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TECHNICAL REPORT

Technical meeting of the EFSA Scientific Network for Risk assessment in Animal Health and Welfare - Bovine Tuberculosis Testing Parma 21 February 2012[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The Animal Health and Animal Welfare (AHAW) scientific network was created in 2009 with the aim to facilitate scientific cooperation in the field of the EFSA's mission by coordinating activities, exchanging information, developing and implementing joint projects, exchanging expertise and best practices. During the AHAW scientific network meeting, in November 2011, the subject of bovine tuberculosis (TB) and in particular diagnostic testing for the purpose of disease control and demonstration of freedom was identified as of common interest. A technical meeting was organised with the objective of sharing information about bovine TB and bovine TB testing data and to assist EFSA in collecting information relevant to future mandates. Representatives from 10 Member States participated in the meeting. Based on the experts presentations a summary of the information concerning the official status on Bovine TB infection of each country and bovine TB testing and surveillance was made. There is large variation on the test and testing protocols used in different MS. These variations refer to the use of both the official bovine TB diagnostic test (tuberculin skin tests) and the ancillary test (IFN-γ BA test) but also and more important on the objectives to achieve. These variations can affect the diagnostic tests accuracy. The different tests and testing protocols are not adequately described resulting in surveillance data that are difficult to validate and compare. he meeting provided an overview of the issues on the disease surveillance and control and gave the opportunity to identify areas of future collaboration.

KEY WORDS

Bovine tuberculosis, testing, IFN-γ test

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BACKGROUND AS PROVIDED BY EFSA

AHAW Network-Mandate and mission

The Animal Health and Animal Welfare (AHAW) scientific network was created in 2009 with the aim to facilitate scientific cooperation in the field of EFSA's mission. In line with the "Decision concerning the establishment and operation of European Networks of scientific organisations operating in the fields within the Authority's mission"[4], the aim of the network is to support the European Food Safety Authority (EFSA) and the Member states in carrying out its mission in accordance with the established standards of scientific excellence, transparency and responsiveness foreseen in Regulation No 178/2002/EC[5]. These include *inter alia* facilitating the development of a scientific cooperation framework by the coordination of activities, the exchange of information, the development and implementation of joint projects and the exchange of expertise and best practices in the fields within the Authority's mission.

During the AHAW scientific network meeting, in November 2011, the subject of bovine tuberculosis (TB) and in particular diagnostic testing for the purpose of disease control and demonstration of freedom was identified as of common interest.

The procedures for gaining, maintaining, suspending, withdrawing or re-gaining the officially tuberculosis free (TBOF) herd status are laid down in Annex A to Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine ("the Directive")[6] and are based on the results of tuberculin skin tests carried out in bovine herds. In addition Member States or regions thereof may be declared TBOF if certain requirements are fulfilled. Annex B to the Directive sets up details of the diagnosis of bovine TB.

It was agreed to organise a technical meeting with the objective of sharing information about bovine TB and bovine TB testing.

Furthermore EFSA received in December 2011 a request from the European Commission (Mandate M-2011-0378) on bovine tuberculosis testing and the technical meeting was a opportunity to exchange information between EFSA and MS.

PARTICIPANTS

The AHAW Network members were requested to confirm their interest in participating in the meeting and appoint one Tuberculosis experts from their countries. The meeting participants and their affiliation are listed in Appendix A.

[4] http://www.efsa.europa.eu/en/networks/supportingunits.htm
[5] Regulation 178/2002/EC, of 28 January 2002, laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002.
[6] Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra community trade in bovine animals and swine OJ L121, 29.7.1964.

DISCUSSION

1. Current work on Bovine Tuberculosis at MS

The experts were invited to give a presentation about the current situation in their country describing if appropriate the following aspects:

- Country status *vis a vis* bovine TB (Free/Not free/ Not Officially free/outbreaks)
- Testing protocols (Skin test / gamma interferon / meat inspection /other, Antigen used, cut-off values, etc)
- Issues related to other infectious diseases potentially affecting bovine TB testing
- TB in wildlife

1.1. The Netherlands

- Country status for bovine TB

Officially free

- Testing protocols

No routine tests are performed.

Single intradermal tuberculin (SIT) test with high dose of Lelystad bovine purified protein derivative (PPD) (5000 I.U/dose) is used in known infected herds.

In the Netherlands, the gamma interferon bovine avian (IFN-γ BA) test is used as ancillary test, after the performance of skin test, in positive herds in order to increase specificity. The use of the IFN-γ test has been investigated particularly in order to find specific antigens and /or peptides. Currently a mix of antigen peptides, produced by Animal Health and Veterinary Laboratory Agency (AHVLA) (UK),is being used.

- Issues related to other infectious diseases potentially affecting bovine TB testing

Problems related to the use of IFN-γ BA bovine test can be the specificity of the antigens used (distinction between paratuberculosis and tuberculosis reactions) .

Paratuberculosis prevalence, in NL, increased a lot since 1950 decade. The problem of possible cross reaction in the interpretation of the skin test become an issue after the discovery of “avian” reactions in the single tuberculin skin test (SIT) during eradication of bovine Tb. For this reason, as a screening test, the single intradermal comparative tuberculosis test (SICTT) interpretation was adapted to reduce false positive results.

- TB in wildlife

The prevalence of tuberculosis in zoo animals is quite high and cases may not be diagnosed and reported to the competent authority. Practical difficulties impair the performance of the SICTT test, the example of a couple of white rhinos that had to be tested prior to movement to Germany was given. Due to obvious anatomical restrictions, the skin test could not be performed. In this case and in similar cases, the use of serological (ELISA) tests should be taken into consideration.

1.2. Poland

- Country status for bovine TB

Officially free since 2009.

Poland started eradication in 1959 when prevalence was between 5% and 32%. In 1975 this was reduced to 0.1% and in 2009 there were only 12 outbreaks with 111 bovines culled.

In 2010 and 2011 there were respectively 18 and 16 outbreaks.

- Testing protocol

Routine tests are performed every 5 years. A SIT test is performed using bovine PPD from Czech Republic. A positive test result leads to the culling of the animal. If the result is inconclusive, a SICCT test is performed 42 days after, i.e. standard test interpretation.

The main problem related to tuberculosis testing in Poland is the number of false positive results of SIT test, in 2009, 111 samples from animals with positive results of skin test were retested and M*ycobacterium bovis* was isolated in 62 cases while 49 cases were negative (44,1% of all cases).

The IFN-γ BA test (using Australian type PPDs) was introduced as a ancillary test, the test is used in a serial way to be performed in case of inconclusive results with the tuberculin skin test . The test is used as a ancillary test and not a stand-alone official one. The results of a comparative study on the use of IFN-γ BA in Poland showed a large number of false negatives, lower sensitivity.

A practical difficulty with the IFN-γ BA test implementation is the need to have a reduced time between the blood sample collection and the delivery to the laboratory.

1.3. Germany

- Country status for bovine TB

Officially free since 1997.

- Testing protocols

In Germany no routine testing is performed.

Disease monitoring is performed at the slaughterhouse with the clinical ante-mortem visit and post-mortem inspection. Every year between 4 to 10 cases of bovine TB are detected at post mortem inspection in slaughterhouses. When positive cases are identified, skin tests are performed in the corresponding herds. To avoid false positive results, SICTT is the test choice.

As from 2010, IFN-γ BA tests are performed in some regions in herds where positive cases were identified. IFN- γ BA test has been used as an ancillary test while performing comparative skin test on suspect animals, parallel use of the test . Germany has requested the test developers to produce a special kit for Germany with positive controls. Germany uses only Lelystad PPD.

From all bovine TB infected animals in Germany approximately 50% are detected at the slaughterhouse, while the remaining 50% is detected by epidemiological follow up of cases, testing for animal movement and/or clinical suspicion. In a region in the south of Germany a surveillance programme using skin test was carried out and some herds not previously identified were detected with bovine TB cases. German authorities are concerned that the sensitivity of meat inspection is not sufficiently high to ensure adequate monitoring.

1.4. Spain

- Country status for bovine TB

Spain it is not an officially free country. There is a national eradication program on-going, co-financed by EU.

From 2006 (2006-2010) herd prevalence decreased from 1,76 to 1,50 and animal incidence from 0,42 to 0,36 %, but some regions still have an high herd prevalence.

- Testing protocols

Spain performs routine tests on live animals, complemented by monitoring at slaughterhouse . The routine test is the SIT test. Surveillance tests have been performed routinely on dairy and beef herds since mid 80´s. From 2006 onwards the programme was modified to increase the diagnostic sensitivity of the programme, mainly in high prevalence areas.

SICCT test is not allowed to be performed as a routine test. It can be used only in those herds in low prevalence regions and where there could be some cross-reactions and low risk of bovine TB.

IFN-γ BA test in Spain is used as a complementary test in parallel with the skin test on all confirmed infected herds for the detection of the maximum number of infected animals and, so far, about 180.000 IFN-γ test that have been performed.

PPDs used in skin test are the same used also in IFN-γ tests.

- Issues related to other infectious diseases potentially affecting bovine TB testing

Possible false positive reactions are not taken into account when the test is applied on confirmed infected herds. The IFN-γ BA is not applied on free herds or “to confirm” the results of the skin test.

- TB in wildlife

Scientific evidence is becoming available on the role of wildlife in some regions of the central-south Spain.

1.5. Ireland

- Country status for bovine TB

Ireland it is not an officially free country. There is a national eradication program on-going and, in 2009, received co-financing from the European Union.

Ten years ago there were 40.000 positive animals, in 2011 the number of positive infected animals was 20.000.

- Testing protocols

In Ireland routines SICCT tests are performed. Avian and bovine PPDs from Lelystad (NL) are used.

The IFN-γ BA test started to be used in the Republic of Ireland in 1990 as a parallel test in high prevalence herds to improve sensitivity of *M. bovis* detection , the interpretation criteria were adapted to optimise sensitivity of detection, to limit the number of false negatives

In the following years the number of samples testing positive as a proportion of the number of samples submitted for testing remained relatively stable (approximately 18%). In 2008 the number of samples

tested was over 20,000 with a higher proportion testing positive (26.4%). This was due to a large-scale sensitivity and specificity field evaluation of the IFN-γ BA test involving parallel testing with SICTT and serology. In 2009 the testing strategy was modified and instead of routinely testing animals from high risk herds (reactor re-tests), the veterinarians had to target the high-risk cohorts in infected herds and to justify the IFN-γ test in these groups based on sound epidemiological principles. A natural consequence is that from 2009 the proportion of positive cases increased (33%) even though the number of tests decreased and the national tuberculosis prevalence was decreasing each year. Animals that were IFN-γ test positive / skin test negative were determined to have a 10-fold greater risk of becoming skin test positive within 18 months, if left in the herd, compared with IFN-γ negatives.

In 2010, 5541 animals were tested. 1138 were positive at the first skin test. There was a 79% correlation between skin test positive and IFN-γ positive animals. Of 287 animals with clinical lesions, 257 (90%) were also IFN-γ positive.

The Department of Agriculture, Food and Marine has applied several quality control steps to the Irish Bovine Tuberculosis Programme. The controls focus on the quality of the comparative skin test which is particularly important considering the large number of tests that are performed every year and the multiple factors that can bias the result.

Every year the potency of each batch of bovine tuberculin is initially checked on guinea pigs sensitised with *M.bovis* and 2-3 assays are also performed on naturally infected cattle.

Nevertheless a lot of other problems can bias the result of the test: kits can be expired or just stored in inappropriate places, there can be problems performing the test on the animal, interpretations of the results can be different in accordance of who is checking the reaction etc. Skin test is very robust test but it is necessary to be performed following standardised procedures.

The use of IFN-γ BA test has helped in gaining specificity but there is the issue of time passing between the collection of the blood sample and the delivery to the laboratory that usually is after one day.

Monitoring at the slaughterhouses identifies approximately 1/3 of positive animals. Once the relevant herds have been checked, the results are very consistent on finding 20% of positive ones and 80% of negative.

1.6. UK

- Country status for bovine TB

Scotland is an officially tuberculosis free region since October 2009. England and Wales are not free. Herd incidence is highest in the South west and West Midlands of England and Wales. Sporadic cases in the North and East of England are usually associated with the movements of infected cattle from the endemic regions.

- Testing protocols

The SICCT skin test, with avian and bovine tuberculins from Prionics (Lelystad, Holland), is the primary screening test for bovine TB. From 2006, the skin test in GB has been supplemented with the IFN-γ BA test in some specific scenarios. Between 2001 and 2006 the IFN-γ BA test was also used on a voluntary basis as part of a field trial in infected herds, as well as on an ad hoc basis in herds outside the field trial.

About 30,000 IFN-γ BA tests are performed every year. In the 99.25% of the cases, the IFN- γ BA test is performed in herds with confirmed *M.bovis* infection using 'parallel' interpretation in conjunction with theSICCT test, whereby animals that prove positive to either test are slaughtered.

The use of IFN-γ BA test is mandatory in the following situations:

1. In all herds with confirmed *M.bovis* infection (herds with cases with free status withdrawn) in an area of low bovine TB incidence. The IFN-γ BA test is carried out as soon as possible after confirmation of infection in the herd. This scenario accounts for the majority of blood tests performed in GB.

2. To inform decision-making around possible depopulation of herds with severe or chronic bovine TB breakdowns, ie. cases reoccurrence;

3. Rapid re-testing of some persistent inconclusive skin test reactors in known infected herds (Wales only)

There are other situations in which the use of the IFN-γ BA test is discretionary, rather than mandatory, for instance in herds that suffer chronic TB breakdowns (OTF status withdrawn) located in regions of endemically high TB incidence.

IFN-γ test is usually performed 1 day after the blood sample collection. Their published studies demonstrated that an increase in specificity was noted the longer a sample was left prior to testing (8h versus 24h). All samples are processed in a dedicated laboratory of the AHVLA in the Midlands (England)

Sensitivity of IFN-γ BA is identical regardless whether skin test positive or skin test negative infected animals are tested.

Use of Lelystad avian and bovine PPD for parallel testing, additional use of ESAT-6 and CFP-10 in serial tests.

Additional defined antigens (apart from ESAT-6 and CFP-10) are under development and have been shown to increase overall sensitivity of the IFN-γ.

In GB a study in which the animals were skin tested every 60 days was performed: the result was that there is a loss of skin reaction after repeated short interval skin testing. This does not affect IFN-γ BA test responses and sensitivity. The same result was obtained in a similar study in the Netherlands.

- Issues related to other infectious diseases potentially affecting bovine TB testing

High prevalence of non-specific sensitisation led to the adoption of the SICCT test in the late 1940s, instead of the SIT test. Estimated high herd-level prevalence of paratuberculosis infection in dairy herds, but the overall impact of this infection on the sensitivity and specificity of the TB skin testing regime is not well understood.

- TB in wildlife

The Eurasian badger (*Meles meles*) is a recognised reservoir of *M.bovis* and an important source of (re-)infection for cattle herds in large areas of England and Wales. It has been estimated that infected badgers account for approximately 50% of new cases in the endemic TB areas.

1.7. France

- Country status for bovine TB

Officially free since 2001.

The control and eradication program started at the end of the 50s. In the last 5 years prevalence has increased from 0,1% to 0,8% and there is an interested in find out if it's related to better detection or to a real worst health situation. The most problematic areas are Dordogne, Cote d'Or and Camargue.

- Testing protocols

Tests are performed with different frequency according to the regions estimated prevalence (no routine tests, every year, every 2-3 or 4 years). The general bovine TB testing protocol includes initially a SIT test, after 6 weeks, if positive, a SICCT test is also performed. If the result is also positive the animal is culled and after 3 month histology and culture results are also available.The large time interval between the first screening and the confirmation of the infection constitutes a serious problem.

In 2006, IFN-γ BA test was introduced in parallel to skin test for increasing diagnosis sensitivity in detecting positive animals in infected herds and in a serial manner to discriminate within positive skin tests. About 5000-10000 animals per year per concerned region are tested with test. A PCR test was also introduced for direct diagnosis. These changes result in a lower number of herds with suspension of the free-herd-qualification.

Dordogne and Cote d'Or are considered low prevalence regions and the use of IFN-γ BA contributed to a gain in testing specificity. In Camargue the animals tested are fighting bulls and performing skin tests is quite difficult,in this region the IFN-γ BA test is being used to increase diagnostic sensitivity.

Some technical modifications were applied to Bovigam® in order to use very exclusively Lelystad bovine and avian PPDs. The specific antigens CFP10/ESAT6 are mainly used in conventional herds.

The French food safety and environment agency (ANSES) is addressing mandate on performance of IFN-γ BA test that was received in January 2012. Main questions are related to (i) the choice of antigen combinations and cut-off value of the test depending on the purpose of usage and (ii) a comparative risk evaluation of different protocols for decision making following serial usage of TB diagnostic tests. The mandate focuses on the use of the IFN-γ BA as an ancillary test for surveillance and control programs, not as a stand-alone test for demonstration of TB free herd status or certification.

1.8. Hungary

- Country status for bovine TB

Not an officially free country (but very low prevalence since 1981).

- Testing protocols:

Routine tests are performed once a year, SIT test for all animals older than 3 months and SICCT test for confirmation in officially free herds.

Since 2010, the SICCT test is recommended as a first test in herds with a high number of non-confirmed reactors.

So far IFN-γ BA test has been used in a low number of herds: the reason is the high cost that has to be covered by the farmer and the lack of agreement with results from SICCT test .

Monitoring is also performed in the slaughterhouses but vast majority of cases are diagnosed with in vivo tests .

All confirmed reactors are slaughtered, necropsy, histology and bacteriology tests are performed.

- Issues related to other infectious diseases potentially affecting bovine TB testing

Hungary has a high prevalence of *Mycobacterium spp.* infections causing non specific reaction to skin test and unnecessary culling and movement restrictions.

Some herds were found infected by both paratuberculosis and *M. bovis subsp. caprae,* and, due to the lack of reliable differentiation all the animals from these herds were culled.

For this reason there is a strong interest in high specificity and sensitivity tests.

- TB in wildlife

Before 2005 some cases of *M.bovis* infection in wildlife animals were reported but no action was taken.

In 2006 *M. bovis subsp. caprae* was isolated from wildlife animals and cattle in the same time and regions. The link was confirmed and a wildlife surveillance program was set. Up to now, more than 1500 samples have been collected from deer and wild boars and those samples indicate that wildlife tuberculosis is endemic in some regions of Hungary (especially in two border regions where, as a consequence, there have been outbreaks in beef herds).

1.9. Italy

- Country status for bovine TB:

Not an officially free country but with officially free regions/provinces. There is a national eradication program on-going and, since 2009 the program received co-financing from the European Union.

Tuberculosis prevalence 0.50% (data referred to 2010), but the situation is quite different in the various regions.

- Testing protocols:

SIT tests are performed with different frequency according to the regions situation: no routine tests (Friuli Venezia Giulia), every year, every 2-3 or 4 years.

The IFN-γ BA test has been used at the IZSLER (Brescia) and at IZSPLV (Torino) since1993-1995 and there are six different Istituti zooprofilattici in Italy performing it (Brescia, Torino, Perugia, Salerno, Sassari, Roma).The IZSLER (National Reference Centre for TB) perform the IFN-γ BA tests with Bovigam® kit using the Australian PPDs.

In 2011, 5406 IFN-γ BA tests have been performed on cattle and buffalo. Some IZS use Bovigam® test with double stimulation (Australian PPD and Italian PPD)

The IFN-γ BA test is used during outbreaks in addition to the skin test. In some regions, the test is also used in case of suspect herds (eg. suspect lesion at slaughter-house, epidemiological related herds).

For research purposes the test has also been used with recombinant antigens (ESAT6, CFP10).

1.10. Belgium

- Country status for bovine TB

Officially free since 2003

- Testing protocols

No routine tests are performed but, in case of outbreaks, intensive testing is applied.

SIT is performed for purchase, import, raw milk dairies, tracing-on and tracing-back in suspect herds.SICCT is performed also 6 weeks after a non-negative SIT. IFN-γ BACE test is performed since 2006, combining the PPDs used for SIT and SICCT (Synbiotics), and ESAT-6/CFP-10 peptide cocktails, on a Life Technologies/Fisher Scientific/Invitrogen/DIAsource-BioSource kit.

PCR is also performed since 2006.

The budget spent for skin test is very large (50% of the entire budget designated to bovine health), but most of the time it is not enough to indentify and prevent outbreaks. Tests must be associated in order to have a good level of sensitivity and specificity (specificity of combination of tests can reach 98,9-99,4%).

In 2011, research looked into the performance of ELISA serological test but a poor performance was found.

1.11. Summary and conclusions

The information collected at the meeting and additional information obtained from the Summary Zoonoses report of 2009[7] and contact with national reference laboratories is summarised in table 1. Country status regarding bovine TB and the surveillance system in use are described. In general countries being OTF maintain a passive surveillance system based on notification of clinical cases or detection and confirmation of bovine TB lesions at the slaughterhouse, when cases are detected the herd of origin must be tested and a epidemiological investigation conducted. The routine testing protocols and tests used are described for all countries in table 1 In conclusion:

- There is large variation on the test and testing protocols used in different MS. These variations refer to the use of both the official bovine TB diagnostic test (tuberculin skin tests) and the ancillary test (IFN-γ BA test) but also and more important on the objectives to achieve. These variations can affect the diagnostic tests accuracy.

- The different tests and testing protocols are not adequately described resulting in surveillance data that are difficult to validate and compare.

[7] European Food Safety Authority. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. EFSA Journal 2011;9(3):2090. [378 pp.]. doi:10.2903/j.efsa.2011.2090. Available online: www.efsa.europa.eu/efsajournal

Table 1: Summary Table

	Country status[8]	Surveillance system[9]	Skin test [10]	Interval between routine tuberculin tests	Use of IFN-γ test [11]
NL	OTFC	Passive only	SIT with high dose bovine PPD (5000I.U/dose) in known infected herds		Additional test, after SIT in positive herds to increase specificity. Peptides cocktail, produced by AHVLA (UK), is used.
PL	OTFC	Passive only	SIT with bovine PPDs (from Czech Republic) SICTT after 42 days if first result is not clear	Every 5 years	The IFN-γ BA test (Australian type PPDs) as a control test to be performed in case of doubtful results with the skin test
DE	OTFC	Passive only	SICTT on herds identified by passive surveillance		From 2010, IFN-γ BA tests are performed as an ancillary test with Lelystad type PPDs if some possible cases are identified
SP	COFIN	Passive and routine testing	SIT SICTT only in free herds in low prevalence regions where there could be some cross-reactions	Every year (1 routine testing in low prevalence areas; 2 in high prevalence areas) More frequent in infected herds.	IFN-γ test is used in parallel with the skin test on all confirmed infected herds. PPDs used in skin test are the same used also in IFN-γ BA tests. Cut-off: 0,05
IE	COFIN	Passive and routine testing	SICTT with avian and bovine PPDs from Lelystad.		The IFN-γ BA test started to be used in 1990 as a parallel test in high prevalence herds, the interpretation criteria was adapted to optimise sensitivity of detection, in order to limit the number of false negatives
UK (E&W)	COFIN	Passive and routine testing	SICTT with avian (25,000IU/ml) and bovine (30,000IU/ml) PPDs from Lelystad	Depends on incidence of confirmed herd breakdowns in an area (1, 2, 3, and 4 yearly	IFN-γ tests are routinely performed in GB since Oct 2006, in certain prescribed situations. In the 99.25% of the cases, they are performed in parallel with skin tests in known infected herds (OTF status withdrawn), i.e.

[8] OTFC: Officially tuberculosis free country, OTFR: Officially tuberculosis free region or province, COFIN: Country with eradication programme co financed by EU, NOF: Not Officially free

[9] Surveillance systems can be passive surveillance by meat inspection and notification of clinical cases or include active surveillance with routine testing.

[10] SIT: single intradermal test SICTT: single intradermal comparative tuberculosis test

[11] IFN-γ Bovine-Avian: Gamma - interferon test with bovine PPD and avian PPD diagnostic antigens, IFN-γ Bovine :Gamma - interferon test with bovine diagnostic antigen, IFN-γ CFP10 ESAT6: Gamma - interferon test with CFP10 and ESAT6 diagnostic antigens, IFN-γ MPB70: Gamma - interferon test with MPB70 diagnostic antigen, IFN-γ BACE: Gamma - interferon test with bovine PPD and avian PPD diagnostic antigens and CFP10 and ESAT6 diagnostic antigens

				herd testing intervals). In herds under restriction: Every 2 months to regain OTF status. Skin herd test repeated 6 & 18 months after restoration of OTF status. Mandatory pre-movement testing (with the skin test) of all cattle moving out of herds that are tested every 1 or 2 years, except for movements off to slaughter.	with compulsory slaughter of any skin test negative/IFN-g test positive animals. The remainder (0.75%) are serial blood tests of skin test reactors (or IRs) in herds where a suspicion has arisen of fraud or non-specific cross-reactions to bovine PPD. Serial IFN-g testing is with a modified BOVIGAM, using specific antigens (ESAT6/CFP10 peptides). The use of IFN-γ BA test is mandatory in some situations (see minutes). IFN-γ test is usually performed the day after blood sample collection (maximum interval of 24 hrs between blood collection on farm and stimulation of samples in the lab).
UK (NI)	COFIN	Passive and routine testing	As UK (E&W)	All herds in Northern Ireland are tested annually. No pre-movement tested mandatory.	
FR	OTFC	Passive only	SIT and, if not clear result, SICTT after 6 weeks.	Tests are performed with different frequency according to the regions situation (no routine tests, every year, every 2-3 or 4 years)	IFN-γ test introduced in 2006 in parallel to skin test for selecting culling in infected herds and in serial to discriminate within positive skin tests. Specific peptides (Lelystad PPDs). The specific antigens CFP10/ESAT6 are mainly used in conventional herds.
HU	NOF	Passive and routine testing	SIT for all animals older than 3 months + comparative test for confirmation in officially free herds. SICTT is recommended as a first test in herds with a high number of non-confirmed reactors (since 2010)	Every year	IFN-γ test has been used in a low number of herds: the reason is the high cost that has to be covered by the farmer and the disconcordant results with SICTT.

IT	OTFR and COFIN	Passive and routine testing	SIT	Tests are performed with different frequency according to the regions situation: no routine tests (Friuli Venezia Giulia), every year, every 2-3 or 4 years	IFN-γ test since 1993-1995 at IZSLER (Brescia) and at IZSPLV (Torino). Actually there are six different Istituti zooprofilattici in Italy performing it (Brescia, Torino, Perugia, Salerno, Sassari, and Roma). Duriong outbreaks in addition to skin test. In some regions, also in case of suspected herds (suspected use of fraudulent matter, suspect lesion at slaughter-house, epidemiological related herds). The IZSLER (National Reference Centre for TB) perform the IFN-γ BA tests with Bovigam® kit using the Australian PPDs. Some IZS use Bovigam® test with double stimulation (Australian PPD and Italian PPD) For research purposes the test has also been used with recombinant antigens (ESAT6, CFP10).
BE	OTFC	Passive and routine testing	SIT for purchase, import, raw milk dairies, tracing-on and tracing-back in suspect herds. SICTT 6 weeks post non-negative SIT.	Every 6 weeks to 6 months	IFN-γ BACE test is performed since 2006, combining the PPDs used for SIT and SICTT (Synbiotics), and ESAT-6/CFP-10 peptide cocktails, on a Life Technologies/Fisher Scientific/Invitrogen/DIAsource-BioSource kit.

2. EFSA Mandate regarding bovine tuberculosis testing

The European Commission, requested the AHAW Panel to deliver a scientific opinion on the use of a IFN-γ test for the diagnosis of bovine TB. The procedures for gaining, maintaining, suspending, withdrawing or re-gaining the officially bovine TB free herd status and for certification for intra Union trade are laid down in Council Directive 64/432/EEC and are based on the results of tuberculin skin test in its various forms. The skin test has a number of drawbacks, both in terms of test characteristics, limited sensitivity at the level of the individual animal and testing logistics.

The mandate terms of reference are:

1. to issue a scientific opinion on the suitability of the IFN test for inclusion amongst the official tests for the purpose of granting and retaining an officially tuberculosis-free herd status as laid down in Annex A to Directive 64/432/EEC and certification for intra Union trade in bovine animals as required in Article 6(2)(a) of that Directive;

2. to issue a scientific opinion on the suitability of other, possibly newer, tests, if any, for their inclusion amongst the official tests for the purpose of granting and retaining an officially tuberculosis-free herd status

3. in the event of a negative opinion to point (1), to advise the Commission on which further validation studies are necessary to evaluate the suitability of the IFN test, or any other new test, for inclusion amongst the official tests for the purpose of granting and retaining an officially tuberculosis-free herd status

Suitability was defined as having sensitivity equivalent or superior to the skin test and have specificity not lower than that of the current standard test with lowest specificity used in the EU. Further, there should be no foreseeable practical difficulties that could compromise test performance.

EFSA has established a expert ad hoc WG and is gathering evidence to answer the terms of reference. Data sources to be considered are: 1) A literature systematic review and meta analysis of diagnostic tests for bovine tuberculosis in cattle performed by AHVLA - in 2009[12] , 2) Member States experience with the use of gamma interferon in control and eradication programs, 3) results from trials conducted by the European Reference laboratory and the 4) data on test results from a public data call foreseen to be launched in March 2012.

A list of possible tests to be considered for evaluation was discussed and the experts agreed that IFN- γ test (bovine avian and specific antigen CFP10 and ESAT6 in particular) was the most relevant to be evaluated. Serology tests evaluation is needed but there was agreement between experts that these tests must be associated with skin tests in order to improve sensibility.

Different datasets are needed to appropriately address the terms of reference , but although data on test performance is available it will be difficult to merge non-standardised data. Estimates can be biased by different testing protocols, different antigens used and cut-offs. In most countries IFN-γ BA tests are performed after the first disclosure skin test, and positive animals to the skin test may have already been culled after this first skin test resulting in sensitivity estimates for the SIT in the first post - disclosure test may be lower than expected.

[12] Veterinary Laboratories Agency (VLA) 2011. Meta-analysis of diagnostic tests and modelling to identify appropriate testing strategies to reduce M. bovis infection in GB herds. SE3238 Final Report. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=16114&FromSearch=Y&Publisher=1&SearchText=SE3238&SortString=ProjectCode&SortOrder=Asc&Paging=10

All experts agreed that there is a strong need of validation of a single or standardised testing protocol in order to avoid the problems related with the use of the skin test, which is highly dependent on the competency of the operators.

The network representatives were asked if they considered IFN- γ BA as a possible replacement for the skin test. Due to logistical, performance (specificity is lower in IFN- γ BA compared to the skin test) and cost reasons the test was assessed by the experts as not suitable.

It was highlighted that the use of the tests presently available cannot fit all situations. IFN- γ test are more suitable for use at an individual animal level, however the use of the skin test is more appropriate at the herd level.

APPENDIX

A. Technical meeting participants

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ABBREVIATIONS

AHAW	Animal Health and Animal Welfare
AHVLA	Animal Health and Veterinary Laboratory Agency
ANSES	Agence Nationale de Sécurité Sanitaire
EFSA	European Food Safety Authority
EU	European Union
IFN-γ	Gamma interferon test
IZSLER	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
IZSPLV	Istituto Zooprofilattico Sperimentale del Piemonte, della Liguria e della Valle D'Aosta
MS	EU Member State
NOF	Not Officially Free
NOOUT	Not officially free but no outbreaks
OTFC	Officially tuberculosis-free country
OTFH	Officially tuberculosis-free herd
OTFR	Officially tuberculosis-free region or province
OUT	Outbreaks or reactors in the past 2 years in the same herds
PCR	Polymerase Chain Reaction
PPD	Purified protein derivative
SICTT	Single Intradermal Comparative Tuberculosis Test
SIT	Single intradermal tuberculin test
Skin test	Tuberculin skin test
TB	Tuberculosis
TBOF	Officially tuberculosis free
WG	Ad hoc working group