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Continuous monitoring of bovine spongiform encephalopathy rapid test performance by weak positive tissue controls and quality control charts

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ABSTRACT

Bovine spongiform encephalopathy (BSE) rapid tests and routine BSE-testing laboratories underlie strict regulations for approval. Due to the lack of BSE-positive control samples, however, full assay validation at the level of individual test runs and continuous monitoring of test performance on-site is difficult. Most rapid tests use synthetic prion protein peptides, but it is not known to which extend they reflect the assay performance on field samples, and whether they are sufficient to indicate on-site assay quality problems. To address this question we compared the test scores of the provided kit peptide controls to those of standardized weak BSE-positive tissue samples in individual test runs as well as continuously over time by quality control charts in two widely used BSE rapid tests. Our results reveal only a weak correlation between the weak positive tissue control and the peptide control scores. We identified kit-lot related shifts in the assay performances that were not reflected by the peptide control scores. Vice versa, not all shifts indicated by the peptide control scores indeed reflected a shift in the assay performance. In conclusion these data highlight that the use of the kit peptide controls for continuous quality control purposes may result in unjustified rejection or acceptance of test runs. However, standardized weak positive tissue controls in combination with Shewhart–CUSUM control charts appear to be reliable in continuously monitoring assay performance on-site to identify undesired deviations.

1. Introduction

In view of the epidemic and zoonotic potential of bovine spongiform encephalopathy (BSE), a prion disease in cattle (Prusiner, 1997; Wells et al., 1987), its prevalence is monitored worldwide by surveillance schemes that target clinically BSE suspicious and *per se* unsuspicious cattle slaughtered for human consumption (Oesch et al., 2000) as well as perished animals (Doherr et al., 2001; Doherr et al., 2002). Reliable diagnostic *ante mortem* procedures for BSE are not yet available; the diagnosis relies on *post mortem* immunochemical detection of the disease-associated pathological prion protein (PrP^d) or the microscopic examination for characteristic histopathologic lesions in brain tissue samples (Gavier-Widen et al., 2005). In the past decade numerous rapid PrP^d detection methods have been developed that allow screening of large sample numbers in a reasonable time frame (Biffiger et al., 2002; EFSA, 2004; Grassi et al., 2001; Schaller et al., 1999). However, a positive screening test always results in

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a laboratory BSE suspicion followed by confiscation of carcasses and defined actions of the veterinary services until the results of time-consuming and laborious confirmatory tests (Office International des Epizooties, 2004) are available. In consequence false positive samples are not desirable and therefore a high diagnostic specificity resulting in a high predictive value of a positive test result is required. On the other hand, a high diagnostic sensitivity and thus a high predictive value of a negative test result are essential to estimate the disease prevalence precisely. To assure both most countries require (i) approval of BSE rapid tests before implementation, (ii) batch release testing of test kit production lots by the authorities, (iii) screening laboratories as well as test kit suppliers to work under standardized quality management systems and (iv) participation of the screening laboratories in interlaboratory comparison trials (Buschmann et al., 2004). Indeed, all these measures do not monitor the test performance on-site at the level of individual analytical runs. This can be achieved by the introduction of defined control samples in parallel to the test samples. Because of biosafety concerns and the lack of sufficient amounts of material, BSE positive tissue control samples are neither supplied with the test kits nor provided by most national reference laboratories. Instead, non-infectious recombinant or synthetic PrP peptides are included in the test kits. Most of the current BSE rapid tests first separate PrP^d from its physiological isoform termed PrP^c by limited digestion with proteinase K (PK). This treatment completely degrades PrP^c, but leaves a PK resistant core fragment (PrP^{res}) of PrP^d intact (Prusiner, 1996). In a second step, PrP^{res} is then detected immunochemically by PrP specific antibodies. The positive control peptides provided in the test kits (hereafter referred to as peptide controls) are PK sensitive and thus introduced into the test procedure after the digestion step. Consequently they act as controls for the detection step, but not for the PK digestion.

Assay validation, in addition to assess criteria that identify any undesired deviations at the level of individual test runs, should also involve means to monitor the test performance of analytical runs over time (Jacobson, 1998). The degree of standardization of the kit peptide controls may be variable and is usually not known to the laboratories. It is therefore not clear to which extent such controls have the potential to serve also for continuous quality control purposes.

In order to address these aspects we characterized and subsequently introduced weak positive BSE tissue controls (hereafter referred to as weak positive controls) in two widely used commercial BSE rapid tests, the PrioStrip (Prionics, Switzerland) and the TeSeE ELISA (Bio-Rad, France), as additional controls. Their performance was recorded in test runs in two routine diagnostic laboratories over a period of several months.

The purpose of this study was to assess the suitability of the peptide positive controls by comparison with standardized weak positive tissue controls for a continuous monitoring of BSE rapid tests and to develop a statistics-based early on-site warning system for undesired random or systematic shifts or drifts in the test performance.

2. Materials and methods

2.1. Laboratory facilities

All experiments were conducted according to national biosafety regulations (SECB, 2001). Tissue homogenates for weak positive controls were prepared at the NeuroCentre (Swiss National Reference Laboratory for animal TSE). Rapid tests for routine BSE screening were conducted in frame of the Swiss BSE surveillance program at the NeuroCentre for the PrioStrip and at the Institute Galli-Valerio for the TeSeE ELISA. The latter joined the study at a later stage and therefore the number of tests runs for the PrioStrip ($n = 160$) was higher than for the TeSeE ($n = 59$). Both laboratories perform BSE rapid tests under a quality management system according to ISO 17025.

2.2. Reference samples

Individual brainstem tissue homogenates of six different BSE confirmed cattle originated from Switzerland and were prepared as follows. For the PrioStrip three brainstem samples (5 g each) were homogenized in 50 ml PrioStrip homogenization buffer with an Ultra Turrax T25 blender (IKA, Germany) at 24,000 rpm. Similarly for the TeSeE three different brainstem samples were homogenized in 50 ml of a 320 mM sucrose solution. Serial dilutions of two of these homogenates in BSE negative homogenate-pools were prepared for the two test respectively and analyzed in sequential test runs. For both rapid tests a large stock of the highest dilution that scored consistently positive was prepared as weak positive control, homogenized in a second round and stored at −20 °C until use in 120 μl ($n = 300$) and 270 μl ($n = 300$) aliquots for the PrioStrip and the TeSeE, respectively. In each of the test runs under investigation an aliquot was thawed and treated similar to field samples homogenates. The homogeneity of the PrP^d concentration in the undiluted homogenates and the weak positive controls was assessed by analyzing replicates of 10 in the same test run of the respective test format. In addition, triplicates of the weak positive controls were included in a blinded set of 16 BSE positive and negative homogenates that was sent to BSE screening laboratories in Switzerland (PrioStrip: $n = 3$, TeSeE: $n = 4$) as part of biannual interlaboratory comparison trials. All laboratories analyzed the samples in parallel in single test runs. The results and the raw data were reported to the reference laboratory.

2.3. BSE rapid tests

The PrioStrip is an immunochromatographic assay for the detection of PrP^d in fresh brainstem tissue samples. Briefly, after sample collection, a homogenate is prepared from a defined piece of tissue of the obex region in the *medulla oblongata*. The homogenate is PK-treated at 47 °C for 60 min to degrade PrP^c. After a pre-treatment, the remaining PrP^{res} is then complexed to a PrP specific monoclonal antibody (mAb) that is conjugated to blue latex microbeads. By capillary forces, the sample con-