Appendix E—Criteria for Evaluating Experimental Tuberculosis Test Performance for Official Test Status, 2008 revision of 1995 guidelines developed by the Diagnostic Test Review Subcommittee of USAHA's Committee on Tuberculosis

CRITERIA FOR EVALUATING EXPERIMENTAL TUBERCULOSIS TEST PERFORMANCE FOR OFFICIAL TEST STATUS

The purpose of these criteria is to provide guidelines to the TB Scientific Advisory Subcommittee for the evaluation of diagnostic tests for the detection of *Mycobacterium bovis* infected animals. It is incumbent upon the sponsor of the test to define the intended purpose of the test (i.e., as a presumptive, supplemental, and/or primary diagnostic test), the proposed interpretation standards, specific application and intended species. Defining the purpose of the new test is critical in establishing the benchmarks for evaluation. The new test will be evaluated according to guidelines described below for Phase I, II, and III. After the evaluation process has been initiated, the sponsor may not make substantive changes in reagents or methods for conducting the test. If substantial changes in the new test are made, the sponsor must reevaluate the test beginning at Phase I. Variances from these guidelines must be approved by the Tuberculosis Committee of the United States Animal Health Association or its designate.

Test Submission and Approval Process

Results may be submitted for review to the Chair of the TB Scientific Advisory Subcommittee (TB SAS) at any time. The TB SAS will review the data by conference call/email or at the regularly scheduled subcommittee meeting at the annual USAHA meeting. The recommendation of TB SAS will be submitted to the Chair of the TB Committee. If needed, the TB SAS will develop and submit a resolution to the TB Committee Chair. The TB Committee Chair will determine whether the recommendation will be immediately released to the TB Committee or held until the annual TB Committee Meeting. At the next TB SAS and TB Committee meeting the results of the TB SAS recommendations will be reported to the TB Committee in its annual report.

PHASE I: Preliminary evaluation for diagnostic sensitivity and specificity

The objective of this Phase is to determine if the proposed test has sufficient diagnostic sensitivity (DSe) and diagnostic specificity (DSp) to be fit for its intended propose.

Diagnostic Sensitivity
The new test must be evaluated on *Mycobacterium bovis* infected[1] animals by the submitting organization and the results submitted to USDA/APHIS/VS for statistical evaluation of test performance. The new test must be evaluated on a sufficient number of animals to reasonable demonstrate that the sensitivity of the new test is equivalent to or better than that of the test currently used in that situation. The formula in Appendix A can be used to estimate the number of animals needed to evaluate DSe with a margin of error of 5% (e =5%). Estimates of DSe and DSp in the US are listed in Appendix B. In this part of the preliminary evaluation, laboratory work (histopathology and/or bacteriology) necessary to determine *M. bovis* infection will be conducted at the NVSL, OIE Veterinary TB Reference Laboratories, National Veterinary Reference Laboratories or a laboratory acceptable to the USDA.

Diagnostic Specificity

[1] As outlined in Definitions section.

The new test must also be evaluated from at least 10 herds from accredited-free states (or herds that are accredited-free and those with no history of exposure to *M. bovis*) to demonstrate that the test is equivalent to or better than that of the test currently used. The formula in Appendix A can be used to estimate the number of animals needed to evaluate DSp with a margin of error of 2% (e =2%). The testing will be done by the submitting organization and the results will be submitted to the USDA for statistical evaluation of test performance. Herds should be representative of the target industry and be diverse in regard to geographic location, breed/species and age.

Results of Phase I trials will be presented to the TB SAS. If the proposed test has sufficient DSe and DSp to fulfill its intended purpose, the test will be recommended for Phase II.

PHASE II: Side by side blind comparison

The objective of Phase II is to determine if the proposed test is fit for its intended purpose by directly comparing the current test side-by-side with the proposed test. This phase should provide sufficient data to show, with confidence, that the proposed test will meet program needs.

Diagnostic Sensitivity
Both the new test and official test will be evaluated in animals from at a sufficient number of *M. bovis*-infected herds to ensure that the new test is equivalent to or better than that of the test currently used in that situation. The formula in Appendix A can be used to estimate the number of animals needed to evaluate DSe with a margin of error of 3% (e =3%). Both tests will be applied to each animal. Whole herds or randomly selected animals from a herd must yield at least one infected animal, which has been subjected to side-by-side testing. Results of testing with one test will not be available to those responsible for determining the results of the other test. All tested animals will be examined at slaughter for detection of tuberculous lesions. Tissues from different organs and lymph nodes will be examined using histopathologic and bacteriologic procedures. Laboratory work (histopathology and bacteriology) will be conducted at the NVSL, OIE Veterinary TB Reference Laboratories, National Veterinary Reference Laboratories or a laboratory acceptable to the USDA. Results of antemortem testing will not be available to the laboratory. The submitting organization will be responsible to run the new test. At least 50% of the samples must be of North American origin and the origin of samples must be delineated in data presented to the TB SAS Subcommittee.

Diagnostic Specificity
Both the new test and currently used test will be evaluated on animals from at least 10 accredited-free US herds to ensure that the new test is equivalent to or better than that of the test currently used in that situation. The formula in Appendix A can be used to estimate the number of animals needed to evaluate DSp with a margin of error of 2% (e =2%). In this part of the blind comparison, preferably all, or a predetermined random sample, of at least 25% the total animals must be tested side by side. Herds should be representative of the target industry and be diverse in regard to geographic location, breed/species and age.

Results of Phase II trials will be presented to TB SAS. If the proposed test has sufficient DSe and DSp to fulfill its intended purpose, the Committee will recommend that the test be approved for Phase III testing. If the USDA determines that the new test performance in Phase II is equivalent to or better than the current official test, then the new test would be recommended for conditional/temporary approval as an official test for a period of 1 to 5 years, with annual reviews for continuation. The sponsor of the new test must have completed the USDA, Center for Veterinary Biologics requirements for licensure prior to conditional approval.

PHASE III: Field trial of use of new test

The primary objective of Phase III is to determine if the proposed test is sufficiently robust to be used under field trials. In addition DSe and DSp will continue to be evaluated.

During a 1 to 5 year trial, the new test will be performed by accredited veterinarians and/or certified laboratories, under natural field conditions. The USDA will assess the performance of the new test on routine samples.

Diagnostic Sensitivity and Specificity will continue to be evaluated by the USDA. Data from side by side comparisons (in Phase II) between the new test and current official test, for the proposed use, can be applied to complete Phase III. If the new test performance evaluates equivalent to or better than the currently used test, it will be referred to the TB Committee for recommendation as an official test.

Definitions:

Criteria for defining infection with *Mycobacterium bovis*

Infected: An animal will be considered infected when *Mycobacterium bovis* has been isolated from one or more tissues AND/OR the animal has mycobacteriosis compatible lesions with the presence of *M. bovis* confirmed by PCR. Culture and histology will be performed following the current protocols at the National Veterinary Services Laboratories (NVSL), OIE Veterinary TB Reference Laboratories, National Veterinary Reference Laboratories or a laboratory acceptable to the USDA.

Exposed: Animals not meeting the criteria for Infected but residing in a herd that has confirmed *M. bovis* infected animals will be defined as exposed and will not be used to determine specificity.

Non-infected: For determining specificity, an animal will be considered non-infected when it comes from an accredited TB-free herd or from a herd with no history of exposure to *M. bovis*.

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Appendix A: Calculation of Sample Size

Sample size for Phase I and II should be calculated using the method of Griener and Gardner[2] or Jacobson[3] using the formula:

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are needed to see this picture.

Where: *e*=margin of error and θ = estimate of sensitivity or specificity from pilot or preliminary study. Both terms are calculated using the decimal form.

The following table provides an example:

	Margin of Error					
Estimated DSe or DSp	10%	8%	5%	3%	2%	1%
80%	61	96	246	683	1537	6147
82%	57	89	227	630	1418	5670
84%	52	81	207	574	1291	5163
86%	46	72	185	514	1156	4625
88%	41	63	162	451	1014	4057
90%	35	54	138	384	864	3457
92%	28	44	113	314	707	2827
94%	22	34	87	241	542	2167
96%	15	23	59	164	369	1475
98%	8	12	30	84	188	753
99%	4	6	15	42	95	380

[2] Greiner M, and I.A. Gardner. Epidemiologic issues in the validation of veterinary diagnostic tests. Preventive Veterinary Medicine 2000 45:3-22

[3] Jacobson RH. Validation of serological assays for diagnosis of infectious diseases. Rev. sci. tech. Off. Int. Epiz. 1998 17:469-486

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Appendix B: DSe and DSp of current Tests

Types and Validity of Official Tuberculosis Tests

Family	Test	DSe	DSp
Bovidae	CFT	82%	96%
	CCT	74%	96%
	SCT	92%	85%
Cervidae	SC-DS	80-85%	61-98%
	CCT	95%	95%

CFT= Caudal Fold Test

CCT= Comparative Cervical

SCT=Single Cervical

SC-DS = Single Cervical Double-strength

From: *Assessment of Risk Factors for Mycobacterium bovis in the U.S. 1992*. USDA-APHIS-VS Centers for Epidemiology and Animal Health, Fort Collins, CO 80521