**Rabies vaccination and level of protection**

“Am I protected?” This is a question that often comes up after a person has received pre- or post-exposure rabies vaccination. Though a natural and valid question, to define and to measure protection from rabies is not as straightforward as some would like.

The antibody level recommended by the World Health Organization (WHO) as an adequate response to vaccination is 0.5 IU/mL. Assays advocated by WHO are the Rapid Fluorescent Focus Inhibition Test (RFFIT) and ELISA--if the RFFIT is not available. The 0.5 IU/mL value is not a level of protection but rather the minimum antibody level determined after evaluation of peak responses in early human clinical trial studies. The RFFIT measures rabies virus neutralizing antibody (RVNA) levels in serum. Using the RVNA level to assess the vaccine response is supported by studies establishing RVNAs as the most significant immune component in preventing rabies after exposure. Animal rabies challenge models show 0.5 IU/mL to be a robust level of protection, though not absolute—as some animals survive experimental challenge with RVNA levels below 0.5 IU/mL, implying that other immune effectors are involved in protection. Other factors in real life exposures, such as the location and severity of bite, the virus variant and the amount of virus received can also influence the strength of immune response required to prevent rabies

Because RVNA levels are a marker, not the sum of protection, establishing a set level of protection is difficult; additionally the assays used to measure the vaccine response are inherently variable. Though RFFIT is the primary WHO endorsed method, there are several method modifications that can be made that may lead to discrepant (or even inaccurate) results if important elements are not standardized and controlled. These elements include: the challenge virus strain and dose, the standard reference serum, and the reading/calculation technique used. However, laboratories following published/standardized procedures will produce results in good agreement. It should be kept in mind that because RFFIT is a cell-based serological assay relying on biological elements (cells, antibodies, and virus), the precision will be less than with chemical assays. Because of this, even in the most accurate laboratories a sample with a result of 0.5 IU/mL in one testing may produce a result ranging from 0.3 IU/mL to 0.8 IU/mL when tested subsequently in the same laboratory. Results produced by different methods can also be discrepant. RFFIT measures the level of RVNA and ELISA anti-rabies binding antibodies. ELISA detects one class of immunoglobulin (IgG) and RFFIT detects IgG and IgM. Individuals vary in their antibody response to vaccination due to genetic factors resulting in differing levels of each neutralizing antibodies and binding antibodies. This variation, combined with different classes of immunoglobulin predominating at various time points after vaccination (IgM early and IgG later), helps explain why one person may have a high response as measured by ELISA but a low RFFIT response, while another may have results that are opposite (low ELISA/high RFFIT levels) and a third have results approximately equal. An established recognized level of adequate rabies vaccination is useful for the standard evaluation of rabies biologics and basis of medical decisions; yet understanding the origins of the level and the methods used to measure it is vitally
important in the evaluation of protection. As with all laboratory testing, the most useful results are obtained by the method that is ‘fit for purpose’; the RFFIT measures the neutralizing function of the antibodies produced in response to rabies vaccination and is best for estimating the protection provided by that response.

Contributed by Susan M. Moore, Kansas State University Rabies Laboratory, Manhattan, Kansas, USA