United States Department of Agriculture
Marketing and Regulatory Programs
Animal and Plant Health Inspection Service
Veterinary Services

Approval of Laboratories to Conduct Tests for Equine Infectious Anemia

1. Purpose and Background

This document outlines the procedures to initially approve laboratories to test for equine infectious anemia (EIA), the requirements to maintain approval, and the requirements for laboratories performing EIA tests.

This guidance document represents the Agency's position on this topic. It does not create or confer any rights for or on any person and does not bind the U.S. Department of Agriculture (USDA) or the public. Veterinary Services (VS) may make this information available to the public. While this document provides guidance for users outside Veterinary Services, VS employees may not deviate from the directions provided herein without appropriate justification and VS Equine Health Team concurrence.

2. Document Status

A. Review date: February 27,2023

B. This document cancels and replaces VS Memorandum No 555.16.

3. Reason for Reissuance

VS has revised the laboratory approval requirements.

4. Authority and References

A. Authorities (U.S. Code (U.S.C.) and *Code of Federal Regulations* (CFR)):
- 21 U.S.C. 151-159
- 7 CFR 371.4
- 9 CFR part 75
- 9 CFR 130.30

B. References:
- VS Form 10-11, Equine Infectious Anemia Laboratory Test
- Veterinary Services Processes Streamlining (VSPS) electronic Form 10-11, Equine Infectious Anemia Laboratory Test
- National Veterinary Services Laboratories Application for Laboratory Training
- NVSL SOP-EO-0034, "Requirements for Equine Infectious Anemia Agar Gel Immunodiffusion (Coggins) and Enzyme-Linked Immunosorbent Assay Testing at Veterinary Services Approved Laboratories." Obtain this document by calling the National Veterinary Services Laboratories (NVSL) at (515) 337-7551 or emailing at NVSL.DVL.Equine_Ovine@aphis.usda.gov.
- Standardized format for reporting monthly data from EIA laboratories.
- National Veterinary Accreditation Program Reference Guide: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide

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- Table of contents: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Table-of-Contents
- Equine section: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Equine
- Equine ID: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Animal-Identification/Equine-Identification
- Appendix: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Appendix
- Equine teeth and aging: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Appendix/Equine-Teeth-and-Aging

C. Definitions:

1) Official test

Any test for the laboratory diagnosis of EIA that uses a diagnostic product that is:

a. Produced under USDA license and found to be efficient for that diagnosis under the Virus-Serum-Toxin Act of March 1913, and subsequent amendments; and

b. Conducted in a laboratory approved by the Animal and Plant Health Inspection Service (APHIS) Administrator per 9 CFR part 75.

All EIA tests are official tests; VS strictly prohibits screening or preliminary tests. Approved personnel must conduct all official EIA tests at approved facilities, certified in accordance with the procedures outlined in this document.

2) Official test form

The VS Form 10-11 is the official Federal form for EIA test requests. The VS Form 10-11 serves as the reference standard for all other official EIA test forms. Revisions and changes to the official VS Form 10-11 will reflect the most current information and data points required, and will supersede the requirements outlined in this document.

a. Information/data points required on the VS Form 10-11:

Form Serial Number (official use only).
1. Laboratory Accession Number (laboratory use only).
2. Date Blood Drawn.
3. Test Requested By Veterinarian: ELISA vs. AGID.

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4. Reason for Testing: Interstate Movement, Within State Use/Annual Testing, Change of Ownership/Sale, International Import/Export, Illness/Clinical Suspect, Investigation/Exposure.
5. Location of Equine at Blood Draw (name, address, and phone number).
6. County of Equine at Blood Draw.
7. Owner (name, address, and phone number).
8. Accredited Veterinarian:
   a) Accredited Veterinarian Name, Address, and Phone Number.
   b) Federal Category II Veterinary Accreditation number.
   c) Original Signature of Submitting Veterinarian and the Statement (*I certify I am a Category II Federally Accredited veterinarian, authorized in the State where the sample was obtained, by me, from the animal described below*) from the collecting veterinarian.
   d) Signature date.

(Note: VS considers all official EIA form submissions made through the electronic VSPS system digitally signed although they may lack a physical signature.)

9. Tube Number (if used).
10. Tag/Tattoo/Brand Number (if present).
11. Name (if named).
12. Color.
13. Horse Breed or Equine Species.
14. Age (XX Y or XX M) or Date of Birth (MM/DD/YYYY).
15. Gender/Sex (M-Male Intact, F-Female Intact, G-Gelding, FS-Female Spayed).
16. Microchip, Breed or Registration Number(s) (if present).
17. Written narrative description is REQUIRED. Include distinctive markings, unique and permanent forms of identification, when present: Brands, tattoos, scars, cowlicks, or whorls and blemishes; 17. Head, 18. Neck/body, 19. Left forelimb, 20. Right forelimb, 21. Left hind limb, 22. Right hind limb.

- Record all present unique and permanent forms of identification on the form, including, but not limited to brands, tattoos, scars, whorls, electronic identification/microchip number(s), and biometric identifiers/measurements.

- Line drawings (silhouettes) are valuable and can help to accurately identify equines.

- Digital photographs, sufficient to identify the individual, may be used; they should be of high quality, with sharp focus, adequate lighting, and proper perspective. They should include at least three views including frontal, left side, and right side. All three views must have all body parts of the animal visible, and no part of the body covered or obscured.

- If any field is “none,” write “none” or line through; do not leave fields blank.

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More than one animal may be included on one form if the samples come from equids with the same owner and from the same location, collected by the same veterinarian on the same date, and received by the laboratory on the same date. The submitting veterinarian must provide all the required general information (as above, # 1-9) as well as individual animal identification data for each animal/sample (which includes, as above, # 10–22 and the narrative written description of unique and permanent forms of identification, when present; including but not limited to brands, tattoos, scars, whorls, electronic identification/microchip number(s), and biometric identifiers/measurements).

VS will consider approving forms produced by individual States, laboratories, or other organizations as official EIA test forms. Official EIA test forms, both paper and electronic, **must contain identical information/data points** as the VS 10-11 described above and be approved by VS officials at both the Equine Health Team and the NVSL.

Use of these forms must comply with the provisions of this document and must yield copies for distribution to the submitting veterinarian, animal owner, and regulatory officials, as well as a copy to retain in the EIA testing laboratory. For the purposes of this document, the terms “VS Form 10-11” and “official test form” are interchangeable.

Paper and electronic EIA test forms, produced by individual States or other entities and existing when VS publishes this document, will be granted a 6-month grace period following publication of this document, during which they are acceptable and valid for submission to a laboratory.

3) Laboratory director

   The responsible party in a private, Federal, State, university, or military laboratory designated as the laboratory director and with supervisory or procedural authority over the operation of and testing in the EIA laboratory. In a private laboratory, it may be the owner, veterinarian, or other person with legal, financial, or supervisory authority over EIA testing and operations.

4) State, Federal, university or military laboratory

   An EIA test facility under the direct oversight of a State animal health official, a Federal animal health official, a university laboratory director, or a U.S. military laboratory director.

5) Private laboratory

   An EIA test facility with no direct oversight by a State, Federal, or university laboratory official.

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6) Mobile laboratory:

A private, non-stationary, EIA test facility meeting the same requirements for approval as a stationary facility.

7) Satellite laboratory:

An alternate laboratory facility, operating with the approval authority of an existing approved stationary EIA laboratory, for use at offsite equine sales venues, auction markets, or event facilities to meet the transfer of ownership or interstate movement requirements for EIA testing. Satellite laboratories may be within the physical structure of the sales venue or may be a mobile unit owned and operated at equine sales venues by the approved stationary EIA laboratory.

5. Audience

Approved and prospective EIA laboratories, VS employees, other Federal and State agencies, accredited veterinarians, and members of the public.

6. Guidance

A. Application Process for Initial Laboratory Approval

1) The laboratory director provides a written statement (see VS 10-16: Application to Conduct Laboratory Equine Infectious Anemia (EIA) Testing) certifying that he or she has read and understands the requirements described in this document and that the laboratory:

a. Has adequate and appropriate facilities as described in this document and per the attached inspection checklist (Attachment 1).

b. Has technical personnel suitable to perform official EIA testing after completing prescribed NVSL training.

c. Will accept only samples submitted by a Category II federally accredited veterinarian, authorized in the State where the samples were obtained, with a complete and legible official EIA test form.

d. Understands all EIA tests are official tests; VS strictly prohibits screening or preliminary tests.

e. Will use only USDA-approved diagnostic test kits with licensed antigen. Will conduct all testing in accordance with the official protocols described in this document, provided or prescribed by NVSL, or provided in literature accompanying the diagnostic test kits.

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f. Understands all non-negative test results (positive, suspect, discrepant, or equivocal) will be considered preliminary results and the samples must be submitted to NVSL for confirmation.

g. Will be expected to seek proficiency (check) tests and materials, and meet annual (check) test proficiency requirements prescribed by NVSL.

h. Expects to perform at least 500 EIA tests per year as necessary to maintain testing competency.

i. Has regulatory obligations regarding reporting of all official test results. Has the resources available and allocated to provide adequate recordkeeping and to meet the reporting and summary data requirements described in this document.

j. Receives a satisfactory official annual inspection as required to maintain approval.

k. Will maintain current contact information and respond to official requests and inquiries.

2) The VS Area Veterinarian in Charge (AVIC) and the State animal health official (SAHO) will review the written statement, any veterinary accreditation violations known to APHIS officials, and any other information the State wishes to consider, as is its prerogative, to arrive at a consensus to deny or accept the request. The AVIC will notify in writing those with denied requests and they may re-apply. Accepted requests will continue for further processing, as described below.

3) A Federal animal health official (with a SAHO, if available) meets with the laboratory director to review the regulatory and technical requirements for conducting EIA tests and reporting results. The Federal animal health official reviews with the laboratory director: The inspection checklist, standards for accepting samples, conducting testing, reporting of results, and the reporting of summary data.

4) The laboratory director, in an interview with the AVIC or his or her delegate (with a SAHO, if available), demonstrates a thorough working knowledge of the requirements detailed in this document, using the VS Form 10-15, Agreement to Conduct Equine Infectious Anemia (EIA) Testing as a guide. The laboratory director acknowledges these responsibilities by signing the agreement and providing a copy to the AVIC and the SAHO.

5) A Federal animal health official (with a SAHO, if available) performs the initial inspection of the proposed physical laboratory facilities and records the inspection results on the checklist (Attachment 1). Laboratory inspections are subject to user fees for travel and inspection time at the hourly rate as described in 9 CFR 130.30.

6) After completing steps 1-5 above, the AVIC and SAHO must submit a jointly signed Memorandum of Recommendation and Justification for the laboratory, including the

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originals of completed VS Forms 10-15 and 10-16. Mail the memorandum and attachments to: Director, NVSL, 1920 Dayton Avenue, Ames, IA 50010. Laboratory personnel are only eligible for EIA training after submitting the Memorandum of Recommendation and Justification.

B. Application Process for Laboratory Personnel Training

1) Applicants submit a completed VS Form 4-11, NVSL Application for Laboratory Training, to the appropriate AVIC. The application form is available here.

2) The AVIC reviews the VS Form 4-11 application in consultation with the SAHO and they must jointly recommend personnel for training. If acceptable, the AVIC forwards the application to NVSL and sends a copy to the SAHO. If the AVIC or SAHO have questions or concerns regarding the application, they will return it to the laboratory with comments for revision.

3) Upon notification of final approval for training, the applicant must contact NVSL (see final section #7 Inquiries below) for inclusion on the (waiting) list of scheduled future training opportunities.

4) Once training dates become available NVSL contacts the applicant to offer training.

5) The laboratory is responsible for all costs associated with the NVSL training course.

C. Final Approval

1) After satisfactorily completing *6. A. Application Process for Initial Approval (steps 1-6*) and the *6. B. Application Process for Laboratory Personnel Training (steps 1-5),* the laboratory is eligible for final approval by the NVSL Director on behalf of the APHIS Administrator. If approved, the NVSL Director notifies the laboratory, the SAHO, the AVIC, and the Equine Health Team by letter.

D. Training Requirements

1) Laboratories must pay all costs associated with the NVSL training courses.

2) At all times, in any given physical, mobile, or satellite laboratory, at least one laboratory employee actively involved in EIA testing or oversight must be NVSL-trained and authorized.

3) Private laboratories

a. All employees performing or overseeing EIA testing must be NVSL-trained and authorized.

4) Federal, State, military, and university laboratories

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a. Upon request and pending consensus approval from NVSL, the AVIC, and the SAHO, personnel trained and authorized by NVSL to conduct EIA testing may train others at that same Federal, State, military, and university laboratory to conduct EIA testing. After completing the in-house training, NVSL will provide individual proficiency tests (standard user fees apply) to newly trained personnel. Upon successfully completing all the requirements described in this document, NVSL will authorize the individual to conduct EIA testing only in that same laboratory. If the in-house trained individual fails the individual proficiency test he or she must retake the training subject to additional fees. If any individual fails the proficiency test two successive times, he or she must wait 1 year before NVSL will again offer proficiency testing to that individual.

b. For in-house training of personnel at a Federal, State, military, or university laboratory, the laboratory's annual proficiency panel may not substitute for an individual's proficiency test. Each newly trained individual must take and pass an individual proficiency test and have received a letter from NVSL authorizing them to perform EIA testing.

c. If a Federal, State, university, or military laboratory no longer has any NVSL-trained personnel on staff, the laboratory must notify NVSL immediately and arrange to send staff to NVSL for EIA training as soon as practicable; within 6 months at the latest. However, the laboratory may continue to operate with the in-house trained and authorized personnel during that time. NVSL will notify the AVIC and the SAHO of the laboratory's status.

E. Training and NVSL Authorization Procedures

1) Whether conducted in-house (if permitted) or by NVSL, the training should cover all aspects of testing including accepting samples, diagnostic testing, forms, signatures, and reporting results and summary data. Training will include the importance of the laboratory's obligations as set forth in this and other pertinent regulations and guidance.

2) Whether conducted in-house (if permitted) or by NVSL, laboratory personnel must successfully complete an NVSL-administered individual proficiency test upon completion of the training.

3) After personnel successfully complete the individual proficiency test, NVSL issues a certificate (for NVSL trained) or letter (if trained in-house) authorizing them to conduct EIA testing.

4) NVSL-authorized personnel who have not performed EIA testing for more than 1 year must retake an individual proficiency panel. The laboratory's annual proficiency panel may not substitute for this panel. User fees apply.

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5) Laboratories must notify NVSL if they no longer employ personnel authorized to conduct EIA testing. NVSL will suspend or revoke a private laboratory's approval if NVSL-authorized personnel are not available to conduct the tests. NVSL will notify the AVIC, the Equine Health Team, and the SAHO regarding the laboratory's status.

F. Laboratory Standards for Performing EIA Testing

1) The approved EIA laboratory may only test samples collected in the United States and submitted by:

   a. A federally accredited veterinarian (Category II) authorized to perform accredited duties in the State where the sample originated; or

   b. A State or Federal animal health official.

2) Samples should be collected, handled, stored, and transported to the laboratory so they arrive in good condition, in sufficient volume, and free from heat damage, hemolysis, or other degradation or contamination. Samples should be submitted to the laboratory as soon as possible after collection. Laboratories must reject submissions of samples older than 30 days, as they do not reflect current exposure status.

3) A complete and legible official test form (VS 10-11 or VS-approved equivalent as described above), which includes all of the data specified in *Section C. Definitions, 2.) Official Test Form*, must accompany samples submitted for EIA testing. At the laboratory's discretion, it may process samples accompanied by incomplete forms, but a laboratory will **not release results until the form is properly completed**.

4) Only APHIS-approved and licensed diagnostic test kits can be used for testing.

5) All EIA tests are official tests; VS strictly prohibits screening and/or preliminary tests.

6) Conduct tests according to the test protocols described in the diagnostic test kit literature unless official NVSL protocols direct otherwise. NVSL provides these protocols during training and notifies all laboratories when it significantly updates them. Include the appropriate control samples as specified in the instructions accompanying the diagnostic test kit for each test performed. In addition, include a weak positive sample, obtained from either NVSL or an approved commercial source, each time you conduct an agar gel immunodiffusion (AGID) test. Keep a written log or electronic record of all tests conducted, including sample ID, test type, test kit company name and lot number, results for both control and test samples, and the name of the person or persons conducting the test.

7) The laboratory assigns each submission an accession number (specific to that laboratory) so it can unequivocally identify each sample. Laboratories may assign a single accession number to multiple samples only if the samples come from equids with the same owner and from the same location, collected by the same veterinarian

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on the same date, and received by the laboratory on the same date. Each such sample in the accession number must be uniquely identified (by animal ID) on official test forms and on all laboratory records.

8) All samples testing non-negative (positive, suspect, discrepant, or equivocal, as defined in the diagnostic test kit or NVSL protocols) on any of the licensed EIA diagnostic tests must be immediately recorded on the submission form as preliminary results and submitted to NVSL for confirmation.

   a. NVSL may conduct ancillary testing on these samples or may request additional samples, if necessary. NVSL will bear the cost of confirmatory or ancillary testing on initial or additional samples, but not the costs of obtaining or shipping samples.

   b. The referring laboratory, at its expense, must submit the following to NVSL within 48 hours of obtaining a positive, suspect, discrepant, or equivocal (preliminary) result:

      1. At least 2 ml of serum sample.

      2. A photo or electronic copy of the official test form (VS Form 10-11) with referring lab's preliminary results recorded.

      3. A completed NVSL submission form (VS Form 10-4) documenting the test kit name and lot number(s) and the result(s) obtained at the referring laboratory for each referred sample.

   c. The referring laboratory retains the original official EIA test form (VS Form 10-11) and all paper duplicates until NVSL reports the final result.

   d. If NVSL determines a negative final result (e.g., ELISA classified as a false positive with the AGID negative):

      1. The NVSL-authorized EIA technician at the referring laboratory marks the form AGID negative, records the date of the AGID result, and signs the official EIA test form (VS Form 10-11).

      2. The referring laboratory technician adds: *"Result confirmed by NVSL. Test Result is (input: positive or negative). This form is not valid without NVSL report attached."* The form must also contain the 8-digit NVSL accession number (e.g., NVSL 15-001234) in the Remarks section.

      3. The referring laboratory appends NVSL results to each copy of the official test form. **The official EIA test form must include the appended NVSL result page to be valid**.

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4. NVSL keeps the photocopy of the official test form filed with the corresponding submission form (VS Form 10-4).

5. The referring laboratory must provide a complete, valid copy of the official test form, including the appended NVSL result page, to the submitting veterinarian.

8) On each test result report (VS 10-11), include the official name, city, and State of the approved laboratory that conducted the test, the test result, and the type of test performed (ELISA vs. AGID). Reports must include the handwritten signature (or secure electronic signature) of the technician who performed the test and of the technician who reported the NVSL result. Stamped or perforated signatures are not acceptable. The technician's initials are acceptable (in lieu of a signature) if the initials unequivocally identify the person who performed the test. All laboratory information and signature/initials must be legible on all copies of the official test form. Complete testing and record official results before the reporting technician signs the official EIA test form.

9) Laboratories must maintain an original paper or an electronic copy of each completed EIA animal test form at the laboratory for at least 24 months after the test completion date.

10) Before distributing the final results the submitting veterinarian may request changes to the VS Form 10-11 (including address corrections or spelling mistakes) at the laboratory's discretion. The laboratory must document the request and retain that document with the laboratory copy of the VS Form 10-11.

11) After distribution of the final results the information recorded on the original VS Form 10-11 cannot be changed. In that case, take a new sample and submit a new form.

12) Laboratories must retain, and maintain in good condition, all samples received and tested for at least 30 days from the date they report results.

G. Reporting Test Results

1) Negative Test Results: Laboratories should return official negative test results to the submitting veterinarian as soon as possible after completing the test. Laboratories can mail, email, fax, or otherwise electronically return results to the submitting veterinarian. However, if the veterinarian submitted the samples with OMB-approved, official paper VS 10-11 forms, then the laboratory must mail paper copies of PART 1 - VETERINARIAN/SUBMITTER and PART 3 – OWNER to the submitter within 30 days. Some venues or importing countries may require these paper forms. Some practitioners may feel these forms are more secure and prefer their use.

Laboratories should forward official negative test results to the SAHO and AVIC where the animal resides and to the submitting veterinarian at least monthly after completing the tests. The laboratories can also email, fax, or otherwise electronically

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forward results, at the AVIC's or SAHO's request, provided the information remains available for epidemiological investigations and results can be obtained within 2 working days. Back up these files weekly.

The laboratory must document the reporting described above and retain this documentation along with a paper or electronic copy of the negative test results for the annual inspection.

2) Non-Negative Test Results: The referring laboratory should immediately record results (positive, suspect, discrepant, or equivocal) on the official VS 10-11 as preliminary. All samples forwarded to NVSL for confirmation (positive, suspect, discrepant, or equivocal on any EIA test) require the laboratory to immediately (no more than 24 hours after the test is completed) notify the SAHOs and the AVIC of the affected States.

If NVSL determines final results on referred samples to be positive, NVSL immediately reports the results to the SAHOs of the affected States, the AVIC, the referring laboratory, and the Equine Health Team. The AVIC coordinates with the SAHO and ensures the submitting veterinarian receives notification. NVSL provides copies of all positive test results as soon as possible to the affected SAHOs.

If NVSL determines final results on referred samples to be negative, NVSL communicates the results to the referring laboratory, the SAHO, and the AVIC. The referring laboratory's NVSL-authorized EIA technician will amend *(as detailed in E. 8) d. Laboratory Standards for Performing EIA Testing)* the original official test form (VS Form 10-11) and initial and date the amendment, which reflects the final negative result. On receiving the negative laboratory result form from NVSL, the EIA technician attaches the NVSL report to all copies of the official test form. It then becomes part of the official record for distribution with the official test form.

H. Reporting Summary Data

Laboratories must provide the VS Equine Health Team and the relevant SAHO with reports within 30 days of the close of the previous month. These reports should include monthly totals of negative and positive EIA tests, grouped by test type (e.g., AGID vs. ELISA) and by the sample origin State, using the standardized reporting spreadsheet or approved equivalent provided by the VS Equine Health Team. Email reports to equine.health@usda.gov or electronically transmit to the Equine Health Team via a method approved by them. The laboratory must document the reporting and retain that documentation for the annual inspection.

I. Requirements for Mobile and Satellite Laboratories

1) Mobile laboratories

a. VS does not have to approve a mobile facility.

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b. VS inspects the facility annually. The facility must meet all requirements for a stationary laboratory as set forth in the checklist. The facility specifically must be able to maintain the appropriate controlled environment for testing and/or storage, as indicated in the inspection checklist (Attachment 1).

c. VS conducts annual proficiency testing at this facility.

d. Mobile laboratories performing fewer than 500 EIA tests per year (as documented by official test chart records) should justify their approval or renewal to the AVIC and SAHO and may be subject to additional inspections or proficiency panels initiated by NVSL or the SAHO. If the mobile facility fails to maintain standards or proficiency, VS will withdraw approval. Use of a mobile facility is restricted to the State VS approved, unless it obtains written authorization from both the SAHO and the AVIC of each alternate State or States. Submit that authorization to NVSL.

e. Laboratories with both a stationary and mobile capability must be able to distinguish laboratory test records for EIA tests completed at the mobile laboratory from tests conducted at the stationary laboratory.

f. Upon request, and at the time of inspection, the laboratory director must provide the SAHO, inspecting Federal official, and the appropriate AVIC a chronological log or clear documentation of all instances and locations of mobile operations.

2) Satellite laboratories

a. VS does not have to approve a satellite laboratory.

b. VS inspects the facility annually. The facility must meet all requirements for a stationary laboratory as set forth in the checklist. The facility specifically must be able to maintain the appropriate controlled environment for testing and/or storage, as indicated in the inspection checklist (Attachment 1).

c. Conduct proficiency testing at the main or home laboratory. This may include the satellite location; there is no separate annual requirement for the satellite laboratory.

d. The 500-test minimum guidance applies to the main or home laboratory; there is no separate minimum for the satellite laboratory unless the satellite laboratory technician does not also perform testing at the main or home laboratory.

e. Use of the satellite facility is restricted to the State VS approved, unless it obtains written authorization from both the SAHO and the AVIC of each alternate State or States. Submit that authorization to NVSL.

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f. Laboratories must be able to distinguish laboratory test records for EIA tests completed at the satellite laboratory from tests conducted at the main or home laboratory.

g. Upon request, and at the time of annual inspection, the laboratory director must provide the SAHO, inspecting Federal official, and the appropriate AVIC a chronological log or clear documentation of all instances and locations of satellite operations.

J. Maintaining Approval

1) Each approved laboratory must be able to demonstrate and document compliance with the requirements in this document; failure to meet these requirements will be grounds for withdrawal of laboratory approval.

2) The approved Laboratory Director must know and meet NVSL's deadlines. He or she must request the annual proficiency test, obtain the testing samples or materials, and must follow all instructions from NVSL to take and pass the annual proficiency test in the allotted time frame. Each laboratory will be proficiency tested once a year regardless of how many approved technicians work at the laboratory. Conduct proficiency testing for satellite laboratories at the home or main laboratory. This may include the satellite location, but additional proficiency testing is not required for satellite locations unless the technician does not also perform testing at the main or home laboratory. Laboratories must pay for the annual proficiency testing. Laboratories must maintain accurate contact information and respond promptly to inquiries and requests from NVSL.

3) Laboratories that VS recently approved (within 6 months of the proficiency test distribution date) are exempt from participating for that approval year only. Laboratories that fail the annual proficiency test will lose approval or undergo other corrective action as determined by NVSL. Failing an annual laboratory proficiency test twice in 1 year is grounds for immediate laboratory removal. Laboratory personnel must retake and pass the NVSL EIA laboratory technician course to regain approval. NVSL provides laboratory proficiency final results to the SAHO and AVIC for laboratories approved in the State.

4) Laboratories must meet their reporting and summary data requirements without prompting. They must respond promptly to any NVSL inquiries regarding the number of tests performed, ordering proficiency testing, reporting proficiency results, and other requested information.

5) Federal, State, university, military, private, mobile, and satellite laboratories must undergo a satisfactory official annual inspection conducted by Federal personnel or a cooperative team of Federal and State personnel to retain approval. The AVIC or SAHO may request additional inspections. Satellite laboratories require inspection and approval of each operating site or venue.

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Official laboratory inspections are subject to user fees. Laboratories are responsible for payment before, or at the time of, inspection. 9 CFR 130.30 lists user fees for time and travel dedicated to laboratory inspections. User fees specific to EIA lab inspection are charged under APHIS service code 2005 “hourly fee (EIA) (BT) (BL)” and reported in APHIS Import/Export functional area AP00UFVSIMPEXP00 under account code ZXVSICZZZZVSIMPEXEXPTICZZZZ in which “Z” at the beginning of the account code represents the last number of the fiscal year and “ZZZZ” relates to the cost center for the appropriate State.

a. Inspection or certification by other organizations or authorities does not negate the requirement for this inspection. Inspection by Federal personnel or a Federal-State team does not absolve laboratories from other local or State requirements.

b. Inspectors will:

1. Verify adequate and appropriate facilities as described in this document and per the attached inspection checklist (Attachment 1);

2. Verify the laboratory has technical personnel suitable to perform official EIA testing who have received and completed the prescribed NVSL training and are certified by NVSL;

3. Verify the laboratory accepts only samples submitted by a Category II federally accredited veterinarian, authorized in the State where the samples were obtained, and submitted with a complete and legible official EIA test form.

4. Review documents to ensure all EIA tests are conducted as official tests and there is no evidence of screening or preliminary tests.

5. Verify the laboratory uses only USDA-approved diagnostic test kits with licensed antigen. All testing is conducted in accordance with the official protocols as described in this document, provided or prescribed by NVSL, or in literature accompanying the diagnostic test kits.

6. Verify all non-negative test results (positive, suspect, discrepant, or equivocal) are considered preliminary results and the samples are immediately submitted to NVSL for confirmation.

7. Verify the laboratory meets annual check test proficiency requirements prescribed by NVSL and continues to expect to perform at least 500 EIA tests per year as necessary to maintain testing competency.

8. Verify the laboratory has fulfilled all regulatory obligations regarding reporting of all official test results, adequate recordkeeping, and has met the reporting and summary data requirements described in this document.

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9. Confirm and update laboratory contact information.

10. Provide the SAHO a copy of the inspection checklist within 30 days of completion.

11. Review for proper completion of official test charts and to verify the required reporting of both positive and negative test results is documented.

6) Federal or State officials may inspect an approved laboratory at any time during the laboratory's normal business hours or by appointment. VS may subject laboratories with deficiencies in compliance or procedures to corrective actions, as appropriate. Inspectors will recommend removing approval for EIA testing for laboratories with uncorrected deficiencies.

7) Laboratories performing fewer than 500 EIA tests per year (as documented by official test chart records) should justify their approval/renewal in writing to the AVIC and SAHO and may be subject to additional inspections or proficiency panels initiated by NVSL or at the SAHO's request. If the lab fails to maintain standards or proficiency, VS will withdraw approval.

8) VS tracks EIA lab inspections using an information management system (currently the Emergency Management Response System [EMRS]). The AVIC oversees EIA inspections: monitoring and scheduling, entering inspection data, attaching worksheets in EMRS within 30 days of finishing the inspection, and notifying State animal health officials of the inspection schedule.

9) The VS Equine Health Team, in cooperation with NVSL and State animal health officials, creates and maintains an appropriate EIA laboratory inspection training program, and reviews inspections to keep inspections consistent, timely, and accurate across the country. If a laboratory's inspection is reported (in EMRS) as 30 days past the annual inspection date, VS notifies the AVIC and SAHO.

K. Changes in Laboratory Contact Information, Location, or Ownership

1) The laboratory director must maintain current contact information and inform the AVIC and NVSL immediately of any approved personnel name or employment status changes, changes of address, telephone, or email information. The AVIC notifies the SAHO and VS Equine Health Team of changes.

2) A change in the laboratory director necessitates a new, signed EIA Agreement.

3) If the physical location of the laboratory changes (excluding mobile laboratories) while the ownership and clientele remain the same, notify NVSL and the AVIC as soon as possible. The AVIC notifies the SAHO and the VS Equine Health Team. VS will conduct an official inspection and administer a proficiency test in the new facility within the first 30 days of operation. Laboratories successfully completing a

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proficiency panel within 6 months of the annual test distribution are exempt from the annual proficiency test for that year only.

4) Transfers of ownership, relocations out of State, or other moves not covered above are considered new laboratory applications and will require the concurrence of the SAHO and AVIC as would starting any new approval application.

L. Existing Laboratories

VS will permit approved laboratories operating when VS releases this guidance to continue operation with the following provisions:

1) VS will send existing laboratory directors this guidance document for review.

2) NVSL must receive a new, signed EIA Director's Agreement that acknowledges receipt and an understanding of the requirements before it will make the annual proficiency test available to that laboratory. Existing laboratories will have 6 months to comply with new requirements in this document.

3) VS will grant 6-month waivers from the requirement for a new EIA Laboratory Director's Agreement, upon request, to those labs with less than 6 months until the next annual inspection.

M. Removal of Laboratory Approval

The APHIS Administrator may withdraw laboratory approval when a laboratory fails to meet any of the criteria for approval. In all cases, APHIS will notify the laboratory, the AVIC, and the SAHO in writing. Find the procedures to appeal laboratory removal in 9 CFR 75.4(d).

APHIS will remove approval in any of the following situations:

1) The laboratory requests removal.

2) The NVSL Director receives a written recommendation for removal by concurrence of the AVIC and the SAHO.

3) The laboratory fails to maintain adequate and appropriate facilities as described in this document and per the attached inspection checklist (Attachment 1).

4) The laboratory fails to maintain technical personnel with prescribed NVSL training to perform official EIA testing.

5) The laboratory fails to accept only samples submitted by a Category II federally accredited veterinarian, authorized in the State where the samples were obtained, and with a complete and legible official EIA test form.

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6) The laboratory fails to use only USDA-approved diagnostic test kits with licensed antigen.

7) The laboratory fails to conduct all testing in accordance with the official protocols as described in this document, provided or prescribed by NVSL, or in literature accompanying the diagnostic test kits.

8) The laboratory fails to conduct all EIA testing as official tests or conducts screening and/or preliminary tests.

9) The laboratory fails to treat all non-negative test results (positive, suspect, discrepant, or equivocal) as preliminary results and/or submit the samples to NVSL for confirmation.

10) The laboratory fails to seek and meet annual check test proficiency requirements prescribed by NVSL and/or fails to perform at least 500 EIA tests per year without an official exemption.

11) The laboratory fails to meet the regulatory obligations regarding reporting of all official test results and/or fails to meet the reporting and summary data requirements described in this document.

12) The laboratory fails to obtain a satisfactory official annual inspection.

13) The laboratory fails to maintain current contact information and/or respond to official requests and inquiries.

14) APHIS will also consider any other compliance issues that occurred during the previous 12 months.

N. List of Approved Laboratories

The NVSL Director maintains a current list of laboratories approved to conduct tests for EIA. NVSL updates the list regularly, and it is available here, on the APHIS Animal Health Laboratory Information Services APHIS-Approved Laboratories page.

7. Inquiries

Direct any questions about the laboratory approval process to the NVSL Diagnostic Virology Laboratory by telephone at (515) 337-7551, by fax at (515) 337-6508, or by email at NVSL_concerns@aphis.usda.gov.

Direct all other questions relating to this document to the Equine Health Team at equine.health@usda.gov or by telephone at (301) 851-3558.

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Attachment 1
Inspection Checklist

Laboratory Name:	
Date of Inspection:	
Name and Title/Affiliation of Inspector:	
State Representative (if present):	
Laboratory Director:	
Director's Representative (if applicable):	
Physical Address of Laboratory (street, city, State, and ZIP code (not P.O. box):	
Mailing Address of Laboratory (if different):	
Shipping Address of Laboratory (if different):	
Laboratory Phone #:	
Laboratory Fax #:	
Laboratory Director's Email Address:	
Laboratory/Alternate Email Address:	

List or attach a list of all NVSL-trained personnel currently conducting EIA tests at the laboratory and the date on which NVSL authorized each person.

Name	Date Authorized

List or attach a list of all in-house trained personnel currently conducting EIA tests at the laboratory and the date on which NVSL authorized each person (applies to State, Federal, university, or military laboratories ONLY).

Name	Date Authorized

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Laboratory Inspection Checklist for Equine Infectious Anemia (EIA) Testing

Section	Item	Yes	No	Notes
Building Facility	The building is in good repair and provides a professional appearance inside and outside.	Y	N	
	The building has adequate and functional doors, windows, and screens that maintain a clean and climate-controlled environment appropriate for a laboratory.	Y	N	
	The building has clean, functional restrooms.	Y	N	
	The building appears free of rodents, insects, and other pests.	Y	N	
	Refuse is properly contained, removed and is handled in accordance with local ordinances.	Y	N	
Laboratory Facility	There is a separate and dedicated room reserved for laboratory use where the EIA testing is conducted, with floor-to-ceiling walls and doors delineating that room. The public is denied access to the laboratory space. The laboratory space may also be used to store pharmaceuticals, biologics, or clean medical supplies, for example, but cannot be used for any animal use, eating meals, or other use that could create dust, dirt or excessive traffic; however all other standards will be maintained. While EIA testing is being conducted there will be no non-laboratory concurrent use.	Y	N	
	Adequate open, clutter-free bench space (at least 5 feet) is evident. There is hot and cold running water with a sink in the laboratory area.	Y	N	
	Bright white light is available to the bench space. For AGID testing the facility must be capable of dimming or restricting ambient/daylight to properly read the results.	Y	N	

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Section	Item	Yes	No	Notes
	Laboratory temperature is maintained at all times between 68° and 77 °F (20° and 25 °C) and/or an incubator is available to maintain these temperatures for the testing, when required. The laboratory facility is capable of preventing reagents and supplies from cold damage or overheating in accordance with label instructions.	Y	N	Laboratory temperature at time of inspection: ___°F
	Mobile and satellite laboratories: When operating or supplied with reagents, are equipped with a thermometer capable of high/low temperature memory/recording. A weekly high/low temperature record/log is kept.	Y	N	Attach or copy the temperature log.
	Laboratory and laboratory equipment is clean, functional, and properly stored; records indicate it is properly maintained in accordance with manual of operations.	Y	N	
	Refrigerator is functional, properly maintained, and equipped or supplemented with a thermometer capable of high/low temperature memory/recording, and is labeled for lab use only; no food or drink. A weekly high/low temperature record/log is kept.	Y	N	Attach or copy the refrigerator temperature log.
	Clean laboratory clothes (coats) are available and required to be worn.	Y	N	
	There is no evidence of prohibited activities in the laboratory area, such as: eating, drinking, applying cosmetics, handling contact lenses, or storage of food. Appropriate signage is clearly visible.	Y	N	
Laboratory Supplies and Equipment	**The following equipment must be available and functioning properly for the agar gel immunodiffusion (AGID) test:**			
	High intensity laboratory light that can be focused for reading AGID plates.	Y	N	

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Section	Item	Yes	No	Notes
	Blinds on windows or separate room so light can be reduced to read AGID plates.	Y	N	
	Medium seven-well immunodiffusion template cutter, a center well surrounded by six evenly spaced wells. Wells are 5.3 mm in diameter and 2.4 mm apart.	Y	N	
	A balance designed and functioning to read with accuracy to plus or minus 0.1 gram; maintained and calibrated in accordance with operations manual.	Y	N	
	Method to remove agar plug (such as a vacuum pump).	Y	N	
	Equipment to make agar: Graduate measures, flasks, and additional appropriate glassware.	Y	N	
	Source of heat for agar preparation (microwave, hot plate, autoclave).	Y	N	
	Method for protecting from disturbance and incubating AGID plates on test (a room temperature incubator will suffice).	Y	N	
	Clean or new pipettes for delivery of reagents to wells.	Y	N	
	Distilled water and chemicals for buffer (sodium bicarbonate, boric acid, distilled water).	Y	N	
	Noble agar.	Y	N	
	Disposable 60 mm or 100 mm petri dishes.	Y	N	
	Materials or equipment to accurately measure pH within 0.2 pH unit.	Y	N	
	The following equipment must be available and functioning properly for the enzyme-linked immunosorbent assay (ELISA) test:			
	Incubator (if 37 °C incubation is required for the ELISA test used), functioning and properly maintained in accordance with operations manual.	Y	N	
	Wash bottles, pipetting devices, and plate holders.	Y	N	
	ELISA washer and reader (optional).	Y	N	

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Section	Item	Yes	No	Notes
	Clean or new micropipettes and appropriate tips to deliver reagents to wells.	Y	N	
	Pipettes must be properly calibrated a minimum of every 12 months, preferably every 6 months.	Y	N	
	Maintain pipette calibration records.	Y	N	Attach calibration record log.
Control of Specimens and Reporting	**(Only applicable for approved laboratories)**			
	Laboratory should not be accessible to the general public during testing.	Y	N	
	Records indicate the laboratory assigns an accession number with unique identification (animal name) to each sample. The specimen identity is maintained on the sample, worksheets, on the petri dish and ELISA plates/strips. The accession number is recorded on the official EIA reporting form.	Y	N	Inspector will trace a recent random sample through entire process and verify accountability and document sample number and compliance.
	Test worksheets include appropriate and complete information, including accession number, animal and sample identification, lot numbers and expiration dates of reagents used in the test, date/time of test start, identity of technicians setting up and completing test, and date/time of test completion.	Y	N	
	Maintenance of test worksheets and accession paperwork for a minimum of 24 months. The lab must provide the worksheets at inspection or NVSL request.	Y	N	
	Specimens not appropriately identified are not tested.	Y	N	

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Section	Item	Yes	No	Notes
	Records indicate only specimens with properly filled official EIA submission forms (with name of the owner, name, address, and accreditation number of the submitting veterinarian, location of animal at the time the test sample was obtained, complete animal identification, and signed by the submitting veterinarian) are tested/processed. A minimum of 1 random accession per month for the previous 12 months will be reviewed for completeness and compliance and accession numbers recorded.	Y	N	
	The test results for each sample are recorded on a worksheet which should be made available for review.	Y	N	
	The results are recorded on the reporting form with a copy kept in the laboratory. Results are reported only as negative, positive, or no test.	Y	N	
	Records indicate all positive, equivocal, or discrepant samples are forwarded to NVSL. If a non-negative result was found since the last inspection, the inspector will trace a sample and document NVSL confirmation and appropriate notifications to Federal and State officials.	Y	N	
	Records indicate all tests are reported regardless of results. No unofficial EIA tests are performed to determine the status of the animals before the "official" test is performed. **Each and every EIA test is an official test.**	Y	N	

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Section	Item	Yes	No	Notes
	On a review of reports of test results they include the name, city, and State of the laboratory, the type of test performed, and the handwritten signature (or secure electronic signature) of the technician who performed the test. Stamped or perforated signatures are not in use. The technician's initials unequivocally identify that person. All laboratory information and signature/initials are legible on all copies of the official test form. A minimum of 1 random accession per month for the previous 12 months will be reviewed and documented for compliance.	Y	N	

	Records indicate official test results and summary data are reported to the State and/or Federal animal health officials within the time specified by these officials.
	• Non-negative test results were reported within 24 hours to:
	  ○ SAHO where the animals were sampled and where laboratory is located.
	  ○ AVIC where the animals were sampled.
	  ○ VS Equine Health Team via email.
	• Negative test results were reported monthly to the SAHO where the laboratory is located (as requested or required) and the SAHO where the animals were sampled (as requested or required).
	• Negative test results were reported monthly to the SAHO in the State where the animals were located.
	• Monthly summary data is being reported to the SAHO and the VS Equine Health Team.
		Y	N	

	Records or inspection indicate specimens are held for at least 30 days after results are reported; either refrigerated whole blood or frozen serum with clot removed.	Y	N	Document the dates on a representative number of samples currently stored.

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Section	Item	Yes	No	Notes
EIA Reagents	**(Only available to approved laboratories)**			
	Only reagents licensed by APHIS or supplied by NVSL are to be used. Both unopened and open/partially used reagents must demonstrate current expiration dates and be properly stored/refrigerated in accordance with label directions.	Y	N	Record lot numbers and expiration dates on current reagents or a representative sample.
	Unused or outdated reagents, other chemicals and supplies, and the inoculated EIA AGID or ELISA plates must be appropriately discarded according to local rules and regulations.	Y	N	
Test Procedure	Appropriate SOPs are available in the lab.	Y	N	
	Records indicate the procedures outlined in the appropriate test protocol are being followed.	Y	N	

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ADDITIONAL REMARKS

_____
_____
_____
_____
_____
_____
_____
_____
_____

Laboratory is: ☐ Satisfactory ☐ Unsatisfactory (check one)

_____
Inspector's Name, Signature, and Date

_____
Laboratory Director's Name, Signature, and Date

_____
Laboratory Director's Representative Name (if not same), Signature, and Date

Inspector/Federal VMO: Inspection copies to the following; check when completed:

- ☐ Laboratory Director/Representative
- ☐ Diagnostic Virology Laboratory, NVSL, Ames, Iowa
- ☐ State Animal Health Official
- ☐ Federal District Office
- ☐ Copy uploaded to EMRS

Estimated date of next laboratory inspection: _____

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