EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

OPINION OF THE SCIENTIFIC STEERING COMMITTEE ON THE FIELD TRIAL EVALUATION OF TWO NEW RAPID BSE POST MORTEM TESTS

Results achieved using the LIA Test (Prionics) and the aCDI Test (InPro) in the field trial

Adopted on 6 March 2003

Keywords: Bovine Spongiform Encephalopathy, rapid tests, evaluation, field trial

1. Introduction:

Following a call for expression of interest several parties indicated their wish to participate with their new rapid BSE post mortem tests in an EC evaluation exercise. In a pre-selection, 5 of these tests were selected for participation in a laboratory evaluation exercise conducted by the Institute of Reference Materials and Methods (IRMM) of the European Commission.

The results of this laboratory evaluation exercise were discussed by an expert group on 12 December 2001. On 11 January 2002 the Scientific Steering Committee (SSC) recommended that these tests should undergo an evaluation under field conditions (field trial) prior to approval. The Commission Services invited the SSC to prepare a design for such a field trial, which was adopted by the SSC on 22 February 2002[1] and lays down the precise protocol to be followed in order to establish whether the tests in question can demonstrate their non-inferiority when compared to already approved tests. The already approved tests were used as reference tests for the new rapid BSE tests (new tests) during the field trial evaluation.

It has to be emphasised that the purpose of this field trial was not to rank the sensitivities of approved and new tests or to find out whether the new tests are able to detect BSE in cattle earlier in the incubation period. These issues should be looked at in separate studies.

In order to assess the results and the execution of the field trial, an expert meeting was held on 12 February 2003, which brought together experts who have been already involved in the drafting of the field trial protocol and other experts who were involved in the execution of the field trial using one of the tests under routine conditions in their laboratories.

IRMM, whose task was to collect all field trial data as specified in the field trial protocol prepared internal reports (one for each company) for this meeting summarising relevant data and also giving details on events/problems, which occurred during the execution of the field trial.

Prionics and InPro provided their package inserts, which they claim describe exactly the laboratory work carried out during the execution of the field trial.

The above mentioned documentation served as the basis for the discussion held during the working group meeting.

[1] Opinion of the SSC on Design of a field trial for the evaluation of new rapid BSE post mortem tests http://europa.eu.int/comm/food/fs/sc/ssc/out246_en.pdf

2. Mandate

As stated in the field trial protocol, the performance of any new test should not be statistically inferior to that of the currently approved tests. It was the mandate of the working group to assess on the basis of the field trial data, whether the new tests under consideration fulfil this criterion.

3. Conditions for the field trial

According to the field trial protocol the following had to be examined:

- the sensitivity relative to reference tests: 200 true positive samples that were tested positive using a reference test;
- the specificity relative to reference tests: 10,000 consecutive samples from healthy slaughtered cattle that were tested negative using a reference test;
- the performance of the new tests on low quality samples: 200 poor quality negative samples that were tested negative using a reference test.

Other conditions were:

1. Each new test should be compared to at least two reference tests as regards the sensitivity (200 true positive samples) and the specificity (10,000 negative samples). The maximum number of samples to be tested with one of the reference tests should not exceed 70%.
2. The examination of the 200 true positive samples and the 10,000 negative samples should be carried out in at least two different laboratories located in at least two different Member States or in at least one Member State and Switzerland. The maximum number of samples to be tested in one of the laboratories and one of the countries should not exceed 70%.
3. The estimation of sensitivity (200 true positive samples) has to be carried out in National Reference Laboratories (NRL) or in certain state owned laboratories if they are in the possession of a suitable number of positive samples and if the responsible NRL agrees.
4. The true positive samples should be well documented (origin and age of the sample, e.g. sub-population; condition of the sample, e.g. autolysis etc.; brain region used; storage conditions; duration of storage).
5. The true positive samples should be homogenised following a defined protocol (Lind protocol) or following other equivalent homogenisation protocols if their use is justified to IRMM (companies have to show that their homogenisation protocol does not discriminate the reference test).
6. At least two batches of the new tests have to be included for the estimation of the sensitivity and the specificity.
7. Raw data on the testing of the 200 true positive samples have to be submitted to IRMM at least on a weekly basis.
8. Raw data on the testing of the 10,000 negative samples form routine slaughter will be communicated to IRMM on a daily basis.
9. Description of the test procedure: it was necessary to have clear, stringent and detailed descriptions of the test procedures used during the field trial. The laboratories involved

should meet in order to discuss whether the written test procedures used during the field trials proved to be accurate and fully understandable. If necessary, test procedures might have to be clarified.

The clarified test procedures or if clarification was not necessary, the test procedures used, would then be defined as part of the approval. Later changes in the test procedure would invalidate the approval.

4. SUMMARY OF RESULTS

4.1 LIA Test (Prionics®-Check Luminescence Immunoassay)

Prionics opted to change the homogenisation protocol for the positive samples. This amendment was agreed by IRMM after Prionics demonstrated that the changed homogenisation protocol did not discriminate the reference test.

	ReferenceTest	**number of samples**	**correctly identified**	**%**	**95% confidence limit one sided Poisson**
Specificity	BioRad Platelia	4101	4101	100	
	Prionics Check Western	9781	9781	100	
Total		**13882**	**13882**	100	>99,97%
Sensitivity	BioRad Platelia	153	153	100	
	Prionics Check Western	77	77	100	
Total		**230**	**230**	100	>98,7%
Low quality samples	Prionics Check Western	**200**	**200**	100	>98,5%

The LIA test fulfilled all conditions as laid down in the field trial protocol. All samples were examined between 30 July and 20 December 2002.

During the testing of the specificity of a total of 13,882 samples from healthy slaughtered cattle 12 samples initially tested positive but turned out to be negative upon re-testing according to the field trial protocol.

4.2 aCDI Test (InPro automated Conformation Dependent Immunoassay)

InPro used the Lind protocol for the homogenisation of the positive samples.

	Reference Test	**number of samples**	**correctly identified**	**%**	**95% confidence limit one sided Poisson**
Specificity	Enfer	4482	4482	100	
	BioRad Platelia	5737	5737	100	
Total		**10219**	**10219**	100	>99,97%
Sensitivity	BioRad Platelia	146	146	100	
	Prionics Check Western	72	72	100	
Total		**218**	**218**	100	>98,6%
Low quality samples	Prionics Check Western	**200**	**200**	100	>98,5%

The aCDI test fulfilled all conditions as laid down in the field trial protocol. All samples were examined between 2 October 2002 and 25 January 2003.

During the testing of the specificity of a total of 10,219 samples from healthy slaughtered cattle 15 samples initially tested positive but turned out to be negative upon re-testing according to the testing protocol.

5. CONCLUSIONS

Having assessed the field trial results, the working group concluded that both tests fulfilled the requirements of the field trial and that both have performed at least equally well compared to the reference tests, i.e. they have proven their non-inferiority.

After some necessary amendments to both provided package inserts, the group was satisfied that these test descriptions reflect the test procedures followed by the laboratories involved during the execution of the field trial. These package inserts should be defined as part of the approval and changes should only be introduced after having been notified to, and agreed by, the European Commission.

Both tests are recommended for approval by the European Commission in the framework of Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 ("the TSE Regulation").