tract of the trigeminal nerve (Figure 4)

Robust training and great care are needed in the application of this type of sampling method to ensure that the *initial rapid test* is not compromised by the collection of a sample which does not represent the desired target area.

In cattle approaching or in clinical-stage disease, almost any grey-matter sample will contain sufficient PrP to produce a positive rapid-test (Figure 5)

Figure 4

(This figure is reproduced from the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*)

1=nucleus of the solitary tract; 2= nucleus of the spinal tract of the trigeminal nerve; 3=dorsal nucleus of the vagus nerve

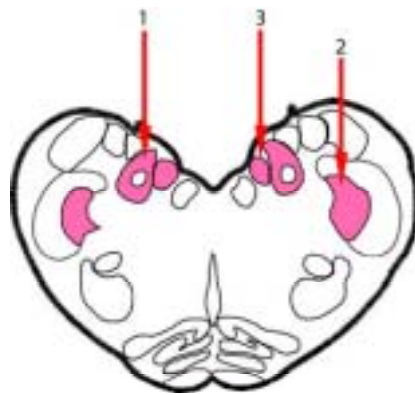


Figure 5

PrP immunostaining at the obex in a clinical case of BSE



However, earlier accumulations of PrP (visualised by immunohistochemistry) –as would be likely in any younger healthy slaughter population - are much

more targeted, and the boundaries of such PrP accumulation are very abrupt (Figure 6)

Figure 6

Boundaries between those neuroanatomical areas targeted by PrP accumulation (to the left of the line) and those which are not, are very clear.



Such cases may be missed by any sampling method that takes a longitudinal core which, if inaccurately directed, might not contain the target areas.

Additional evidence to support the need to sample close to the obex comes from the initial rapid test evaluation data generated by the IRMM in Geel http://europa.eu.int/comm/food/fs/bse/bse42_en.pdf (see summary on p.65)

Sheep

All of the above issues apply also to sheep, with the added complication that PrP distribution patterns vary more than in cattle, and the physical size of the brainstem is much smaller than in cattle.

The principal target area (the dorsal nucleus of the vagus – see Figure 4) lies very close to midline. This means that any approach, whether it is hemisectioning or core sampling, has little tolerance for inaccuracy if the target area is to be sampled from one side and left intact on the other.

Action advised

1. **Each NRL should ensure that all local sampling instructions contain appropriate detailed reference to the cross-sectional and longitudinal anatomy of the structures which require to be targeted.**
2. Additionally, instructions must be included on how to record and sample material which is not optimally collected/oriented or properly identifiable at an anatomical level when it is not possible to correctly position the sampling tool, or accurately identify the obex.
3. It is advised that the accuracy of sampling is monitored by the NRL by review of a randomly-selected proportion of negative cases in addition to any positives which are referred for confirmation, together with a review of any problems documented under point 2.

References

1. Yoshikawa,T. (1968). Atlas of the Brains of Domestic Animals. The Pennsylvania State University Press, University Park and London.
2. Lignereux,Y. (1986) Atlas stereotaxique de l'encephale de la vache frisonne. PhD Thesis, l'Universite Paul Sabatier de Toulouse.

We acknowledge the OIE, Angus Wear, Makoto Haritani and Stefan Roels for some of the illustrations.