# Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens

## APHIS/Veterinary Services Approved Brucellosis Laboratories


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# Glossary

**Active surveillance**: First point testing, herd testing, diagnostic testing, movement testing, wildlife testing, etc.

**APHIS-approved laboratory**: Laboratory approved by APHIS to perform official brucellosis testing.

**Bovine**: Cattle and/or bison.

**Ruminant Health Center (RHC) Veterinary Medical Officer (RHVMO)**: Veterinary medical officer points of contact. RHC will notify the laboratory of the appropriate RHVMO point of contact.

**First Point Test (FPT)**: Testing at the first point where livestock may be concentrated, usually a livestock market buying station or similar location, that is part of active surveillance testing. Specimens collected at a first point of concentration will be recorded on VS Form 4-54 Brucellosis Test Record – Market Cattle Testing Program.

**Identification device (ID)**: Any manmade identification device including, but not limited to, back tags, metal brucellosis ear tags, metal "brite" (silver) ear tags, radio frequency identification (RFID) ear tags, owner management tags, or carcass/house tags (if not removed from the carcass).

**Passive surveillance**: Slaughter surveillance.

**Non-negative test result**: A test result that is not negative (i.e., suspect or reactor range test result).

**Screening tests**: The official brucellosis screening test is the buffered acidified plate antigen (BAPA) test. Greater Yellowstone Area (GYA) State laboratories may use the fluorescent polarization assay (FPA) as a screening test because of high seasonal test demand.

**Secondary tests**: The official brucellosis secondary test is the tube format FPA.

**Supplemental tests**: Brucellosis tests, other than official screening and secondary tests, used to provide additional information for epidemiological investigations.

**Veterinary Services (VS) Form 4-10 (National Veterinary Services Laboratories (NVSL) Contact Information Update Form)**: OMB-approved form used to collect contact information for laboratories submitting specimens to NVSL.

**VS Form 4-33 (Designated Brucellosis Test Record)**: OMB-approved multi-part forms or RHC-approved electronic equivalents.

**VS Form 4-33A (Brucellosis Test Record – Continuation Sheet)**: Continuation sheet for VS 4-33.

**VS Form 4-54 (Brucellosis Test Record – Market Cattle Testing Program)**: OMB-approved multi-part forms or RHC-approved electronic equivalents.

**VS 10-4** (**Specimen Submission Form)**: OMB-approved form that must accompany all specimens submitted to NVSL for testing.

**VS Hub**: VS locations in Raleigh, NC (Eastern Hub); Fort Collins, CO (Western Hub); and Riverdale, MD (National Hub).

# Acronyms

**AIC**: Animal Identification Coordinator or personnel designated by the Area Veterinarian in Charge.

**APHIS**: Animal and Plant Health Inspection Service.

**AVIC**: Area Veterinarian in Charge.

**BAPA**: Buffered acidified plate antigen test.

**BMRT**: NVSL Brucella and Mycobacterium Reagents Team.

**CF**: Complement fixation test.

**RHVMO**: Ruminant Health Center Veterinary Medical Officer.

**DE**: Designated Epidemiologist.

**FPA**: Fluorescent polarization assay.

**FPT**: First point testing.

**ID**: Identification device.

**NVSL**: National Veterinary Services Laboratories, Ames, IA.

**RFID**: Radio frequency identification device.

**RHC**: Ruminant Health Center.

**SAHO**: State animal health official (usually the State veterinarian or State veterinarian's designee)

**SOP**: Standard operating procedure.

**UPS**: United Parcel Service.

**USPS**: United States Postal Service.

**VS**: Veterinary Services.

# 1. Purpose

The purpose of this document is to outline the standard operating procedure (SOP) for properly processing, examining, and reporting results for bovine brucellosis **passive** (slaughter) **and active** (e.g., first point testing, herd testing, diagnostic testing, movement testing, wildlife testing, etc.) surveillance serological submissions. **Bovine brucellosis serological surveillance includes cattle, bison, and cervids.** Brucellosis surveillance serological submissions include any surveillance described by the National Bovine Brucellosis Surveillance Plan submitted for official brucellosis testing at APHIS-approved brucellosis laboratories.

## 1.1 Disease

*Brucella abortus*; *Brucella* sp.

## 1.2 Application and Scope

APHIS intends all approved brucellosis laboratories to implement this SOP to increase efficiency and to facilitate processing, examining, and reporting of brucellosis surveillance serological submissions.

# 2. Specimens and Handling

## 2.1 Specimens Required

Bovine brucellosis slaughter surveillance serological specimens for official brucellosis testing consist primarily of specimens from bulls and cows two years of age and older. The age of animals for specimens for active brucellosis surveillance serological submissions is specified in the brucellosis program regulations and standards.

The Federal or State animal identification coordinator (AIC) works with slaughter establishments and facilities and collectors of active bovine brucellosis surveillance specimens to collect good-quality specimens (a minimum of six to eight milliliters of whole blood) in a USDA Food Safety and Inspection Service- and laboratory-approved blood collection vial.

The AIC trains slaughter establishment/facility staff and other collectors of active bovine brucellosis surveillance specimens in appropriate collection procedures. This includes packaging and shipment of bovine specimens in 40-count cell boxes or on rods, as well as applying tube numbers with permanent ink on all (or as per agreement between the laboratory and the submitter) collection vials. Record information from manmade identification devices (IDs) on a completed VS Form 4-54 (Brucellosis Test Record – Market Cattle Testing Program) if collecting specimens at a slaughter facility or at a first point testing (FPT) facility. The ID accompanies the specimen to the testing laboratory unless an alternative method is described in a signed fee-basis agreement addendum with the slaughter facility for brucellosis specimen collection. (Identification Required)

If the specimens are from active surveillance other than FPT, record their information on a VS Form 4-33 (Brucellosis Test Record) and, if needed, a VS Form 4-33A

(Brucellosis Test Record – Continuation Sheet).

Ship specimens collected at slaughter facilities designated for national surveillance to the APHIS-approved brucellosis laboratory designated in the fee-basis agreement for those facilities.

Ship specimens collected in a Greater Yellowstone Area (GYA) State to the designated APHIS-approved brucellosis laboratory within that State.

Ship all other active surveillance specimens for brucellosis testing (such as epidemiological investigation specimens or international shipment specimens) to an APHIS-approved brucellosis laboratory. If an APHIS-approved brucellosis laboratory is not located within the specimen's State of origin, submit the specimens to an out-of-State APHIS-approved brucellosis laboratory or to NVSL for brucellosis testing. **NVSL will charge a user fee for brucellosis testing not required by the brucellosis program (e.g., specimen testing for international or domestic animal movement, brucellosis-free herd certification, etc.).**

**If the initial test laboratory does not have the capability to perform secondary tests, send all brucellosis non-negative screening test specimens to NVSL for secondary testing. Send all non-negative secondary test specimens to NVSL for confirmatory testing.**

VS Forms 4-10 (NVSL Customer Contact Information Update Form) and 10-4 (Specimen Submission Form) accompany specimens referred to NVSL or shipped directly from the field to NVSL. The VS Form 10-4 can be submitted electronically via the National Centers for Animal Health Portal. Please contact NVSL for more information.

Ship specimens to the APHIS-approved brucellosis laboratory at the end of each day or at least every other day. Samples shipped to NVSL do not require prior arrangement and will be received and stored appropriately over weekends and holidays; however, **do not ship specimens on Fridays, weekends, or holidays unless prior arrangements are made directly with the receiving laboratory**.

## 2.2 Retention of Specimens

Unless forwarded to NVSL, blood serum specimens remain at the laboratory until the laboratory has performed all examinations, as necessary or requested, in response to non-negative screening examinations.

If the **entire** collection submitted from a slaughter facility for a kill/collection date or the **entire** collection for an active brucellosis surveillance collection location/date has negative screening test results, the laboratory disposes of the specimen collection using its standard laboratory procedures.

If a specimen has a screening test positive result, the laboratory retains the nine specimens tested before and the nine specimens tested after the positive specimen until the laboratory supervisor, the designated epidemiologist (DE), and State animal health official (SAHO), or their designees, of the sampled animal's State of origin, and a Ruminant Health Center Veterinary Medical Officer (RHVMO) collaboratively determine

that the specimens may be properly discarded using laboratory procedures.

**Any specimen yielding a non-negative test interpretation on a brucellosis secondary test remains at the laboratory and is preserved within the laboratory's archives unless it is sent to NVSL. Serum from non-negative specimens, as determined by the secondary examination, are stored in a laboratory freezer (-20 degrees Celsius) and archived for no less than one year. NVSL policy is to retain the samples it receives for 40 days.**

## 2.3 Identification Required

Remove all physical identification devices from the animal including, but not limited to, manmade IDs (e.g., back tags, metal brucellosis ear tags, metal "brite" (silver) ear tags, radio frequency identification (RFID) ear tags or devices, owner management tags, carcass/house tags, sale tags, etc.); then record and correlate the IDs with the corresponding blood specimen, if the specimen is collected at slaughter. Place all the animal's IDs into an individual three-hole plastic ID bag correlated to the specimen tube number. **If the carcass tag is not removed from the carcass, record the information so that it can be correlated to the blood specimen**.

The Area Veterinarian in Charge (AVIC) may allow the use of any alternate method for handling, recording, and retaining the IDs (e.g., recording by whom, how, and for how long the IDs must be handled and retained) if the alternate method provides a ready means of identifying and correlating the blood specimen to a specific carcass with its corresponding ID. The alternative method is described in a signed fee-basis agreement addendum with the slaughter facility for brucellosis specimen collection. The AIC assures compliance with the provisions described in the alternative method.

The fee-basis agreement addendum describes when the IDs from animals with negative test results may be discarded, who will notify the slaughter facility regarding when the ID collection may be discarded, and the frequency of the ID disposal notifications. **The IDs for negative specimens are retained at least until all necessary test results are reported to the DE of the specimen's State of origin.**

If the specimens are active brucellosis surveillance serological submissions, record **all** IDs on the appropriate VS form. If the collection is from FPT, use VS Form 4-54; for other active brucellosis surveillance serological submissions, use VS Form 4-33 and, if needed, VS Form 4-33A.

## 2.4 Retention of Identification Collected with and Accompanying Slaughter Blood Specimens

IDs collected and correlated with the blood specimens remain at the laboratory or slaughter facility, per the fee-basis agreement between the AVIC and the slaughter facility, until all secondary and supplemental testing necessary or requested has been conducted on the specimen.

If the **entire** collection submitted from a slaughter facility for a kill/collection date has negative screening test results, the IDs accompanying the blood specimens are properly disposed of using laboratory procedures after test results are reported to the DE of the specimens' State(s) of origin.

If the **entire** collection submitted from a slaughter facility for a kill/collection date has negative screening test results and the IDs are retained at the slaughter facility, the ID collection is properly disposed of using procedures described in the signed fee-basis agreement with the slaughter facility. The fee-basis agreement addendum with the slaughter surveillance facility describes when IDs may be discarded. The addendum includes who notifies the facility and the frequency of the ID disposal notifications.

### 2.5 Retrieval, Recording, and Retention of Animal Identification for Brucellosis Test Non-Negative Specimens

Retrieve, record, and retain any and all IDs of any specimen yielding a non-negative screening test result, regardless of any additional secondary or supplemental test results, along with the IDs of the nine animals tested before and the nine animals tested after the non-negative animal. (Retrieval of ID for Non-Negative Specimens, Recording ID for Non-Negative Specimens on a New VS Form 4-54 or VS Form 4-33, and Retention of Records.)

If the ID did not accompany the blood specimen to the laboratory, the AIC of the specimen's State of origin retrieves the ID of the non-negative animal and the IDs of the nine animals tested before and the nine animals tested after the non-negative animal and sends those IDs to the DE of the animal's State of origin. The AIC will record the identification and send a copy to the laboratory to be kept with the specimen records housed in the laboratory (Retrieval of ID for Non-Negative Specimens, Retention of Records). If the ID does not accompany the blood specimen, the AIC ensures that the ID is retained and can be correlated to specimens as described in the fee-basis agreement addendum.

IDs from screening test non-negative specimens are retained until the DE, in collaboration with a RHVMO, determines that retention is no longer necessary. This applies to IDs collected at approved national surveillance slaughter facilities.

If any active surveillance test specimen yields a non-negative secondary test result, the AIC of the specimen's State of origin, or personnel designated by the AVIC of the specimen's State of origin, verifies any and all IDs correlated with that non-negative specimen and the IDs of the nine animals tested before and the nine animals tested after the non-negative animal. The AIC or designee provides a record of the verification to the laboratory to be kept with the specimen records housed in the laboratory (Retention of Records).

## 3. Examinations

### 3.1 Assessment

All bovine brucellosis surveillance submissions received at the APHIS-approved brucellosis laboratory will have their overall general quality assessed (Assessment) and recorded after specimen processing (Processing).

Bovine brucellosis surveillance specimen submissions that display an untestable quality (such as hemolyzed or degraded specimens, or specimens with an insufficient volume of serum) shall be documented (Reporting of Test Specimen Quality) depicting the

specimens' condition. If the laboratory determines that a specimen is untestable due to extremely poor quality, the laboratory disposes of the specimen using appropriate laboratory procedures (Specimens of Extremely Poor Quality).

Submission of high-quality brucellosis test specimens is vital to obtaining accurate test results and statistically valid data for brucellosis surveillance analysis and other brucellosis testing.

## 3.2 Examination

After assessment, laboratory staff examines the specimens to detect antibodies to *Brucella abortus* using serological examinations in series format; however, serological testing cannot distinguish between *B. abortus*, *B. suis*, and *B. melitensis*. Specimens shall be prepared for examination as described in Preparation.

**Series Testing Format**
Initial serological screening examination is conducted using the Buffered Acidified Plate Antigen (BAPA) Examination, followed by the Fluorescent Polarization Assay (FPA-Tube) as the secondary examination if the BAPA test result is positive. If the FPA-tube test yields a non-negative test interpretation (suspect or positive), test the specimen using the Complement Fixation Test as a supplementary examination. If the FPA and/or CF test is not available at the initial laboratory or if the initial laboratory is not approved to perform the FPA-tube or CF test, NVSL performs the FPA-tube and/or CF tests as necessary when it receives the specimen. The DE of the specimen's State of origin provides a courier (FedEx or UPS) account number or USPS postal frank to cover charges for directing such requests to NVSL.

Record all screening and secondary test results on the appropriate approved VS Form (Codes/Tests Results for Recording on Forms).

The laboratory shall report all negative screening examination test results to the DE of the specimen's State of origin (Reporting Negative Screening Test Results). Report all non-negative secondary examination test results to the DE in the specimen's State of origin (Reporting of Non-Negative Specimens/Collections).

Secondary examination non-negative specimens shall remain archived at the initial testing laboratory for no less than one year (Retention of Specimens).

The laboratory supervisor, an RHVMO, the DE of the specimen's State of origin, or the DE of the animal's State of origin may request an additional secondary test or tests be performed if the specimen is of poor quality, if a non-negative secondary examination result is obtained, or for epidemiological investigation reasons. If the examination is available at the initial APHIS-approved brucellosis testing laboratory, request additional testing from the supervisor of that laboratory. If additional secondary and/or supplemental examinations are necessary or requested and the requested additional examination is not available at the original testing laboratory, submit an official request to the laboratory supervisor and the specimens will be sent from the laboratory to NVSL per the request of the laboratory's supervisor, the DE, or the RHVMO. The requestor provides a FedEx or UPS account number or USPS postal frank to the laboratory to cover charges for directing such requests to NVSL.

In addition to a new VS 4-54 or VS 4-33 (Reporting of Non-Negative Specimens/Collections), the submitting laboratory must enter the specimen's State of origin on the VS Form 10-4 (NVSL Specimen Submission) and the animal's State of origin, if known, to ensure appropriate and timely report distribution.

If supplemental examinations are performed, the testing laboratory reports the examination results to the appropriate brucellosis RHVMO and the DE of the animal's State of origin.

NVSL reports test results through normal APHIS channels to the submitter and to the email groups of the submitter, State of specimen origin, State of animal origin, and State of animal owner (if this information is on the submission form). NVSL routinely tests and reports on specimens it receives within four business days after sample receipt under the posted schedule on the NVSL testing catalog. NVSL may report test results sooner depending on the tests required and the day of the week it received the specimen. Poor quality specimens, incomplete or unclear sample information, holidays, or invalid tests may occasionally cause a delay. To request urgent testing, contact the NVSL Serology Section at (515) 337-7563 or via email at VS.DB.NVSL.DBRL.Sero.Mgmt@usda.gov.

# 4. Calibration Procedures

All instruments used to support examinations conducted in APHIS-approved laboratories undergo routine maintenance and instrument qualification procedures as recommended by the instrument manufacturer's Operator's Manual. NVSL services APHIS-owned FPA test equipment biennially. Other equipment shall be calibrated, at the laboratory's expense, biennially by a source that follows the International Organization for Standardization (ISO) accredited requirements. The source must keep Certificates of Acceptance and Conformity on file. Instruments shall also be calibrated by personnel within the laboratory to manufacturer's specifications if performance of the instrument has been affected.

# 5. Materials

## 5.1 Reagents

The NVSL Brucella and Mycobacterium Reagents Team (BMRT), Ames, IA, provides reagents for the BAPA, as well as the reagents for secondary examinations to the APHIS-approved national brucellosis surveillance laboratories. Other APHIS-approved laboratories may purchase test reagents from the NVSL BMRT.

### 5.1.1 Buffered Acidified Plate Antigen (BAPA) Examination

Antigen: *Brucella abortus* buffered acidified plate antigen (NVSL Reagent Code 2).

Positive Control Sera: *Brucella abortus* complement fixation positive control serum (NVSL reagent 12-H or 12-L 2.0 ml and *Brucella abortus* positive control serum NVSL reagent code 12- series 1.0 ml control sera).
Negative Control Sera: *Brucella abortus* complement fixation negative control serum (NVSL reagent 12-N 2.0 ml and Brucella abortus negative control serum NVSL reagent code 12– series 1.0 ml control sera).

### 5.1.2 Fluorescence Polarization Assay (FPA)

*Brucella abortus* antibody test kit fluorescence polarization assay may be purchased from:

Ellie, LLC
10437 Innovation Drive
Wauwatosa, WI 53226
Phone/Fax: (800) 556-6953
Email: hello@ellielab.com

### 5.1.3 Complement Fixation Test

Antigen: *Brucella abortus* complement fixation (NVSL reagent 3).
Control Sera: *Brucella abortus* complement fixation negative control serum (NVSL reagent 12-N 2.0ml), *Brucella abortus* complement fixation low positive control serum (NVSL reagent 12-L 2.0ml), *Brucella abortus* complement fixation high positive control serum (NVSL reagent 12-H 2.0ml), or *Brucella abortus* 12–series control sera (NVSL reagent 12- 1.0 ml).
Preserved sheep cells
Hemolysin (rabbit antiserum against sheep erythrocytes) Complement (guinea pig)
Cyanmethemoglobin reagents and standards
Sodium barbital and barbital or commercial veronal buffered diluent (VBD)

## 5.2 Supplies

NVSL will provide, upon request, a list of supplies necessary for performing approved brucellosis tests.

## 5.3 Equipment

NVSL will provide, upon request, a list of equipment necessary for performing approved brucellosis tests.

# 6. Reporting

## 6.1 Screening Secondary and Supplemental Examination Results

### 6.1.1 Recording Results

Record results on VS Form 4-54 or VS Form 4-33 in the column for the specific examination performed using the codes as per 6.1.2, Codes/Test Results for Recording on Forms.

### 6.1.2 Codes/Tests Results for Recording on Forms

| BAPA Examination | |
|---|---|
| **Code/Test Result Recorded on Forms** | **Result** |
| N | Negative |
| P | Positive |

| FPA Examination | |
|---|---|
| **Delta mP Result (Recorded on Forms)** | **Test Interpretation (Do Not Record on Forms Except for Animals to Be Exported Internationally)** |
| Delta mP 1 ≤10 | Negative |
| 10 < Delta mP ≤20 | Suspect |
| 10 < Delta mP ≤40 | Suspect<br>Provided the CF test results are negative |
| 20 < Delta mP | Positive<br>Provided the CF test results are positive, anti-complementary, or not available |
| 40 < Delta mP | Positive<br>Regardless of CF test results |

**Note**: Record the FPA test results as the delta mP reading (reading minus the negative control reading) of the specimen. For example, if a specimen had a reading of 125 and the negative control reading was 85, then the delta mP result will be recorded on the VS Form 4-54 or VS Form 4-33 as "40."

| Complement Fixation Test (CF) | |
|---|---|
| **Test Result (Recorded on Forms)** | **Test Interpretation (Do Not Record on Forms)** |
| 1+ 1:10 or lower | Negative |
| 2+ 1:10 through 1+ 1:20 | Suspect |
| 2+ 1:20 or higher | Reactor |

**Note 1:** CF dilution results (e.g., 1+ 1:10, 2+ 1:20, etc.) shall be recorded on the VS Form 4-54 or VS Form 4-33.
**Note 2: Anticomplementary test results should be reported as AC. A titer should not be reported if CF test is AC.** The following note may be attached to the specimen test form (VS Form 4-54 or VS Form 4-33).
**Note 3:** AC = anticomplementary result = indeterminate result. The factors causing an anticomplementary result are not known but the test may be successful with a new sample from the animal. Specimens should be clear serum without evidence of hemolysis for complement fixation or other serological tests. (USDA APHIS NVSL SOP on *Brucella abortus* CF (SOP-SERO-0015)).

## 6.2 Reporting of Test Specimen Quality

Submission of high-quality brucellosis test specimens is vital to obtaining accurate test results and statistically valid data for brucellosis surveillance analysis and other brucellosis testing.

### 6.2.1 Specimen Quality Abbreviations

Record specimen quality via the following abbreviations and note the specific reasons for specimen invalidity or lack of fitness.

| Specimen Quality Abbreviations | | |
|---|---|---|
| **Abbreviation** | **Term** | **Description** |
| **C** | Contaminated | Specimen contains cloudy, "creamy," translucent to opaque serum. |
| **QNS** | Quality Not Sufficient | Not enough serum present to perform examinations; includes specimens that may have leaked if caps not properly sealed, etc. |
| **M** | Missing | Specimen tube number recorded on the test form, but the specimen tube was not present when the specimen shipment was received at the laboratory. |
| **NID** | No Identification | No identification was included with specimen. |
| **H** | Hemolysis | Specimen displays extreme hemolysis: Thick, viscous liquid with no testable serum. |
| **INV** | Invalid | Specimen that may appear to be of good quality yet produces an invalid result. |
| **GEL** | Gel | Specimen displays gelatinous qualities: Solid, "gelled" specimen with little to no testable serum. |
| **B** | Broken | Broken collection tube indicating a shipping problem. |
| **LA** | Laboratory Accident | Laboratory accident: Mishap in the laboratory prevented specimen from being examined. |

### 6.2.2 Brucellosis Test Specimen Quality Submission Reports

Each APHIS-approved national brucellosis surveillance laboratory shall provide, by the 10th day of each month, to the designated RHVMO**,** a monthly Brucellosis Test Specimen Quality Submission Report regarding brucellosis surveillance specimens submitted to the laboratory so the RHC can monitor and audit the quality of test specimens. The total number per month of **missing specimens**, those that are **unsuitable for testing**, and those that **do not provide valid test results** should make up less than **five percent** of the total number of specimens collected and submitted by **any one facility or person**. Fee-basis agreement submitters may not be paid for specimens of untestable quality if the untestable quality is due to unsatisfactory collection, handling, or submission.

The designated RHVMO monitors and audits the quality of brucellosis test specimens submitted to APHIS-approved national brucellosis surveillance test laboratories to assure submission of high-quality, testable specimens. The RHVMO notifies the designated VS District personnel for the specimens' State if the total number of missing specimens, those that are unsuitable for testing, and those that do not provide valid test results exceeds five percent and are thus not to be authorized for payment under the fee-basis agreement.

If a significant number of specimens (**five percent or more of the total number from any one facility or person**) submitted for brucellosis testing are unsuitable for testing, the designated RHVMO forwards a copy of the report to the designated District personnel for the specimens' State of origin. At the direction of the AVIC, the State's DE or AIC investigates the source (i.e., the slaughter facility, FPT facility, or the collecting veterinarian) of the unsuitable specimens to determine the reason(s) for their unsuitable quality and works with the submitting person or facility to improve the quality of future specimens.

If the APHIS-approved brucellosis laboratory is not a national brucellosis surveillance laboratory, the DE reviews, in a timely manner, the brucellosis test forms from APHIS-approved brucellosis laboratories in his/her district to assure quality specimens are submitted for brucellosis testing. The reviewing DE should immediately report collections containing untestable specimens to the DE of the State where the APHIS-approved laboratory that received the specimens is located.

## 6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results

### 6.3.1 Reporting Negative Screening Test Results

The laboratory shall, **within 5 working days** of finishing the brucellosis test(s), **report all negative** brucellosis screening test results to the DE of the specimen-submitting State.

Record negative test interpretation results for bovine brucellosis surveillance specimens on the original VS Form 4-54 or VS Form 4-33.

Laboratories can electronically submit the VS forms or mail them to the DE of the specimens' State of origin.

### 6.3.2 Reporting of Non-Negative Specimens/Collections

**Report non-negative brucellosis test results via telephone and *either* electronic internet communication (e-mail) *or* fax.**

The laboratory shall, **within one working day or sooner** of completing the brucellosis FPA secondary examinations, report all **non-negative** FPA test results to the DE, the AVIC of the specimens' State of origin, and to the designated RHVMOs. The laboratory supervisor shall identify

such specimens with a "withheld" (W) test interpretation notation.

If the specimen's State of origin is not the same as the animal's State of origin, the DE of the specimen's State of origin will, **within one working day or sooner** of receiving the test results, report the findings to the DE of the animal's State of origin.

The DE or AVIC in the specimen's State of origin, DE or AVIC in the animal's State of origin, or an RHVMO may request additional brucellosis testing of the non-negative brucellosis test specimens before classifying the animal's brucellosis status.

Report the brucellosis screening and, if performed, secondary examination test results for the nine specimens tested before and the nine specimens tested following the non-negative specimen. **Retrieve and record IDs (Identification Required) for screening examination and secondary examination of non-negative specimens on a new, separate VS Form 4-54 or VS Form 4-33. Record all test results on the new VS Form 4-54 or VS Form 4-33 in the appropriate column for each examination. A copy of the new VS Form 4-54 or VS Form 4-33 and VS Form 10-4 shall accompany the original forms and be sent (initially electronically, followed by a mailed hard copy), to the DE of the specimen's State of origin**. See Retention of Specimens and Recording of ID for Non-Negative Specimens for additional information regarding retention of specimens and IDs.

**If a reference laboratory (e.g., NVSL) confirms non-negative brucellosis test results**, the reference laboratory shall report, within one working day or sooner of completing the brucellosis test(s), all brucellosis test(s) results to the DE and the AVIC of the specimen's State of origin and to a brucellosis RHVMO.

# 7. Procedures

## 7.1 Receiving Submissions via the United States Postal Service (USPS)

Bovine serological specimens submitted to the APHIS-approved laboratory arrive either by mail courier or are directed to the laboratory's post office box. Specimens should arrive in insulated coolers, typically insulated hard plastic coolers (such as a Coleman brand cooler). A designated technician retrieves specimens directed to the post office box at the beginning of each work shift. Upon returning to the laboratory, the technician counts and records the total number of coolers received.

## 7.2 Receiving Submissions via Mail Courier (FedEx, UPS, etc.)

Bovine serological specimens arriving at the APHIS-approved laboratory via mail or other courier will be received by the designated technician at the laboratory shipping/receiving area. Specimens should arrive in insulated coolers as noted in 7.1. The technician counts and records the total number of coolers delivered, verifies the number with the courier, and signs for their

receipt.

## 7.3 Grouping

A technician sorts and groups each vessel containing bovine serological specimens by collection location or plant/slaughtering facility as well as by kill date if specimens are from a plant/slaughter facility.

## 7.4 Processing

Laboratory personnel retrieve paperwork from each collection and sort "bundles" ("bundle" describes ID bags containing specimens and identification bound by rod, string, etc.; each collection may contain several "bundles" in multiple coolers) in ascending order as indicated by the VS Form 4-54 or VS Form 4-33 that accompanies each collection. Additional forms included with collections (such as kill sheets or lot logs) accompany the VS Form 4-54 or VS Form 4-33 as well as the specimens throughout the remainder of the process.

Bovine whole blood specimens may arrive in 40 cell-count cardboard boxes in ascending order. If specimens do not arrive in 40 cell-count cardboard boxes, the specimens should be removed from the 3-hole plastic ID bags and placed in sequential order into 40-count boxes or into blood tube racks from left to right, front to back.

Place the boxes in sequential order on carts or tables in the laboratory processing area. Number each tube (unless an alternative agreement between the laboratory and the submitter is described in the fee-basis agreement addendum) in sequential order. Centrifuge and examine specimens for specimen quality. After centrifuging specimens, place them in order on a cart and in a refrigerator until they are racked for testing.

Place "bundles" of ID bags containing identification in clear plastic bags with collection location or slaughter facility/plant name, kill/collection date, received date, and total number of specimens clearly labeled on the outside of the bag. Retain the bundles in the laboratory until the specimens complete testing.

## 7.5 Assessment

Bovine slaughter surveillance submissions and active (e.g., FPT, farm/ranch testing, epidemiological testing, etc.) surveillance submissions will have their overall general quality assessed and recorded (assessment recorded on a worksheet that accompanies VS Form 4-54 or VS Form 4-33 after the laboratory processes the specimens).

### 7.5.1 Specimens of Overall Good Quality

Specimens of good quality (collections containing an ample volume of serum per specimen and displaying only mild to moderate signs of hemolysis, gel, or other degradation) will undergo the BAPA screening examination.

### 7.5.2 Specimens of Overall Poor Quality

If a collection displays overall poor quality (such as collections containing

a limited volume of serum, or displaying multiple cases of hemolysis, gel, or other degradation), the entire collection will, at the laboratory supervisor's discretion, be tested using the BAPA as the screening assay test (an alternative screening examination) to reduce any complications associated with poor specimen quality. The laboratory will document the condition of the specimens (Reporting of Test Specimen Quality) and report the quality as described in Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results. The documentation should also contain photographs, if possible, and a brief, written description of the specimen(s) indicating the quality and specifics that may have caused the poor conditions (e.g., no ice packs, delayed shipping, etc.). The laboratory supervisor forwards the documentation to the DE of the specimen's State of origin.

### 7.5.3 Specimens of Extremely Poor Quality

In the event of extremely poor specimen quality (specimens displaying extreme hemolysis, extreme specimen degradation, contamination, containing little to no testable serum, and/or other degradation) resulting in specimens yielding invalid BAPA test results, the specimens will be tested using the FPA secondary examination as the screening test. The laboratory will document the condition of the specimens (Reporting of Test Specimen Quality) and report the quality as described in Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results. The documentation should also contain photographs, if possible, and a brief, written description of the specimen(s) indicating the quality and specifics that may have caused the poor conditions (e.g., no ice packs, delayed shipping, etc.). The laboratory supervisor forwards the documentation to the DE of the specimen's State of origin and determines if the specimens are untestable and should be discarded.

**The laboratory supervisor must immediately report collections not tested and disposed of due to extremely poor specimen quality to the DE and the AVIC in the specimen's State of origin.**

## 7.6 Preparation

### 7.6.1 Preparing the Specimen

Before examinations begin, prepare specimens to ensure the quality of the serum and to allow for accuracy and validity when producing results. Allow refrigerated specimens to warm to room temperature before examination.

### 7.6.2 Centrifuging the Specimen

After specimens have warmed to room temperature, centrifuge each collection or plant/slaughter facility kill date collection to separate serum from any clotting factors remaining in the specimen.

### 7.6.3 Grouping of Specimens

Group specimens by collection location or by plant/slaughtering facility and the kill/collection date. Record corresponding collection information

on a worksheet produced in the laboratory. The information recorded on the worksheet at this step of preparation includes collection location or plant/slaughtering facility name, establishment number, kill/collection date, received date, and collection origin State. All forms accompany the collection from this step of preparation forward.

#### 7.6.4 Disposal

Retain or discard all caps/stoppers removed from each collection tube in preparation for examinations per individual laboratory procedure.

## 7.7 Screening Examination

#### 7.7.1 Screening Secondary and Supplemental Examination Results

Collections assessed and determined to be of adequate quality (i.e., adequate volume of serum per specimen displaying minimal to moderate signs of hemolysis, etc.) shall undergo the BAPA test as outlined by NVSL protocol SOP-SERO-001 as the screening examination. Record BAPA test results per Screening Secondary and Supplemental Examination Results.

Any specimen demonstrating an invalid BAPA reading (uncharacteristic agglutination patterns or unable to distinguish sample artifact from agglutination) will be tested using the FPA-tube test. If the specimen produces an invalid FPA-tube result, record and report information pertaining to the quality of the specimen (Reporting of Test Specimen Quality, Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results).

#### 7.7.2 Positive Reactions

Specimens that produced positive reactions after undergoing a BAPA screening examination shall undergo an FPA-tube secondary examination.

#### 7.7.3 Invalid Results

Specimens that produced invalid results for the BAPA test shall undergo an FPA-tube secondary examination to attempt to obtain at least one result for a screening examination, unless otherwise specified by the laboratory supervisor in collaboration with the DE of the specimen's State of origin.

#### 7.7.4 Invalid Specimen

Specimens determined to be invalid after having undergone a BAPA examination will be noted as such on VS Form 4-54 or VS Form 4-33 using Reporting of Test Specimen Quality.

#### 7.7.5 Use of FPA-Plate for High-Volume Screening Test in States with Brucellosis Endemic in Wildlife

For those State laboratories that have chosen to use the FPA-plate as their screening test because of high seasonal testing demands, the VS

Ruminant Health Center provides a testing algorithm through **VS.SP.Cattle.Health.Center@usda.gov.**

## 7.8 Secondary Examination

Any specimen yielding a non-negative test interpretation on a secondary examination shall remain at the originating laboratory and be preserved within the laboratory's archives. Store serum from non-negative specimens as determined by the secondary examination in a laboratory freezer (-20˚ Celsius) and archive it for no less than one year.

### 7.8.1 Fluorescent Polarization Assay (FPA-Tube)

All specimens that yield a positive BAPA test result shall be tested using the FPA test-tube as outlined by SOP-SERO-0031 (tube test). Record and report results for all specimens that have undergone an FPA-tube examination per Screening Secondary and Supplemental Examination Results on the VS Form 4-54, VS Form 4-33 (Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results).

### 7.8.2 Non-Negative FPA Specimens

All specimens that yield a non-negative test interpretation after having undergone an FPA-tube test shall be retrieved and archived in the laboratory freezer at -20˚ Celsius.

If a specimen yields an FPA-tube non-negative test interpretation, the nine specimens tested before and the nine specimens tested after the non-negative specimen, along with the specimens' animal IDs, shall be retained by the laboratory until the laboratory supervisor, in collaboration with the DE of the specimen's State of origin and a brucellosis RHVMO determine the specimens may be properly disposed of using laboratory procedures.

**If a specimen yields a non-negative FPA-tube test interpretation, forward the specimen to NVSL to confirm test interpretations.** The DE of the specimen's State of origin provides a FedEx or UPS account number to cover charges for directing such requests to NVSL.

Record all non-negative secondary examination specimens per Recording ID for Non-Negative Specimens on a New VS Form 4-54 or VS Form 4-33.

## 7.9 Requests for Additional Secondary and Supplemental Examinations

The DE of the animal's State of origin may request additional secondary tests and supplemental examinations (such as the complement-fixation test (CF)) in consultation with an RHVMO. The DE of the animal's State of origin provides a FedEx or UPS account number to cover charges for directing such requests to NVSL if the tests are not available at the initial laboratory.

## 7.10 Retrieval of ID for Non-Negative Specimens

Bovine brucellosis surveillance serological specimens and specimens collected from deceased animals for all testing purposes that have yielded non-negative test results on a screening or secondary examination shall have **all** physical identification including, but not limited to, any manmade identification devices, retrieved from the submitted collection for that specimen. If possible, create and file a photograph or scanned color image of any physical identification that accompanied the specimen with copies of all records within the laboratory. Record or attach all physical identification of any non-negative specimens to the original VS Form 4-54 or VS Form 4-33 and direct from the DE of the specimen's State of origin to the DE for the animal's State of origin. **Do not clean any IDs**, as blood, hair, or tissues present on the ID may be used for DNA testing to correlate the ID to the blood specimen. The ID will be retained until the DE of the animal's State of origin determines, in consultation with an RHVMO, that retention of the ID is no longer necessary.

**In addition**, all IDs that accompanied the nine specimens tested before the non-negative specimen and the nine specimens tested following the non-negative specimen shall be retrieved by the AIC and retained until the DE of the animal's State of origin determines, in consultation with an RHVMO, that retention of the ID is no longer necessary.

If the ID did not accompany the blood specimen to the laboratory, the AIC of the specimen's State of origin retrieves the IDs and sends them to the DE of the animal's State of origin. The animal's State of origin retains the IDs until the DE of the animal's State of origin determines, in consultation with an RHVMO, that retention of the ID is no longer necessary.

## 7.11 Recording ID for Non-Negative Specimens on a New VS Form 4-54 or VS Form 4-33

### 7.11.1 Recording of ID for Non-Negative Specimens

Retrieve and record all ID (Identification Required) from screening examination non-negative specimens on a new, separate VS Form 4-54 or VS Form 4-33. Record all test results on the new VS Form 4-54 or VS Form 4-33 in the appropriate column for each examination.

A copy of the new VS Form 4-54 or VS Form 4-33 and VS Form 10-4 shall accompany the original forms and be sent (initially electronically followed by hard copy by mail) to the DE of the specimen's State of origin. If the IDs did not accompany the specimens to the laboratory, as per the collection fee-basis agreement, the AIC retrieves and retains the IDs until the DE, in collaboration with an RHVMO, determines that retention of the IDs is no longer necessary. If the screening test non-negative specimen is referred to NVSL, a copy of the new VS Form 4-54 or VS Form 4-33 and VS Form 10-4 accompanies the specimen to NVSL.

### 7.11.2 Recording of ID for Specimens Tested Prior to and Following Non-Negative Specimens

Retrieve and record all IDs from the nine specimens tested before and the nine specimens tested following the non-negative specimen in that particular collection, along with all examination results, on the new VS Form 4-54 or VS Form 4-33. The retrieved ID from these specimens shall be retained at the laboratory or directed to the DE or AIC for the specimen's State of origin. If the IDs did not accompany the specimens to the laboratory, the AIC retrieves and retains the IDs. The IDs will be retained until the DE, in collaboration with an RHVMO, determines that retention of the IDs is no longer necessary.

## 7.12 Retention of Records

A copy of all original forms shall be retained or reproduced and stored in the laboratory. This includes the original VS Form 4-54, original VS Form 4-33, VS-Form 10-4, and the new VS Form 4-54; photographs and scanned images of all IDs correlated with non-negative specimens; any kill/log/lot sheets; and laboratory work sheets. Archive all digital and physical records related to non-negative specimens in the originating laboratory or a location designated by the AVIC for three years.