The vaccination of domestic carnivores and wildlife against rabies is a powerful tool to prevent, control and eliminate this disease. The presence of rabies-neutralising antibodies in blood is considered a reliable indicator for assessing the efficacy of rabies vaccination and as proof of protection against the disease.

The OIE reference methods, i.e., the fluorescent antibody virus neutralisation (FAVN) test and the rapid fluorescent focus inhibition test (RFFIT), require an international positive reference control to validate the test, and to obtain harmonised titres between laboratories worldwide for mutual recognition of test results. This is why the OIE recommends the use of the OIE reference serum of dog origin to express the titre of sample in IU/mL.

For many years, the production and determination of the titre of the OIE antirabies-positive reference serum of dog origin has been carried out by ANSES-Nancy, the OIE Reference Laboratory for Rabies (Malzéville, France).

Experiments and husbandry were conducted according to European Directive 2010/63/EU and French regulations on ethics in animal experimentation. The protocol of dog immunisation was proposed to the OIE Reference Laboratories for Rabies as well as to the OIE Biological Standards Commission for approval. To more closely approach the real use of the reference serum (mainly to test the level of antibodies in vaccinated animals), three inactivated monovalent antirabies veterinary vaccines, based on the most used vaccinal strains (PV and Flury LEP strains) in the world, were used to immunise dogs. The immunisation protocol was the same for each veterinary vaccine. Five naïve dogs were vaccinated with each of the three vaccines (a total of 15 dogs in all). Serological monitoring was carried out on each dog to observe the level of rabies-neutralising antibodies produced over a period of several weeks. When the level of antibodies produced was considered satisfactory, the blood was sampled from each dog and centrifuged to obtain the serum. The distribution in vials and freeze drying were performed by a specialised company. Before and after the freeze-drying step, several sterility controls and serological titrations were performed at ANSES-Nancy.

The titre of this reference serum was determined by the OIE Reference Laboratories for Rabies during a ring trial. This inter-laboratory test was organised by ANSES-Nancy and five OIE Reference Laboratories for Rabies (from France, Germany, South Africa, the United Kingdom and the United States of America) were involved. Each received a panel of 12 coded samples: one sample containing a naïve serum; three samples containing the positive reference serum whose titre was to be determined, and eight samples corresponding to the different dilutions of the second WHO International Standard for Anti-Rabies Immunoglobulin, of human origin, titrating 30 IU/mL. Each laboratory was invited to titrate the panel of samples in three independent runs by using one of the two sero-neutralisation tests prescribed by the OIE, either the FAVN test or the original RFFIT. Different statistical tests were carried out by ANSES-Nancy on the results given by participating laboratories to determine the consensus value. The consensus titre of the second batch of the OIE reference canine serum for rabies is equal to 5.59 IU/mL.

The OIE Biological Standards Commission (February 2014) agreed to adopt the serum as an OIE-approved standard. This OIE-approved reference reagent is now available and may be requested from our laboratory – ANSES-Nancy.