Minimum acceptable rabies antibody level

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PrEP and minimum acceptable rabies antibody level

- Not indicated for general U.S. population
- Indications
  - Risk of unrecognized exposures
  - Potential for high potency or unusual exposures
  - Opportunities for frequent contact with potentially rabid mammals
  - Travel to canine rabies endemic country without timely access to acceptable PEP
- Minimum acceptable antibody level is the target for level for:
  - Primary immunogenicity 2-4 weeks after completion of primary vaccination schedule
  - Maintenance antibody level checks

Proposed revisions

Risk category	Nature of Risk	Typical Population	Disease Biogeography[‡]	Primary Immunogenicity PrEP	Long-term immunogenicity
#1: Elevated risk for unrecognized and recognized exposures and	Risk of virus exposure is continuous. Exposure is often in high concentrations and may go unrecognized. Direct and indirect exposures.*	Laboratory personnel working with live rabies virus in research, diagnostic, or vaccine production capacities (e.g., necropsy of suspect rabid animal or working with rabies virus cultures)	Laboratory	Goal: Same primary series for 3 risk groups	Goal: Titers maintained high in case of unrecognized exposures
#2: Elevated risk of both unrecognized and recognized exposures	Risk of virus exposure is episodic. Exposure typically recognized but could be unrecognized and is greater than for those in the #3 risk group. Direct exposures and rarely indirect exposures	Persons who frequently handle bats or at frequent risk for coming into contact with bats because of entrance to high density bat environments (e.g., bat biologist)	All geographic regions where bats are a reservoir for rabies**		
#3: Elevated risk of recognized exposures	Risk of virus exposure greater than population at large. Exposure is a recognized one. Direct exposures.	Persons who work with animals • Animal care professionals (e.g., veterinarians, technicians, animal control officers) • Others who repeatedly handle terrestrial reservoir species (e.g., wildlife biologists, rehabilitators, and trappers) • Spelunkers • Veterinary students • Short-term / volunteer hands-on animal care workers where increased risk is expected for short time periods*	All geographic regions where terrestrial and non-terrestrial mammals are reservoirs for rabies		Goal: Anamnestic response elicitation to recognized exposure
		Travelers who will be performing activities (e.g., occupational or recreational) that put them at increased risk for exposure to rabid dogs and may have difficulty getting access to safe PEP (e.g., in rural area). Children may receive PrEP depending on the country to which they will travel (see CDC Traveler's Health destination pages)*	• Geographic regions internationally with canine rabies		
#4: Low risk of exposure / (i.e., general population)	Risk of virus exposure is uncommon. Bite or non-bite exposure	U.S. population at large	• Nationwide	• No pre-exposure prophylaxis • No serologic monitoring	n/a

*Direct exposures are bite and non-bite (e.g., contamination of fresh open wound or mucous membranes with saliva), Indirect exposures (i.e. droplet)

[‡]For questions about the disease biogeography of the region where an exposure occurred, please contact your local or state health department

[†] ates except Hawaii

Why do we care about minimal antibody levels?

- Indicates successful primary PrEP series
- Used to monitor for evidence of immunity
- Current minimal antibody levels differ between ACIP and WHO
  - Causes confusion
  - Levels difficult to interpret

KANSAS STATE UNIVERSITY

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Kansas State Veterinary Diagnostic Laboratory

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RFFIT Tests

About the RFFIT Test

Current Turnaround Time: RFFIT test results are normally available in 3 to 4 weeks from the date that the sample is received here at the Kansas State

- Kansas State University is one of 3 places that perform rabies serology testing
- Inordinate amount of time and energy spent on explaining interpretation of test results
- Websites for all 3 places provide detailed information because of the frequency asked questions

Human Result Interpretations

What is "the RFFIT"?

The acronym RFFIT stands for Rapid Fluorescent Focus Inhibition Test. The test measures the ability of antibodies that may be present in a sample to neutralize and block rabies virus from infecting the cells used in the test. These antibodies are called rabies virus neutralizing antibodies (RVNA). In the test, serum (the non-cellular portion of a blood sample) is first diluted fivefold (1 part serum in 4 parts diluent). Further (serial fivefold) dilutions are performed, each of which contain less and less of the sample. The serum dilutions are mixed with a standard amount of live rabies virus and incubated. If RVNA are present in the sample, they will bind to the virus. Tissue culture cells are then added and incubated with the test sample and virus. Whatever rabies virus that may not have been neutralized by the antibody in the sample will then infect the cells. These foci of infection in the cells can then be seen under the fluorescent microscope. If there are a lot of infected cells, there is very little antibody; conversely, if antibodies are high, the cells will have very little evidence of infection. The endpoint titer is calculated from the percent of virus-infected areas observed within the wells containing the various dilutions of the sample on the slide.

What does your result tell you?

The RFFIT result can be reported in either a titer which is a ratio (e.g., 1:50) or as a standardized concentration represented as international units (IU) per mL of serum (e.g., 0.5 IU/mL). The IU value is calculated from the titer by comparing it against a standard reference serum. We use the following formula: sample titer divided by the reference serum titer, multiplied by the IU/mL value of the reference serum.

Because the RFFIT test is a biological system using live cells, infectious virus, and antibodies, the reference serum can vary in titer level for each batch of testing (within an established acceptable range). Therefore the calculation of IU/mL depends on the titer of the reference serum measured in the batch tested. In general, you can take the titer value divided by 100 to get a rough estimate of the IU/mL value. To obtain the exact value you must use the calculation with the reference serum titer value that resulted from that batch of tests.

According to World Health Organization guidelines, a rabies antibody level of greater than or equal to 0.5 IU/mL demonstrates an adequate response to vaccination (1). If the level falls below this value, a booster dose of rabies vaccine may be recommended for people who are at frequent risk of rabies virus exposure. In contrast, the ACIP guidelines state that evidence of complete neutralization at a serum dilution of 1:5 in RFFIT testing (corresponding to 0.1-0.2 IU/mL in our laboratory) is considered an adequate response to rabies vaccination (2). The lowest antibody level that can be accurately and precisely measured by the RFFIT in our laboratory is 0.1 IU/mL; below this level, there is uncertainty as to the specificity of the result. Because the ACIP level is close to the assay threshold, the level of 0.5 IU/mL is more conservative for guiding human vaccination decisions and applicable in most situations.

Points that should be considered as to whether a person should receive a

Antibody level and protection

- Rabies is 100% fatal
- It is preventable with vaccines
- High circulating antibody *against virus glycoprotein is* key to survival after rabies virus exposure
- Human challenge studies are not feasible or ethical
  - Animal rabies vaccine challenge studies are used surrogates
- No definitive titer is known to be universally protective

Minimum Rabies Virus Neutralizing Antibody (RVNA) level associated with survival in animals

T.O. Bunn and H.D. Ridpath, 1984: Using statistical analysis of pre challenge titers and survival:

- 1:17 titer, ~0.2 IU/mL = 95% survival rate
- 1:44 titer, ~0.5 IU/mL = 99% survival rate

Other summary studies in dogs, cats and wildlife gave similar results regarding practical significance of rabies antibodies, also commenting strongly on the variability of the test method.

Current ACIP and WHO RVNA cut-off values for adequate vaccine response

- World Health Organization (WHO)
  - 0.5 IU/mL
- ACIP
  - Minimum acceptable antibody level is “complete neutralization at a 1:5 serum dilution by the rapid fluorescent focus inhibition test (RFFIT)”
    - Loosely converts to anywhere from 0.1-0.3 IU/mL
    - No cases of rabies have occurred in the US with this cut-off
      - Misleading because many factors contribute to survival
    - Recommends rabies serology results be reported in IU/mL, but does not provide relationship of the recommended level to how results are reported. ***It is neither a titer value or an IU/mL value.***

Agency/Year	Booster vaccination recommended if level is below:	Method of Testing:
WHO		
1992	0.5 IU/mL	MNT or RFFIT; ELISA only with caution
2005	0.5 IU/mL	RFFIT or FAVN; ELISA if RFFIT not available
2013	0.5 IU/mL	RFFIT or FAVN; ELISA
2018	0.5 IU/mL	RFFIT or FAVN; ELISA
ACIP		
1976	None, boosters recommended every 2 years	None stated
1980	1:16 titer or booster every 2 years	RFFIT
1984	1:5 titer per CDC; 0.5 IU/mL per WHO	RFFIT
1991	1:5 titer *	RFFIT
1999	Complete neutralization at a 1:5 serum dilution†	RFFIT
2008	Complete neutralization at a 1:5 serum dilution‡	RFFIT

***Recommended response 2-4 weeks after either pre- or post-exposure vaccination is complete neutralization at a 1:25 serum dilution which is equivalent to the WHO level of 0.5 IU/mL**

†Recommended response 1-2 weeks after post-exposure vaccination is complete neutralization at a 1:5 serum dilution

‡RVNA titer most properly reported according to a standard as IU/mL

The ACIP cut off value is confusing and hard to interpret against the RFFIT value reported for determination of booster vaccination.

Mouse Neutralization Test=MNT; Rapid Fluorescent Foci Inhibition Test=RFFIT; Fluorescent Antibody Virus Neutralization= FAVN; Enzyme Linked ImmunoSorbant Assay=ELISA

Test conditions cause variability in Rapid Fluorescent Focus Inhibition Test (RFFIT) titer results

A titer is the serum dilution that neutralizes a standard dose of live rabies virus.

- Titer values are variable due to the inherent variability in cell based serological assays. Virus, antibody and cells are all affected by multiple conditions that are difficult to control such as pH, temperature, humidity etc.

Factors causing titer variability of test samples will affect the reference serum titer similarly.

- Each time a test is run a unique set of variable are at play and will affect all sample titers in the same manner, including the reference serum

Solution to variability: Reporting RFFIT results in International Unit per mL (IU/mL)

IU/mL value calculation from the RFFIT Test Data

50% End Point Titer determination

- Number virus positive fields per 20-field count
- Calculate titer value using Reed and Muench formula to obtain the 50% endpoint titer:

  - The ACIP cutoff level of complete neutralization at a 1:5 serum dilution calculates to a 1:11 titer value

  - Formula to convert titer to IU/mL:
  - $\frac{\text{Sample serum titer value}}{\text{Reference serum titer value}}$ X IU/mL of the Reference serum (2 IU/mL)

Example: 50/200 X 2.0 IU/mL = 0.5 IU/mL

The ACIP level (1:11 in titer value) can range in IUs from 0.1 to 0.3 in IU/mL

Practical example of the benefit of using IU/mL versus titer--

Virus dose			RVNA result		Rabies Vaccine Boost Recommendation	
			Titer	IU/mL	ACIP 1:11	WHO 0.5IU/mL
Lab A	Low Challenge Virus Dose 10 $TCID_{50}$	Mary	1:24	0.2	NO	YES
		John	1:90	0.6	NO	NO
		Reference Serum	1:300	2.0		
		Negative Serum	1:2	0.0		
Lab B	Medium Challenge Virus Dose 50 $TCID_{50}$	Mary	1:10	0.2	YES	YES
		John	1:37	0.6	NO	NO
		Reference Serum	1:125	2.0		
		Negative Serum	1:2	0.0		
Lab C	High Challenge Virus Dose 100 $TCID_{50}$	Mary	1:4	0.2	YES	YES
		John	1:10	0.6	YES	NO
		Reference Serum	1:35	2.0		
		Negative Serum	1:2	0.1		

Conclusion: In different labs, titer results are different but results in IU/mL is the same between labs, thus the recommendation for vaccine booster or not is the same

Lower Limits of Rabies Antibody Detection

The ACIP cut-off range for RFFIT (as converted to IU/mL) represents the lower limits of quantitation in most laboratories and can overlap with non-specific neutralization (false positives)

Range of IU/mL values associated with ACIP cur-off (titer of 1:11)

	False Positive Titer Results			
IU/mL	Study 1	Study 2	Study 3	**Average**
>/=0.1	2.4%	4.3%	1.3%	**2.7%**
>/=0.2	1.2%	2.9%	0.8%	**1.6%**
>/=0.3	1.2%	2.9%	0.4%	**1.5%**
>/=0.4	0.0%	0.0%	0.4%	**0.1%**

Results are from 3 clinical trial studies, samples drawn before vaccination (day 0) samples

Implications for a change in antibody cut-off level for adequate response to vaccination

Rabies Titer checks at Veterinary Conferences and Schools

	% of people with RVNA ≥0.5 IU/mL	% of people with RVNA ≥ ACIP cut-off	% people needing a booster at ACIP cutoff	% of people needing a booster at 0.5 IU/mL cutoff	Fold increase of people needing a booster if cut-off changes
Las Vegas 2000	62	78	22	38	1.7
Boston 2001	76	89	11	24	2.2
Nashville 2002	77	89	11	23	2.1
Denver 2003	86	95	5	14	2.8
Philadelphia 2004	88	97	3	12	4.0
Minneapolis 2005	82	94	6	18	3.0
Dallas 2008	95	99	1	5	5.0
Dallas 2015-2018	93	98	2	7	3.5
6 Vet Schools 2005-2014	80	97	3	20	6.7

On average 2.5-fold increase (range 1.7 – 6.7) in people recommended a booster between current ACIP cut-off and 0.5 IU/mL

Summary: 0.5 IU/mL is a better alternative to the current ACIP cut-off

- 0.5 IU/mL is robustly associated with survival from challenge
- 0.5 IU/mL is the RVNA level that assures minimal false positives in the RFFIT

- The IU/mL unit of measurement
  - Is more precise and accurate among and within laboratories performing titer checks globally
  - Is understandable in relation to how results are reported
  - Major labs already report RFFIT results in this unit.

Proposal: Change cut-off from ‘complete neutralization at a 1:5 serum dilution’ (~0.1-0.3 IU/mL) to 0.5 IU/mL

Advantages:

- Reduces confusion for high stakes infection
- Increase in precision and accuracy of RVNA results within and between laboratories
- Decreased risk in reporting false RVNA positive results
- A more robust level that accounts for method variability

Disadvantages:

- Based on previous RVNA antibody monitoring data more people would be recommended to receive a booster vaccination.

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