

SAMPLE COLLECTION, PROCESSING,  
SHIPPING & RECEIVING of RFFIT SAMPLES

DoD FOOD ANALYSIS AND DIAGNOSTIC LABORATORY

STANDARD OPERATING PROCEDURE

SOP D-076 - SAMPLE COLLECTION, PROCESSING, SHIPPING  
and RECEIVING of RFFIT SAMPLES

Date Prepared: 21 January 2021  
Date of Original: 21 January 2021  
Schedule for Review: Biennial  
Author: MAJ Melissa Kottke and Edwin Cooper  
Distribution: Molecular Diagnostic Section

APPROVAL:

Diagnostics Section Supervisor:

*Donald A. Beasley*

DONALD A. BEASLEY  
CPT, MS  
20 January 2021

Clinical Laboratory Director:

*Edward L. Mazuchowski*

EDWARD L. MAZUCHOWSKI, MD, PhD  
LT COL, USAF, MC  
21 January 2021

QA Section Chief:

*Marisol S. Castaneto*

MARISOL S. CASTANETO, PhD  
LTC, MS  
21 January 2021

Revision History:

Edition Date	Revision (Section & Description)	Reviewer Initials
21 Jan 2021	New SOP Created	EC/DAB/MS

SAMPLE COLLECTION, PROCESSING, SHIPPING & RECEIVING of RFFIT  
SAMPLES**I. PURPOSE**

The purpose of this Standard Operating Procedure (SOP) is to provide guidance to supplement local SOPs, state and federal requirements for collection, processing, shipping and receiving of patient serum for the Rapid Fluorescent Focus Inhibition Test (RFFIT), a rabies virus neutralization assay.

**II. PRINCIPLE**

To establish uniform specimen processing, shipping, and receiving procedures for the Department of Defense (DOD) Food Analysis and Diagnostic Laboratory (FADL) and to establish a common reference point for these procedures.

**III. SAFETY PRECAUTIONS**

**A.** Universal precaution shall be practiced with face shields, eye protection, gloves and laboratory coats worn during this procedure IAW Occupational Safety and Health Administration (OSHA) register, April 2012, 29 CFR Part 1910.1030.

**B.** Title 29, CFR 1910.1030(d)(4)(ii)(A), cleaning of contaminated work surfaces after completion of procedures is required to ensure that employees are **not** unwittingly exposed to blood, blood products, or other infectious materials remaining on a surface from previous procedures.

- 1.** This paragraph requires contaminated work surfaces to be cleaned with an "appropriate disinfectant".
  - a.** Appropriate disinfectants include a diluted bleach solution and EPA-registered tuberculocides (List B),
  - b.** Sterilants registered by EPA (List A), products registered against HIV/HBV (List D) or sterilants/ high level disinfectants cleared by the FDA.
  - c.** The lists of the EPA registered products are available from the National Antimicrobial Information Network on it's website at <http://npic@ace.orst.edu/> or at (800) 858-7378. The sterilants and high level disinfectants cleared by the FDA can be

found at

<http://www.fda.gov/cdrh/ode/germlab.html>.

- d. Any of the above products are considered effective when used according to the manufacturer's instructions, provided the surfaces have not become contaminated with agents or volumes of concentrations of agents for which higher level disinfection is recommended.

**NOTE:** The EPA lists contain the primary registrants' products only. The same formulation is frequently repackaged and renamed and distributed by other companies. These renamed products will not appear on the list, but their EPA registration number must appear on the label. Products cleared solely by the FDA will not have an EPA number.

- C. Fresh solutions of household bleach made up daily (every 24 hours) are also considered appropriate for disinfection of environmental surfaces and for decontamination of sites following initial cleanup (i.e. wiping up) of spills of blood or other potentially infectious materials.
  1. Contact time for bleach is generally considered to be the time it takes the product to air dry.
  2. Solutions of bleach should not be stored in glass containers, but in material such as the plastic in which the bleach, the consumer product, is packaged in.
  3. Household bleach (5.25% sodium hypochlorite), a one to ten (1:10) dilution of ordinary household bleach and water is recommended. Combine approximately 1-1/2 cups of bleach to one (1) gallon of water.

#### IV. **SAMPLE SUITABILITY/SAMPLE IDENTIFICATION**

Specimens for the RFFIT assay shall follow local phlebotomy SOPs and collect whole blood into collections tubes (vacutainers) without anticoagulants, RED top tubes. All specimens will be identified through labeling or traceable barcodes that contain patient name, DoD ID or other identifying number, date and time of collection, phlebotomist initials and a unique accession number for

test.

It is recommended that at least 0.5 mL of clear serum are collected for samples.

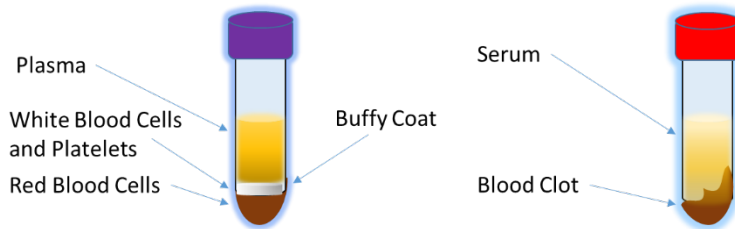
**V. SPECIAL INSTRUCTIONS/CERTIFICATION**

N/A

**VI. EQUIPMENT/MATERIALS and STANDARDS**

- A. See local Phlebotomy Guidance
- B. Blood Collection Tubes (Vacutainers)
- C. Centrifuge

**VII. PROCEDURAL NOTES**



- A. **Whole Blood:** Composed of plasma and formed elements.
  1. Plasma is the fluid portion of blood that contains proteins, ions, nutrients, hormones, antibodies, metabolites, enzymes, and clotting factors.
  2. The formed elements of blood are red blood cells, white blood cells, and platelets.
  3. When collected in tubes without anticoagulants and permitted to clot, the collected liquid product following centrifugation is called serum. The extraction of serum is difficult and time consuming.
- B. **Blood Plasma:** A yellowish colored watery intravascular fluid component of blood in which all blood cells are suspended. Contains serum, water, albumin, proteins, and clotting factors.
- C. **Serum:** Isolated from whole blood that was collected in specialized serum separator tubes or blood

collection tubes without anticoagulants and permitted to clot.

- D. **Hemolysis:** Hemolysis occurs when cellular membranes in red blood cells (RBC) are disrupted and hemoglobin and other intracellular components escape into the serum or plasma.
- E. **Venipuncture:** Puncture of a vein through the skin in order to withdraw blood for analysis.
- F. **RFFIT:** Rapid Fluorescent Focus Inhibition Test
- G. **Red Top Tube:** Contains no anticoagulant or preservative. Use for collection of serum or clotted whole blood.
- H. **Serum Separator Tube (SST):** Contains no anticoagulant or preservative, but has a gel that will separate the serum from the cells upon centrifugation. Use for collection of serum or clotted whole blood.

#### VIII. PROCEDURE

- A. Specimens for the RFFIT assay shall be collected in **red** top blood collection tubes (vacutainers) or in serum separator tubes and should sit upright after the blood is drawn at room temperature for a minimum of 30 minutes to a maximum of 60 minutes to allow the clot to form.
  - 1. All specimens will be centrifuged at the end of clotting time (in accordance with local guidance) to ensure clotted materials and serum have been separated.
  - 2. If the blood is not centrifuged immediately after the clotting time, the tubes should be refrigerated (2°C-8°C) for no longer than four (4) hours.

**WARNING:** Excessive centrifuge speed (over 2000g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated.

For an on-line calculation tool, please refer to:  
<http://www.changbiosciences.com/cell/rcf.html>

- B. Use a pipette to transfer the serum.
1. Pipette serum into labeled vial.
  2. Aliquot volume to the recommended 0.5 to 1 mL.
  3. Close the caps on the vials. This process should be completed within one (1) hour of centrifugation.
  4. Clotted blood will be discharged according to local procedures.

**NOTE:** Be very careful **not** to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.

5. Any grossly hemolyzed specimens must be document, discarded, and redrawn.
- C. Specimens that cannot be analyzed and or shipped immediately can be stored at 2°C-8°C for up to seven (7) days.
- D. **SPECIMEN PROCESSING AND HANDLING:**  
All local policies and procedures for safe processing and handling will be followed while working with patient sera.

- E. **SUBMISSION FORM:**  
A submission form or manifest **must** be filled out with:
1. Patient's Last Name
  2. Patient's First Name
  3. Date of Birth
  4. Gender and
  5. DOD ID number

- F. **SPECIMEN PACKING and SHIPPING:**  
Submitters are responsible for shipping specimens

**SAMPLE COLLECTION, PROCESSING,  
SHIPPING & RECEIVING of RFFIT SAMPLES**1<sup>st</sup> Version

in conformity with all safety and labeling regulations.

1. Package specimens to avoid leakage or breakage. All specimen mailing containers **must** meet current Department of Transportation (DOT) requirements.
  
2. Specimens shall be packed in an appropriately sized insulated container with a sufficient number of ice packs to ensure the internal temperature of the shipping container is maintained at 2°C to 8°C for a 24 hour shipping period.

If the specimens are shipped for overnight or next-day delivery, the refrigerants or gel packs are **not** required.

3. **In order to ensure the satisfactory receipt and proper testing of your specimens it is necessary to:**
  - a. Ensure each tube is labeled as stated in sample identification instructions listed above. The information should match the shipping manifest and/or laboratory request forms.
  
  - b. Layer of packing material, i.e. absorbent pads or bubble wrap>
  
  - c. Completed manifest or submission form is placed in a zip-lock bag to remain dry.
  
  - d. Ship the samples by a carrier that will deliver them within 24-72 hours, e.g., FedEx, UPS, or DHL.

**NOTE:** The USPS does **not** deliver to the laboratory.

- e. **SHIP TO:**  
Attention: DIAGNOSTIC  
FOOD ANALYSIS DIAGNOSTIC LABORATORY  
2899 Schofield Road, Suite 2630  
JBSA Ft Sam Houston, TX 78234-7583

**IX. REFERENCES:**

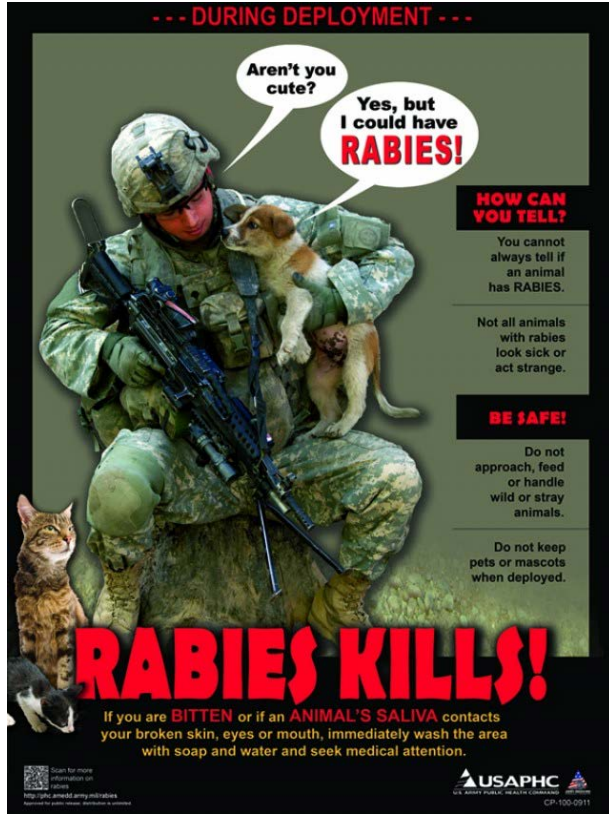
- A. **(ED-006)** - Laboratory Methods for Detection of Rabies; pages 109-141; September 1981; US Department of Health and Human Services; Center for Disease Control; Atlanta, GA 30333
- B. **(ED-008)** - Smith, J.S., Yager, P.A and Baer, G.M; Chapter 15 - Laboratory Techniques in Rabies; pages 181-192; (1996); 4th Edition; Bulletin World Health Organization; Geneva
- C. **(ED-178)** - Bird, B.R. and Forrester, F.T.; 1981; Basic Laboratory Techniques in Cell Culture; General Procedures for the Subcultivation of Cell Line Monolayers; pgs. 104-115; USHHS; Centers for Disease Control, Atlanta, GA 30333
- D. **(ED-439)** - Bloodborne Pathogen Exposure Control Plan Memo 40-169; 27 January 2020

**X. APPENDIX**

**APPENDIX A - Screening Patients for Rabies Antibodies:  
The Rapid Fluorescence Foci Inhibition Test (RFFIT)  
Protocols for Health Care Providers**



## APPENDIX A

Screening Patients for Rabies  
Antibodies: the Rapid Fluorescence  
Foci Inhibition Test (RFFIT) Protocols  
for Health Care Providers

## Whom to Test?

Rabies neutralizing antibody tests, such as the RFFIT are used to monitor antibody levels in persons that may have an occupational risk of rabies virus exposure (e.g. veterinarians, rabies virus laboratory workers, animal control officers, vivarium technicians, etc.). Serological testing may also be used to check the immune response of a person undergoing rabies post exposure prophylaxis when major deviations in vaccination schedule occur, or there are concerns about a patient's immune status.

For most persons, completing pre-exposure or post exposure prophylaxis routine serological testing is not necessary to document seroconversion unless:

- the person is immunosuppressed
- significant deviations of the prophylaxis schedule have occurred
- the patient initiated vaccination internationally with a product of questionable quality; or
- the person's antibody status is being monitored routinely due to occupational exposure to rabies virus.

## Testing:

The RFFIT is a rabies neutralization test performed in cell culture to determine the rabies virus neutralizing antibody level in human or animal sera. It is the gold standard serological assay recommended by the Advisory Committee on Immunization Practices (ACIP) and the World Health Organization (WHO). The Department of Defense (DoD) Food Analysis and Diagnostic Laboratory (DoD FADL) performs RFFIT for all DoD Services Members and their beneficiaries. The testing method used by staff was originally developed by Centers for Disease Control (CDC). Other serological tests, such as enzyme-linked immunosorbent assay are more appropriate for research and are not recommended for samples requiring clinical decision making by clinicians based upon current ACIP and WHO recommendations.

## Test Description:

The RFFIT is performed by mixing different dilutions of test sera with a constant amount of rabies virus and adding the mixture to cultured cells. Patient test sera

rabies neutralizing antibodies will bind to active virus and neutralize the pathogen.

Any non-neutralized virus will replicate in cells and be detected by the assay. Cells are incubated for ~20 hours before they are fixed and stained with fluorescent dyes to detect any rabies virus production. The immunofluorescent staining of infected cells is used as an indicator of rabies virus replication, with infected fields used to determine rabies virus neutralizing titer.

A rabies antibody titer is essentially an estimation of an immune response against rabies virus (either through vaccination or exposure). Current ACIP recommendations outline frequency for checks of persons with an occupational risk of rabies virus exposure. Complete neutralization of rabies virus at a serum dilution of 1:5 (~0.11 IU/mL) is recommended by the ACIP as evidence that the individual still has a detectable level of rabies virus neutralizing antibodies. At this level, an immune competent individual would be expected to mount a rapid response to a booster dose of rabies vaccine in the event of an exposure, precluding the need of rabies immune globulin during post exposure prophylaxis.

If the individual does not have evidence of rabies virus neutralizing antibody at a serum dilution of at 1:5 (~0.11 IU/mL) then they should receive a single booster dose of rabies vaccine.

#### Laboratory Services:

The Department of Defense Food Analysis and Diagnostic Laboratory (DoD FADL) performs RFFIT for all DoD service members and beneficiaries. For service animals, household pets, and specimens of interest the DoD FADL offers the Fluorescent Antibody Virus Neutralization (FAVN) test.

#### Submission information for Providers:

##### Submission Information for Providers

As referenced from Technical Guide 361; Visit the APHC Website and under FADL Diagnostics Forms complete FADL Form D-158, Serological Request and Report Form (Human Serum). Contact the laboratory by calling DSN: 421-4387/4010/4605; Com: 210-295-4387/4010. Or Email

[usarmy.jbsa.medcom.list.phc-rabies-favn-sa@mail.mil](mailto:usarmy.jbsa.medcom.list.phc-rabies-favn-sa@mail.mil) to request a copy of our

current standard operating procedure for collecting and sending a RFFIT sample to the FADL.

**We request all submitted samples refrain from the use of Social Security Numbers and utilize DoD Identification Numbers.**

Before shipping your samples, please email the listed email above to assist laboratory personnel in tracking your shipment.

RFFIT Turn Around Times are currently 10-14 days.

#### Sources

Manning SE, Rupprecht CE, Fishbein D, Halon CA, Lumlertdacha B, Guerra M, Meltzer MI, Dhankhar P, Vaidya SA, Jenkins SR, Sun B, Hull HF. Human Rabies Prevention - United States 2008. Recommendations of the Advisory Committee on Immunization Practices (ACIP).

Briggs DJ, Schwnke JR. Longevity of rabies antibody titer in recipients of human diploid cell rabies vaccine. Vaccine. 1992; 10: 125-9.

Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies neutralizing antibody. Bull World Health Org. 1973; 48: 535-541.