

Rabies Serology – Vaccine Titer Response**What is measured?**

Rabies virus neutralizing antibodies (RVNA) are antibodies with the ability to neutralize rabies virus and prevent the virus from infecting cells. In the test, serum (the non-cellular portion of blood) is first diluted in a diluent (e.g. 1 part serum in 4 parts diluent). Further (serial fivefold) dilutions are performed, each of which contain less and less serum. These serum dilutions are mixed with a standard amount of live rabies virus and incubated. Whatever RVNA is present will neutralize the virus. Next, some tissue culture cells are added and the serum/virus/cells are incubated together. Whatever rabies virus is left (i.e., that which has not been neutralized by the antibody in serum), will infect the cells and this can be seen under the microscope through the use of specific staining. Calculation of the endpoint titer is made from the percent of virus infected cells observed on the slide.

What does the result mean?

The result of this test can be expressed in two ways: as a Rabies virus neutralizing antibodies (RVNA) endpoint titer (e.g., 1:50) or as a value for RVNA potency (e.g., 0.5 IU). The IU stands for international unit and is calculated from the titer by comparing it against the titer of 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.

Example: The sample titer is 1:100, the reference serum titer is 1:200, and the value of the reference serum is 2.0 IU/mL. Using the formula:

$$(100/200) \times 2.0 \text{ IU/mL} = 1.0 \text{ IU/mL}$$

the potency of RVNA in the sample serum is therefore 1.0 IU/mL.

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 measured reference serum titer value.

What does the level of Rabies virus neutralizing antibodies (RVNA) mean?

When we measure antibodies to rabies virus in serum (the liquid part of blood), this measurement provides an indication of an immune response to rabies. The test is not able to differentiate whether the immune response is in response to rabies virus exposure or to rabies vaccination. Titer levels or IU/mL values, especially high ones after vaccination, provide evidence of a good immune response. Although the presence of antibodies after vaccination is important, a specific level may not directly correlate with complete assurance of protection against the disease in every individual because the quality of antibodies (their ability to stop infection) and their duration (how long they remain high) is different for every individual. This is because there are other immunological factors which are involved in the protection from rabies infection. An overview of rabies challenge studies indicates RVNA levels predict survival on more a qualitative rather than quantitative basis [1]. Whether an animal requires a rabies booster vaccination is not determined by the level of RVNA in the serum, but by local regulations. As stated in the compendium of animal rabies prevention and control report by the CDC, RVNA levels are not to be used in place of current vaccination for either management of rabies exposure or for determination of booster vaccinations for animals [2].

References

1. Aubert MF. Practical significance of rabies antibodies in cats and dogs. Rev Sci Tech 1992;11:735-60.
2. Centers for Disease Control and Prevention. Compendium of animal rabies prevention and control., National Association of State Public Health Veterinarians, Inc.(NASPHV), 2008.