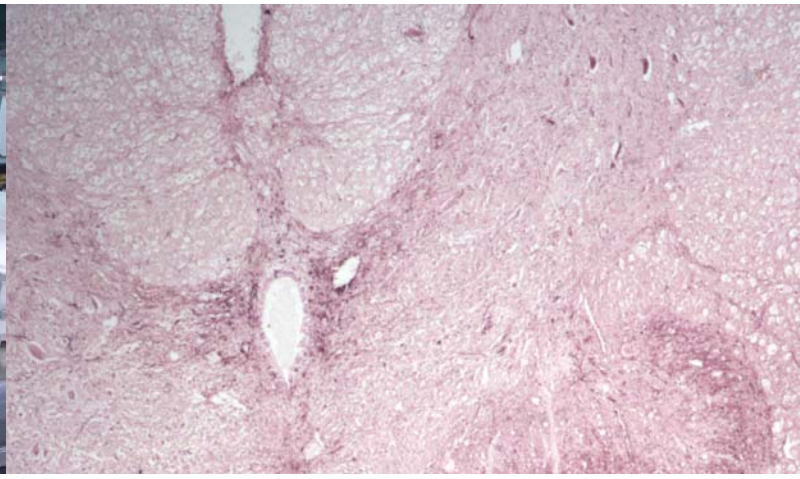
Automated IHC analysis of tissues on slides

Automated IHC analysis of swine fever, TSE, BSE and scrapie on paraffin-embedded and frozen tissues



Introduction

Immunohistochemistry (IHC) is a detection technique used not only for research, but also diagnostics of animal epidemics. The method is established in the international and EU regulations for the diagnosis of notifiable animal epidemics. For the diagnosis of classical swine fever (KSP), the use of the fluorescent antibody test (FAT) on cryosections is laid down in the OIE Manual (literature) and in the EU diagnostics manual (1, 4).
For the diagnosis of transmissible spongiform encephalopathy (TSE), bovine spongiform encephalopathy (BSE) and scrapie, the use of immunohistochemical detection methods (IHC) on formalin-fixed tissue is required according to the OIE Manual (5, 6). The OIE method is rendered mandatory by EU regulation 999/2001.

Materials and Methods

Diagnosis of swine fever (KSP-FAT)
The KSP-FAT is carried out according to the method recommended by the national reference laboratory (3). In preparation of the test, cryosections of tonsil, spleen, lymph node, ileum and kidney are cut, air-dried for 30 minutes and fixed in acetone for 20 minutes. KSP- antigen is detected by the monoclonal antibody BIO 275. In parallel, sections are treated with an unrelated antibody (L42) to serve as a negative control.
Staining and mixing of working solutions are carried out by the GenePaint System: rinsing with PBS pH 7,6 during assembly of the slides into the GenePaint Flow-Through Chambers, blocking with 120µl 5% BSA in PBS for 30 min, washing with 300µl PBS, incubation with primary antibody (dilution according to the type of tissue) for 60 min, 3 washing steps with 300µl PBS each, incubation with FITC-labeled secondary antibody including Evans Blue at a suitable dilution (1:50 to 1:200) for 30 min, 3 washing steps with 300µl PBS each.

Slides are removed from Flow-Through Chambers, washed briefly with distilled water and mounted in DABCO fluorescence conservation buffer. Specimen from the national reference laboratory serve as a positive control. For comparison, both assays are pipetted by hand and incubated in a humid chamber.

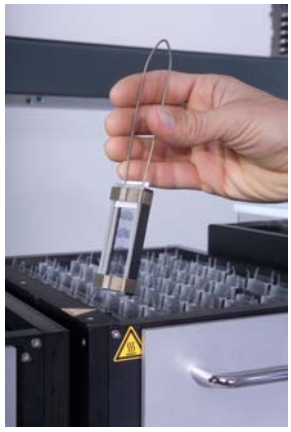
Diagnosis of TSE, BSE and scrapie

TSE-IHC is carried out using the streptavidin-biotin method. The tissue is fixed in formalin. Since formalin stabilises pathological prion proteins, the tissue is treated with concentrated formic acid for 1 hour in order to reduce infectiosity.

The tissue is processed and embedded in paraffin according to a standard protocol. The series of decreasing ethanol concentrations is carried out in the GenePaint pipetting robot, because all of its components are solvent resistant.

All working solutions are mixed by the pipetting robot, except for volumes below 10µl, which are pipetted by hand according to the program's calculations. Endogenous peroxidase is inactivated with H_2O_2 in methanol. Another washing step with TBS is followed by a proteinase K treatment to unmask the epitope (2). Monoclonal antibody L42 is used as primary antibody at a dilution of 1:250 in TBS. Detection is carried out using biotinylated goat anti-mouse antibody (1:300) and streptavidin-peroxidase conjugate (1:300). Suitable substrates are Vector VIP ® (purple) or DAKO DAB (brown). There are 3 washing steps in TBS between each incubation. Specimen from the national reference laboratory serve as a positive control.

Figure 1: GenePaint Chamber Rack holding 48 Flow-Through chambers. The Liquid Handling arm of the robotic system is equipped with eight dispensing tips. It assures automatic and parallel pipetting of all solutions.



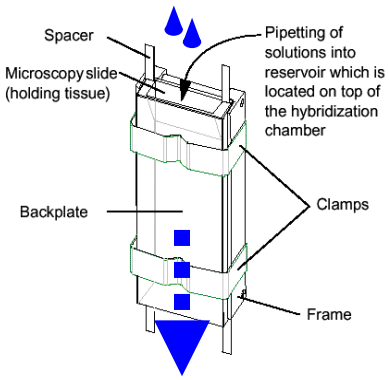


Figure 2: GenePaint Flow Chamber with slide carrying tissue sections. Schematic drawing shows how the slide is assembled in the flow chamber.

Results

KSP-FAT: A positive reaction results in a brilliant, apple green cytoplasmic fluorescence in antigen-containing cells. Figure 1 shows the positive control: a lymph follicle in the lymph node of an infected pig, developed in the GenePaint System.

TSE-Immunohistochemistry: monoclonal antibody L42 labels prion protein PrP in the tissue. Protein particles appear as pronounced intracellular granules, as extracellular plaque-like deposits and as extracellular granules. If VIP ® is used as substrate, deposits are purple (Fig. 2, 4x objective; Fig.3, 40x objective). If DAB is used as substrate, they appear brown (Fig. 4).
Figures 2-4 show the clearly visible signals of the positive controls: paraffin sections of spinal chord of a scrapie-infected sheep.

Discussion

Automation of the immunohistohemical detection method produces results of equal quality to those generated by hand-processing. However, it is important to take care that all solutions are used at incubation temperature, otherwise air bubbles form at the warmer surface of the specimen. These air bubbles may lead to considerable decrease in the quality of results.
A major advantage of automation is that the formulas for calculating working dilutions are integrated in the program, so that individual calculations for different numbers of specimen do not need to be carried out.

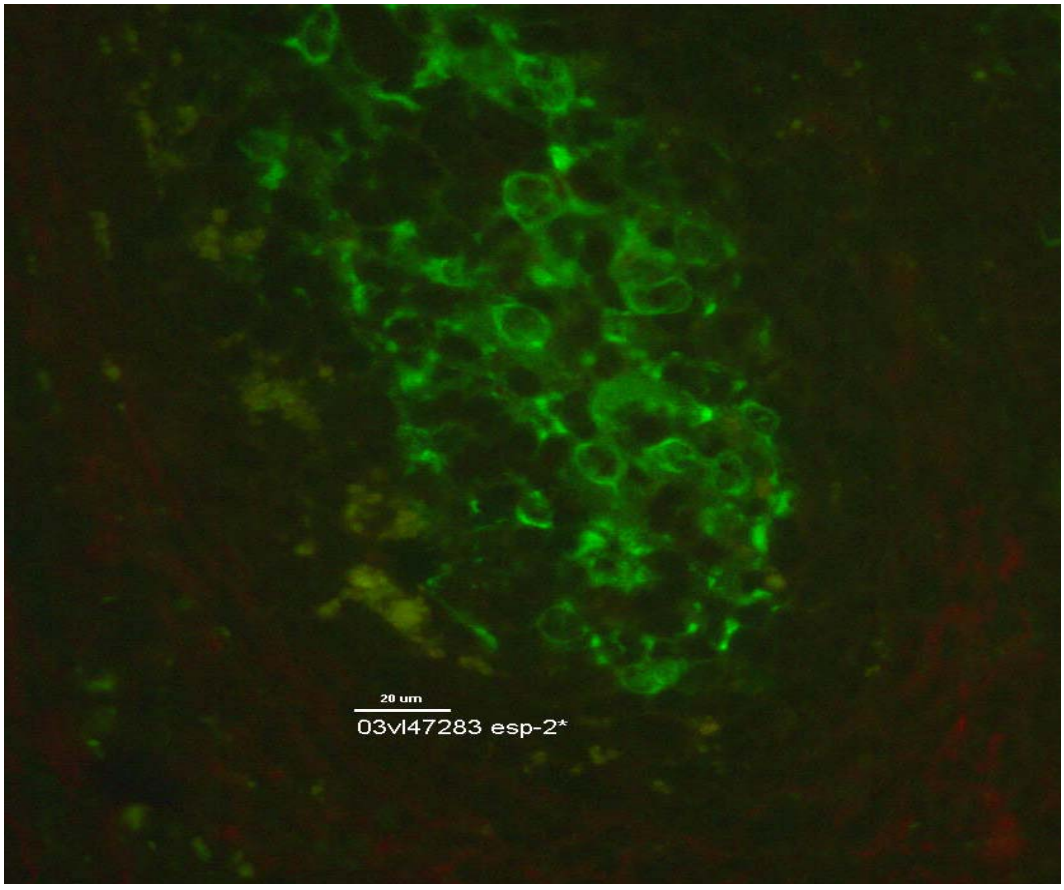


Figure 3: KSP-FAT, BIO 275 (1:50), 40x objective. Lymph node of a KSP-infected pig,positive control.

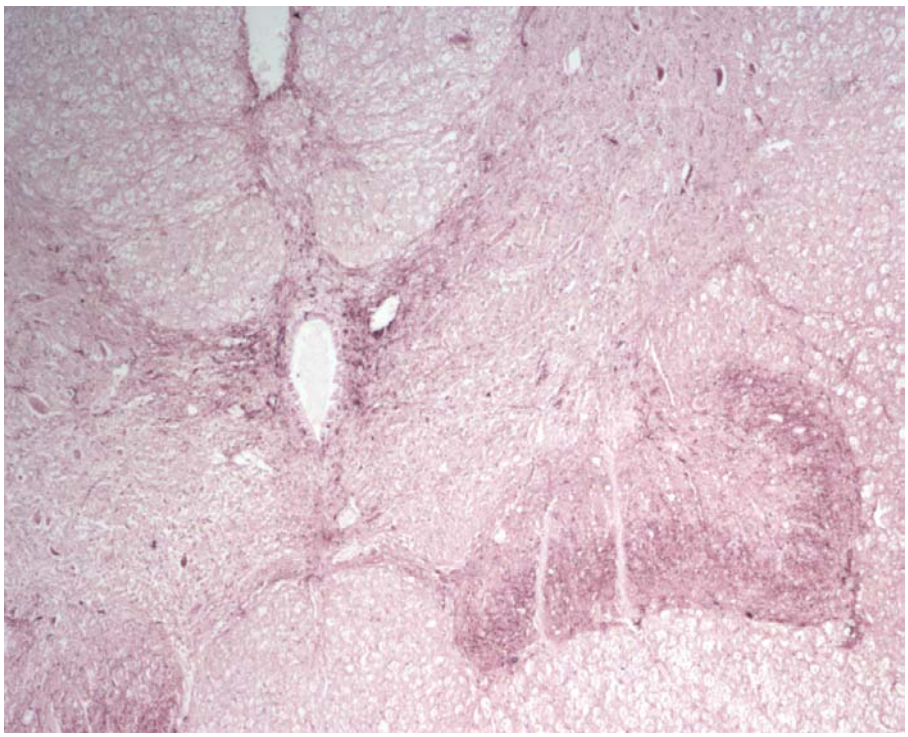


Figure 4: TSE-IHC, L42 (1:250), substrate VIP ®, counterstain 'Hämalaun', 4x objective. Spinal chord of a scrapie-infected sheep, positive control. Areas of PrP deposits are already visible at low magnification.

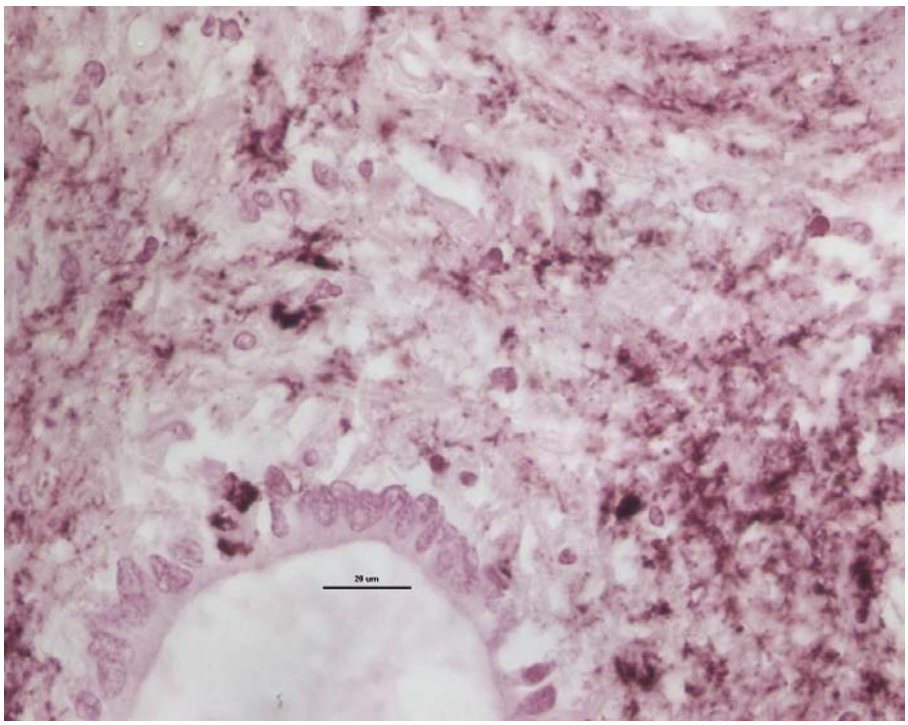


Figure 5: TSE-IHC, L42 (1:250), substrate VIP ®, counterstain'Hämalaun', 40x objective. Spinal chord of a scrapie-infected sheep, positive control

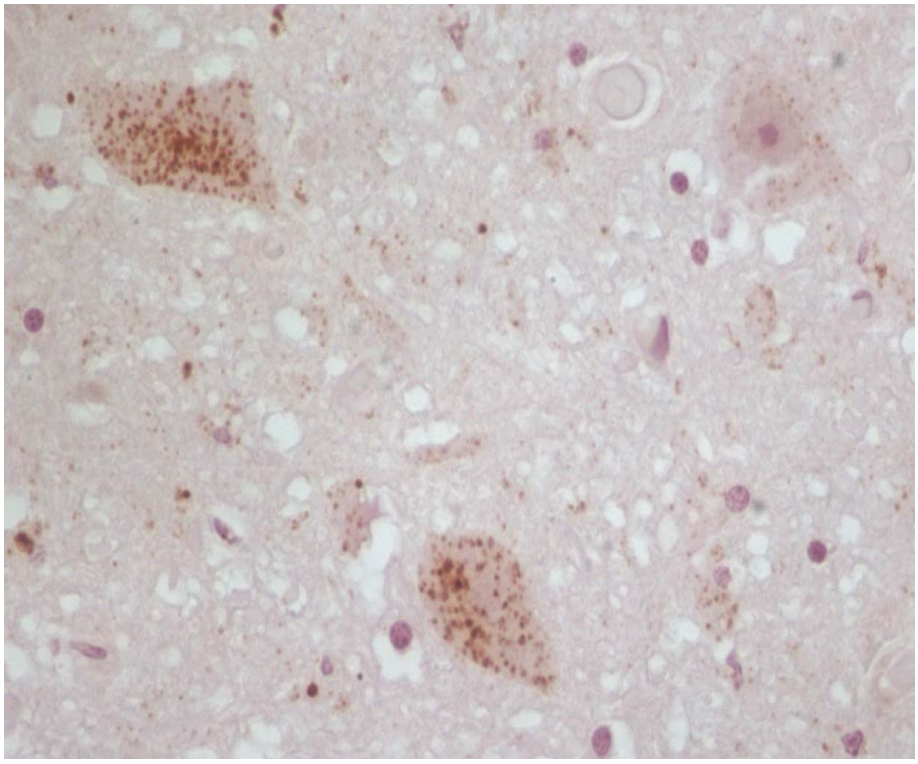


Figure 6: TSE-IHC, L42 (1:250), substrate DAB, counterstain 'Hämalaun', 40x objective. Spinal chord of a scrapie-infected sheep, positive control.

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