TO: Veterinary Services Management Team (VSMT)
FROM: John R. Clifford
Deputy Administrator

SUBJECT: Approval of Laboratories to Conduct Tests for Equine Infectious Anemia

I. PURPOSE

The purpose of this memorandum is to outline the procedures for approval of laboratories to conduct tests for equine infectious anemia (EIA) and the requirements for those laboratories when performing EIA tests.

II. CANCELLATION

VS Memorandum No. 555.16 dated March 7, 2003, is cancelled.

III. DEFINITIONS

A. Official test

Any test for the laboratory diagnosis of EIA that uses a diagnostic product that is: (1) produced under license from the Secretary of the U.S. Department of Agriculture (USDA) and found to be efficient for that diagnosis under the Virus-Serum-Toxin Act of March 4, 1913, and subsequent amendments; and (2) conducted in a laboratory approved by the Administrator of the Animal and Plant Health Inspection Service (APHIS) (see title 9, Code of Federal Regulations (9 CFR), part 75 for additional details). All EIA tests are official tests (i.e., screening and/or preliminary tests are not accepted) and all official EIA tests must be conducted at approved facilities by approved personnel certified in accordance with the procedures outlined in this Memorandum.

B. Official Test Form

The VS 10-11 is the official Federal form for EIA test requests. Electronic forms containing equivalent information to the VS 10-11 may be used. In some States, a State-approved form is acceptable within the originating State. Form VS 10-11, electronic versions of VS 10-11, and State-approved EIA test forms are considered official EIA test forms. Their use must comply with the provisions of this Memorandum 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 Memorandum, the terms “VS 10-11” and “official test form” are used interchangeably.
C. Approved Laboratory

1. State, Federal, or University Laboratory

Any site (facility) under the direct supervision of a State animal health official, an APHIS VS animal health official, a U.S. military laboratory director, or a university laboratory director may apply for laboratory approval to conduct EIA tests. Approval requires that the laboratory meets all of the requirements outlined in this Memorandum. Additionally, at least one laboratory employee must have successfully completed the EIA training course at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, and have current and active involvement with EIA testing.

2. Private Laboratory

A singular test site (facility) with no official supervisory affiliation with a State, Federal, or university laboratory may also apply for approval to conduct EIA tests. Approval requires that the laboratory meets all of the requirements outlined in this Memorandum. Additionally, all laboratory employees who conduct EIA testing must be certified as having successfully completed the EIA training course at NVSL.

Private laboratory test sites (facilities) include the following:

a. Mobile laboratory—A singular facility that maintains the same physical requirements and proficiency requirements as a stationary private laboratory.

   i. States are not obligated to approve mobile facilities.

   ii. The mobile facility may only be used in the State for which it was approved unless written authorization is obtained from the State animal health official and the Area Veterinarian in Charge (AVIC) of the alternate State.

   iii. The laboratory director must keep the State animal health official and the AVIC of the appropriate State advised of all instances and locations of operations.

b. Satellite laboratory—A mobile, alternate laboratory from an existing, approved stationary EIA laboratory used at equine sales facilities or auction markets to meet the transfer of ownership or interstate movement requirements for EIA testing. Testing must be conducted by certified personnel following all of the specifications in this Memorandum.

   i. States are not obligated to approve sales facilities as EIA testing sites.

   ii. The facility must have an appropriate controlled environment for testing.

   iii. Laboratories must have the ability to distinguish test records for EIA tests completed at the satellite laboratory.

   iv. State and Federal animal health officials may withdraw approval to conduct EIA tests at a satellite laboratory at any time.
IV. STEPS FOR LABORATORY APPROVAL

A. A written request must be made to the appropriate AVIC describing the reasons for establishing a new EIA laboratory. The application must include: data on the horse population, intended clientele, planned business hours of operation, estimated number of tests expected per year, and any additional information to justify how the proposed laboratory will serve an unmet need for the EIA program. The AVIC and State animal health official must consider whether EIA testing needs are currently being met through existing laboratories, geographic dispersal of approved laboratories, resources available for monitoring EIA laboratories, and other relevant factors.

B. After review of the EIA laboratory request, the decision to accept or decline a new EIA laboratory will be made through consensus by the State animal health official, the AVIC, and NVSL. The following actions will be taken if approval is authorized:

1. A Federal or State veterinary medical officer will review the regulatory and technical responsibilities and criteria inherent in conducting and reporting EIA tests with the laboratory official. A copy of the inspection check list and standards will be provided to the laboratory director.

2. A Federal animal health official will inspect the physical facilities of the laboratory and record the results on the laboratory inspection checklist (Attachment 1). Laboratory inspections are subject to user fees for travel and inspection time at the hourly rate (see section VI. K.3). Only after the proposed facility is found in compliance may laboratory personnel be eligible for EIA training.

3. The laboratory official will sign the Agreement to Conduct Equine Infectious Anemia (EIA) Testing (Attachment 2) to acknowledge that he or she understands the regulatory and technical responsibilities of the laboratory.

C. After the actions in section IV. A and B have been completed, the AVIC and the State animal health official must submit a jointly signed Memorandum of Recommendation and Justification for the laboratory, including the originals of completed Attachments 1 and 2. The memorandum and attachments must be mailed to: Director, NVSL, 1920 Dayton Avenue, Ames, IA 50010.

Questions in regard to the laboratory approval process should be directed to the NVSL Diagnostic Virology Laboratory by telephone at (515) 337-7551 or by fax at (515) 337-6508.

D. Training applications for personnel to attend the NVSL laboratory course on EIA testing will be accepted only after sections IV A, B, and C have been completed.
E. Final Approval by the NVSL Director
After satisfactory completion of the requirements in section IV and section V.A. (see below), the laboratory is eligible for approval by the Director of NVSL. The laboratory and the AVIC will be notified of final approval by letter from the NVSL Director.

V. TRAINING OF PERSONNEL TO CONDUCT EIA TESTS

A. Laboratory personnel who perform EIA tests must be certified by VS. The AVIC and State animal health official must recommend personnel for training and approval by NVSL. The minimum required training is as follows:

1) Private laboratories: Employees responsible for conducting EIA tests will be trained at NVSL. Laboratory personnel must successfully complete an individual proficiency test administered by NVSL to be certified by VS. All associated costs for the NVSL training course will be paid by the requesting laboratory.

2) Federal, State, and university laboratories: At all times, at least one laboratory employee who is actively involved in EIA testing must have completed the NVSL training course. All associated costs for the NVSL training course will be paid by the requesting laboratory. Personnel previously trained at NVSL may train others in their laboratory to conduct EIA tests at that laboratory with the approval of the AVIC and the State animal health official; training will include regulatory responsibility. Laboratories must provide the names of personnel to be trained in-house to NVSL. NVSL will subsequently provide individual proficiency tests (standard user fees apply) which must be successfully completed in accordance with established NVSL standards for training certification. The laboratory’s annual proficiency panel may not be used for individual certification.

Note: Laboratory officials must arrange to send one or more employees to NVSL for EIA testing training within 6 months if staffing changes occur and there are no longer EIA trained and certified laboratory personnel on staff.

3) Previously trained and certified personnel who have had a lapse in testing greater than 1 year will be required to retake a personal proficiency panel. User fees apply.

B. NVSL will evaluate personnel who do not successfully complete proficiency testing to determine whether additional training at NVSL is necessary, or approval of the laboratory should be canceled.

C. Laboratories approved to conduct EIA tests must inform the AVIC and NVSL when any personnel certified by NVSL to conduct the tests are no longer employed at that
laboratory. Approval of the laboratory will be revoked if certified personnel considered qualified by VS are not available to conduct the tests.

VI. LABORATORY STANDARDS FOR PERFORMING EIA

A. Use only diagnostic test kits that have been approved and licensed by APHIS

B. Conduct tests according to the test protocols described in the diagnostic test kit literature unless otherwise directed by official NVSL protocols. Each time a test is performed, include the appropriate control samples as specified in the instructions accompanying the diagnostic test kits. In addition, include a weak positive sample obtained from either NVSL or an approved commercial source each time an agar gel immunodiffusion (AGID) test is conducted.

C. For EIA testing, accept only samples collected and submitted by:
   - An accredited veterinarian
   - A State or Federal animal health official, or
   - A military veterinarian

Approved EIA laboratories agree to accept appropriate samples from any accredited veterinarian for official EIA testing.

D. Each equid sample submission must be accompanied by an individual animal identification form (written legibly or submitted electronically) that includes the following information:
   1. Name, address, and phone number of the submitting veterinarian
   2. Signature and license/accreditation number of the veterinarian who collected the sample
   3. Name and address of the owner
   4. Location (including county) of the equine when the sample was obtained
   5. Identification of the equine sampled, including name, age, breed, sex, color, markings, and tattoo or registration number.
   6. Sample collection date: The age of samples acceptable for submission is at the discretion of the laboratory; however, it is recommended that samples should not be accepted for testing if submitted greater than 30 days from the collection date.

Note: The signature of the owner or owner’s agent on the official EIA test form is optional, but may be required at the discretion of the submitting veterinarian.

E. Each submission will be assigned a unique accession number (to that laboratory) in a manner that will allow each sample to be identified in the laboratory.
F. On each report of test results, include the name and location (including the city and State) of the laboratory that conducted the test and the type of test performed. Reports must include the handwritten signature (or secure electronic signature) of the technician who performed the test. Stamped or perforated signatures are not acceptable. The technician’s initials are acceptable (in lieu of a signature), provided that the initials unequivocally identify the person who performed the test. All laboratory information and signatures/initials must be legible on all copies of the official test form.

G. Reporting results
1. Report negative test results (electronically or via hard copy) to State and/or Federal animal health officials as instructed by those officials in both the State where the laboratory is located and the State in which the animals were sampled.

2. If horses are positive to the EIA test, notify the State and/or Federal animal health official immediately (or no more than 24 hours after the test results are completed).

3. When requested, provide the State or Federal animal health official with timely reports of monthly totals of EIA AGID and EIA enzyme linked immunosorbent assay (ELISA) tests performed by the laboratory.

4. Report EIA test results to the veterinarian submitting the sample within 48 hours of completing the test.

H. Maintain an original copy or an electronic file of each completed EIA individual animal test form at the laboratory for at least 24 months after the test completion date.

1. Changes cannot be made to the VS 10-11 after results are completed and the forms have been distributed. A new submission will be necessary unless the AVIC and submitting veterinarian agree to void the current test paperwork (by collecting and destroying any distributed paperwork) and resubmit with changes.

2. Changes to the VS 10-11 (including address corrections or spelling mistakes) made prior to final results may be made at the discretion of the laboratory through a request from the submitting veterinarian. The laboratory must retain documentation of the request with the laboratory copy of the VS 10-11.

I. All EIA positive ELISA test results must be confirmed by AGID. Samples that test positive by ELISA must be forwarded to a State, Federal, or university laboratory that conducts AGID testing. Samples with discrepant test results (for example positive ELISA/negative AGID) at any laboratory must be forwarded to NVSL for confirmation testing. NVSL may conduct ancillary testing on discrepant samples or may request additional samples, if necessary.
J. The AVIC and NVSL must be informed of any change of address, other contact information (e.g., telephone number) or a change in the laboratory director who signed the EIA agreement. If the physical location of the laboratory changes (excluding mobile and satellite laboratories) an official inspection and completion of a proficiency test in the new facility must be conducted within the first 30 days of operation (see below). Laboratories that successfully complete a proficiency panel within the 6 months prior to the annual test distribution are exempt for that year only.

*Note:* The relocation requirements are intended for facilities that change physical location but continue to maintain current ownership and serve the same clientele. Transfers of ownership or long distance relocations are not permitted. Such situations require AVIC approval and may be handled as a new laboratory application.

K. Continued approval will require that the following obligations are met:

1. Each approved laboratory must pass an annual proficiency test (one test per laboratory) even if multiple approved EIA technicians are working at that laboratory. NVSL will supply the samples for personnel to test and will evaluate the test results. Laboratories that are approved within 6 months of the proficiency test distribution date are exempt from participating for the approval year only. Laboratories that fail the annual proficiency test are subject to the appropriate corrective action as determined by NVSL, including additional proficiency testing and/or removal. Laboratories that fail the proficiency test twice in 1 year will be recommended for removal.

2. Laboratories must respond to inquiries from NVSL regarding the number of tests performed, ordering proficiency testing, reporting proficiency results, or other requested information in a timely manner.

3. Continued approval for Federal, State, university, private, mobile, and satellite laboratories will require an official inspection conducted by Federal personnel at least every 2 years. Additional laboratory inspections may be conducted at the discretion of the AVIC if there are performance or compliance concerns. Official inspections of laboratories are subject to user fees.

Laboratories are responsible for payment before, or at the time of, inspection. User Fees for time and travel dedicated to laboratory inspections are listed in 9 CFR, section 130.30 and on the APHIS Web site at http://www.aphis.usda.gov/mrpbs/fmd/vs_import_export_fees.shtml. User fees are charged under USDA APHIS service code 2005 “Hourly Fee (EIA) (BT) (BL)” and reported in APHIS accounting reporting category number 699. Inspection by VS
(Federal) personnel does not absolve laboratories from other requirements established by local or State agencies.

The Federal inspecting official will verify the following:
- Compliance with this Memorandum
- Determine whether tests are being conducted according to official protocols
- Determine whether personnel conducting the tests are certified by VS to perform EIA tests
- Review the completion of official test charts and verification of reported annual EIA tests performed

An approved laboratory is subject to inspection by Federal or State animal health officials at any time during the laboratory’s normal business hours. Laboratories with deficiencies in compliance or procedures are subject to corrective actions, as appropriate. Laboratories with severe or uncorrected deficiencies will be recommended for removal.

4. Each laboratory must show active involvement in serving the EIA control program, compliance with the specifications of this Memorandum, and successful completion of a proficiency panel. Laboratories that perform fewer than 200 EIA tests per year (as documented by official test chart records) are subject to approval withdrawal. Exceptions to the minimum testing expectation must be justified to APHIS by letter from the AVIC and State animal health official.

VII. REMOVAL OF LABORATORY APPROVAL

The APHIS Administrator may withdraw laboratory approval when any of the criteria for approval have not been met. In all cases, written notification in regard to the removal of laboratory approval will be provided to the laboratory. The procedures to appeal laboratory removal are listed in 9 CFR, section 75.4(d).

Approval will be removed in any of the following situations:
- The laboratory requests removal.
- The NVSL Director recommends removal.
- The NVSL Director receives written recommendation for removal by concurrence of the AVIC and the State animal health official.
- The laboratory fails to adequately meet the proficiency testing or inspection requirements.
- The laboratory does not meet annual testing numbers as determined by NVSL, the AVIC, and the State animal health official.
Laboratories that fall under circumstances two through five (listed above) will be informed of the removal recommendation by letter from the APHIS Administrator or his/her designee.

VIII. LIST OF APPROVED LABORATORIES

The NVSL Director will maintain a current list of laboratories approved to conduct tests for EIA. The list will be updated regularly and will be available on the APHIS Web site at www.aphis.usda.gov/animal_health/lab_info_services/approved_labs.shtml.
Laboratory Inspection for Equine Infectious Anemia (EIA) Testing

Laboratory name __________________________________________________________
Phone # ______________________ Fax # ______________________
Laboratory or director e-mail address _______________________________________
Laboratory address (physical location-not P.O. Box)
____________________________________________________________________
____________________________________________________________________
Mailing address (if different from above)
____________________________________________________________________
Shipping address for supplies and proficiency tests (if different from above)
____________________________________________________________________
Name of laboratory staff escort for the inspector
____________________________________________________________________
List all personnel currently conducting EIA tests at the laboratory and the date certified for each person.
Name __________________________________ Date Certified
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
Inspector __________________________ Title ________________________________
Inspector’s Telephone # ________________________________________________
Laboratory number __________________________________ (To be assigned by NVSL)
Laboratory Inspection for Equine Infectious Anemia (EIA) Testing

NOTE: Sections I, II, and VI apply to all laboratories. Sections III, IV, and V apply only to laboratories that are approved and performing EIA tests.

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**EIA Laboratory Inspection**

<table>
<thead>
<tr>
<th>Section</th>
<th>Item</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Laboratory</strong></td>
<td>Separate room or portion of room is used for testing.</td>
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<td></td>
<td>Adequate open bench space (at least 3- to 5- feet) and lighting is available to perform test.</td>
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<td>Sink is available in same area with hot and cold running water.</td>
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<td>Laboratory temperature is maintained at all times between 68° and 77 °F (20° and 25 °C) or an incubator is available to maintain these temperatures for the tests when required.</td>
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<td>Laboratory and laboratory equipment is clean and properly stored.</td>
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<td>Appropriate disposal facilities are available in accordance with local rules and regulations.</td>
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<td></td>
<td>Only laboratory equipment and supplies are within the laboratory. No eating, drinking, applying cosmetics, handling contact lenses, or storage of food is allowed in the lab.</td>
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<tr>
<td><strong>II. Laboratory Supplies and Equipment</strong></td>
<td>The following equipment must be available and functioning properly for the agar gel immunodiffusion (AGID) test:</td>
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<td></td>
<td>High intensity light that can be focused for reading AGID plates</td>
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<td></td>
<td>Blinds on windows or separate room so light can be reduced to read AGID plates</td>
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<td></td>
<td>Medium 7-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</td>
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<td>A balance designed to read with accuracy to plus or minus 0.1 gram</td>
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<td>Method to remove agar plug (a vacuum pump is suggested)</td>
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<td></td>
<td>Equipment to make agar: graduate measures, flasks, and additional appropriate glassware</td>
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<td></td>
<td>Source of heat for agar preparation (microwave, hot plate, autoclave)</td>
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<td></td>
<td>Refrigerator labeled for lab use; no food or drink</td>
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<td>Containers for incubating AGID plates on test (incubators suffice)</td>
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<td>Pipettes or pipette tips for delivery of reagents to wells</td>
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<td></td>
<td>Distilled water and chemicals for buffer (sodium bicarbonate, boric acid, distilled water)</td>
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### Laboratory Inspection for Equine Infectious Anemia (EIA) Testing

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<tr>
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<th>Item</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
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<tr>
<td></td>
<td>Noble agar</td>
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<td>Disposable 60 mm or 100 mm petri dishes</td>
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<td></td>
<td>Materials or equipment to accurately measure pH within 0.2 pH unit</td>
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<td><strong>The following equipment must be available and functioning properly for the enzyme-linked immunosorbent assay (ELISA) test:</strong></td>
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<td>Incubator (if 37 °C incubation is required for the ELISA test used)</td>
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<td>Refrigerator labeled for lab use; no food or drink</td>
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<td>Wash bottles, pipetting devices, and plate holders</td>
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<td></td>
<td>ELISA washer and reader (optional)</td>
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### III. Control of Specimens and Reporting
(Only applicable for approved laboratories)

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<thead>
<tr>
<th>Section</th>
<th>Item</th>
<th>Yes</th>
<th>No</th>
<th>Notes</th>
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<tbody>
<tr>
<td></td>
<td>Laboratory should not be accessible to the general public during testing</td>
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<td></td>
<td>Laboratory assigns a unique accession number to each sample. The accession number is recorded on the official EIA reporting form.</td>
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<td>Specimens that are not appropriately identified should not be tested.</td>
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<td>Specimens are received with proper submission form*(with name of the owner, name and address of the submitting veterinarian, location of animal at the time the test sample was obtained, complete animal identification, and signed by the submitting veterinarian).</td>
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<td>The specimen identity is maintained on the worksheets and on the petri dish and ELISA plates/strips.</td>
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<td>The test results for each sample are recorded on a worksheet which should be made available for review.</td>
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<td>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.</td>
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<td>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. <strong>All EIA tests are official tests.</strong></td>
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<td>Official test results are reported to the State (s) and/or Federal animal health officials within the time specified by these officials.</td>
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<td>Specimens are held refrigerated or frozen for at least 2 weeks after results are reported and preferably for at least 30 days.</td>
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*VS Form 10-11 is the official form accepted in all States. Some States allow State-specific EIA test forms.
# Laboratory Inspection for Equine Infectious Anemia (EIA) Testing

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<th>Yes</th>
<th>No</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IV. EIA</td>
<td>Reagents (Only applicable to approved laboratories)</td>
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<td></td>
<td>Only reagents licensed by USDA APHIS or supplied by NVSL are to be used (check inserts for expiration dates).</td>
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<td>Unused portions must be refrigerated.</td>
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<td>Unused or outdated reagents and the inoculated EIA AGID or ELISA plates must be appropriately discarded according to local rules and regulations.</td>
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<td>V. Test Procedure</td>
<td>The procedure that is outlined in the appropriate test protocol must be followed.</td>
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<td>VI. Building</td>
<td>and Cleanliness The building is in good repair and provides a professional appearance inside and outside.</td>
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<tr>
<td>and Cleanliness</td>
<td>Adequate doors, windows and screens are provided and are in good repair.</td>
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<td>The laboratory is separated from the office, storeroom and unused areas by partitions with doors.</td>
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<td>Adequate lighting is available.</td>
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<td>Restrooms are available, clean and in good repair.</td>
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<td>Laboratories are free of rodents, insects and other pests.</td>
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<td>All refuse is placed in proper containers and removed frequently.</td>
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<td>Clean laboratory clothes (coats) are worn.</td>
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**ADDITIONAL REMARKS**

Laboratory is: Satisfactory Unsatisfactory (circle one)

__________________________________________________________________________
Inspector’s signature __________________________ Date

__________________________________________________________________________
Inspector’s name (printed)

__________________________________________________________________________
Laboratory escort’s signature __________________________ Date

__________________________________________________________________________
Laboratory escort’s name (printed)

- Copy of completed laboratory inspection given to laboratory.
- Copy of original inspection to be maintained at area office.
Agreement to Conduct Equine Infectious Anemia (EIA) Testing

I, _____________________________________________, have read and understand Veterinary Services (VS) Memorandum 555.16. I understand my responsibilities as outlined in the Memorandum and agree to abide by the guidelines therein. The guidelines and requirements include, but are not limited to, the following:

1. The individual(s) responsible for conducting EIA tests must have completed training in the proper techniques. The training will be conducted at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa; however, personnel in a State, Federal, or university laboratory who received previous EIA test training at NVSL may conduct training for other laboratory staff in his or her facility provided that the training is approved by the Area Veterinarian in Charge and the State animal health official. All trained personnel must successfully complete an individual proficiency test prior to conducting an official EIA test.

2. All testing must be conducted in accordance with the official protocol for the test as provided by NVSL or as described in literature accompanying the diagnostic test kits. Only diagnostic test kits that have been approved by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) will be used.

3. Annual laboratory proficiency tests must be completed satisfactorily and in a timely manner for the laboratory to maintain approval.

4. All results of EIA tests conducted must be signed appropriately and reported promptly to the State and/or Federal animal health officials in the State where the laboratory is located and in the State in which the animals were sampled.

5. Laboratory officials must comply with inspections and APHIS VS animal health official requests regarding EIA testing activities.

I understand that the laboratory will lose its approval if personnel trained to conduct EIA tests are no longer available to conduct EIA testing.

Signature of Laboratory Director _______________________________________________________

Laboratory Name _________________________________________________________________

Laboratory e-mail address ____________________________________________________________

Laboratory Address _________________________________________________________________

_________________________________________________________________

Telephone number ___________________________      Date ___________