

Rabies Serology – RFFIT testing

What is “the RFFIT”?

RFFIT stands for Rapid Fluorescent Foci Inhibition Test. It is a serum neutralization (inhibition) test, which means it measures the ability of rabies specific antibodies to neutralize rabies virus and prevent the virus from infecting cells. These antibodies are called rabies virus neutralizing antibodies (RVNA). The RFFIT does not measure the amount of rabies specific antibodies but the neutralizing activity of the antibodies present in the serum. In the test, your serum (the non-cellular portion of your blood) is first diluted fivefold (1 part serum in 4 parts diluent). Further dilutions are performed, each of which contain less and less of your 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 your 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 your titer tell you?

The RFFIT test can be used in two ways: to determine a RVNA titer (e.g., 1:5) or to determine a value for RVNA concentration (e.g., 0.5 IU). The IU stands for international unit and is calculated from the titer by comparing it against a standard reference serum. If you do not have RVNA present in your serum it will not neutralize the virus and the titer will be LESS THAN 1:5 (the 1:5 diluted serum did not prevent the virus from infecting the cells). RVNA antibody will neutralize rabies virus to an “endpoint titer” – to a specific dilution where the virus is neutralized. For example, if you have a little RVNA in your serum low dilutions (e.g. 1:5 or 1:25) will neutralize the virus, but higher dilutions will not. In contrast, if you have a lot of antibody in your serum the virus will be neutralized by high dilutions of your serum (e.g. 1:1000 or 1:7000) and not infect the cells. Therefore the further your serum can be diluted and still neutralize virus, the more RVNA you have in your serum. Current ACIP regulations recommend evidence of complete neutralization at serum dilution of 1:5 is considered an adequate response to rabies vaccination (1). If your serum diluted to 1:5 cannot neutralize virus a booster dose may be recommended (for people determined to be at risk of rabies virus exposure - see ACIP recommendations). In summary, the bigger the denominator in the reported titer, the more antibody in your blood (e.g., a titer of 1:125 has more antibody than a titer of 1:5 because it still neutralized at a much higher dilution) and a reported RVNA titer of greater than 1:5 is considered adequate.

REFERENCES

1. Centers for Disease Control and Prevention: Recommendations of the Advisory Committee on Immunization Practices (ACIP). 57: 1-36. 2008. United States. Morbidity and Mortality Weekly Report.