WHO Immunological Basis for Immunization Series

Module 17: Rabies
Update 2017

Immunization, Vaccines and Biologicals

World Health Organization

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Contents

Abbreviations and acronyms

ABLV	Australian bat lyssavirus
ACIP	Advisory Committee on Immunization Practices
ARAV	Aravan lyssavirus
ART	Antiretroviral Therapy
APC	Antigen-presenting cells
BBLV	Bokeloh bat lyssavirus
CCV	Cell culture rabies vaccine
CNS	central nervous system
dRIT	direct Rapid Immunohistochemical test
DPT-IPV	Combined diphtheria, tetanus, whole-cell pertussis and inactivated poliomyelitis vaccine
DUVV	Duvenhage lyssavirus
EBLV-1	European bat lyssavirus type 1
EBLV-2	European bat lyssavirus type 2
ELISA	Enzyme-linked immunosorbent assay
ERIG	Equine rabies immune globulin
FAT	Fluorescent antibody test
FAVN	Fluorescent antibody virus neutralization
GBS	Guillain-Barré syndrome
HAART	Highly Active Antiretroviral Therapy
HDCV	Human diploid cell rabies vaccine
HIV	Human immunodeficiency virus
HRIG	Human rabies immune globlulin
ICTV	International Committee on Taxonomy of Viruses
ID	Intradermal
IKOV	Ikoma lyssavirus
IM	Intramuscular
IRKV	Irkut lyssavirus
JEV	Japanese encephalitis vaccine

KHUV	Khujand lyssavirus
LBV	Lagos bat lyssavirus
Mab	Monoclonal antibodies
MNT	Mouse neutralization test
MOKV	Mokola lyssavirus
NTV	Nerve tissues vaccines
PCECV	Purified chick embryo cell rabies vaccine
PCR	Polymerase chain reaction
PDEV	Purified duck embryo cell rabies vaccine
PEP	Post-exposure prophylaxis
PIKA	Polyinosinic Polycytidylic Acid Based Adjuvant
PrEP	Pre-exposure vaccination
PVRV	Purified Vero cell rabies vaccine
RABV	Rabies lyssavirus
RFFIT	Rapid fluorescent focus inhibition test
RIG	Rabies immune globulin
SHIBV	Shimoni bat lyssavirus
SOT	Solid organ transplantation
VNA	Virus neutralizing antibodies
WCBV	West Caucasian bat lyssavirus
WHO	World Health Organization

Preface

This module is part of the WHO series The Immunological Basis for Immunization, which was initially developed in 1993 as a set of eight modules, comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization, i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance. The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis of vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to the individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, published in the WHO vaccine position papers.*

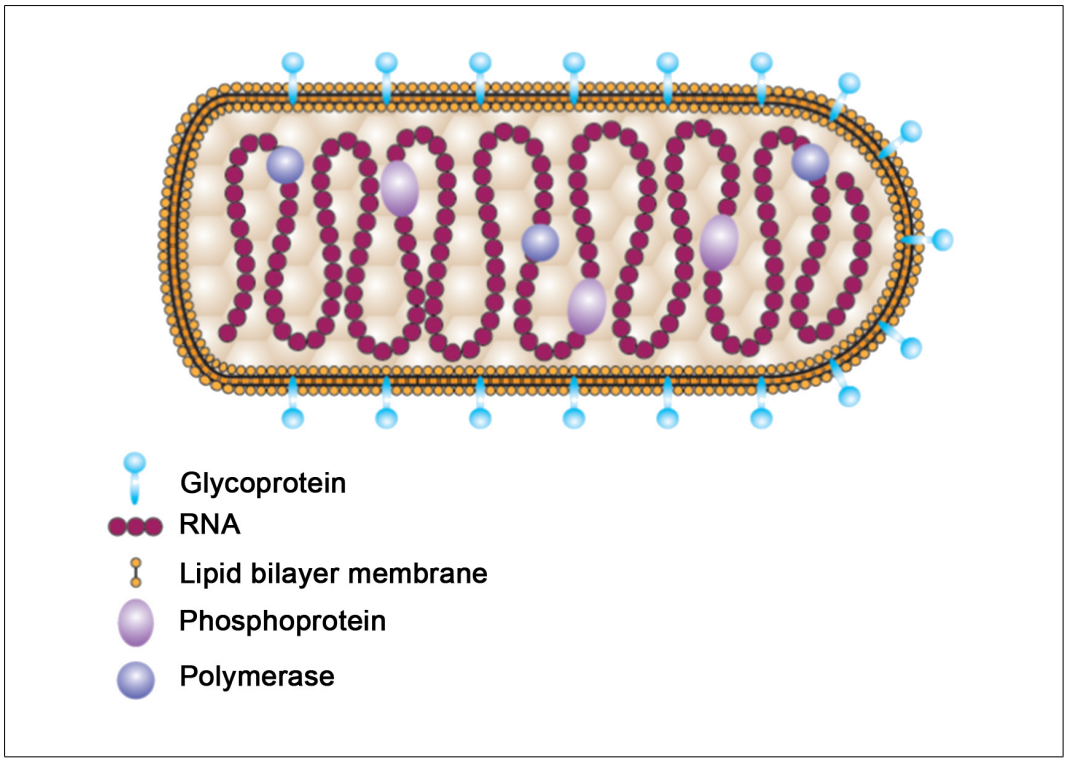
* See: *http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*, accessed November 2017.

1. Rabies virus and other lyssaviruses and disease

1.1 Structure

The etiological agents that cause rabies are enveloped, rod-shaped viruses containing a single-strand negative sense non-segmental ribonucleic acid (RNA) genome. These viruses have a simple genome organization that encodes for five structural proteins, including: a large RNA-dependent RNA polymerase (L), nuclear protein (N), phosphoprotein (P), matrix protein (M), and a surface glycoprotein (G). The G protein induces the production of virus-neutralizing antibodies (VNAs) that are the major immune effectors in protecting against an infection with a lyssavirus *(1,2)*. The ribonucleoprotein complex, consisting of the N, P, L and negative-strand genomic RNA, has been reported to induce the cellular immunity required to augment VNA production as well as to establish immunologic memory and long-lasting immunity *(3)*.

Figure 1: Diagram of a lyssavirus



1.2 Classification

The etiological agents that cause rabies belong to the genus *Lyssavirus* in the family *Rhabdoviridae*, consisting of a total of 13 genera *(1,4)*. According to the International Committee on Taxonomy of Viruses (ICTV),[1] as of 2017 at least 14 species were classified under the *Lyssavirus* genus (Table 1) *(5)*.

Rabies virus is the most important member of the genus. Besides rabies virus, viruses belonging to all other known lyssavirus species have been demonstrated – i.e. Australian bat lyssavirus (ABLV), Duvenhage lyssavirus (DUVV), European bat lyssavirus type 1 (EBLV-1), European bat lyssavirus type 2 (EBLV-2), Irkut lyssavirus (IRKV), Mokola lyssavirus (MOKV) – or can be expected to cause an acute, progressive lethal encephalitis in humans. Other lyssaviruses have not as yet been reported in humans – i.e. Aravan lyssavirus (ARAV), Bokeloh bat lyssavirus (BBLV), Ikoma lyssavirus (IKOV), Lagos bat lyssavirus (LBV), Khujand lyssavirus (KHUV), Shimoni bat lyssavirus (SHIBV), West Caucasian bat lyssavirus (WCBV) *(6)*. Although some of the rabies vaccines that are currrently being produced have been tested against a few of the viruses listed under the *Lyssavirus* genus, not all of the vaccines have been tested against all of the viruses. Currently available rabies vaccines would be more likely to produce cross-neutralizing antibodies against those viruses that are more closely related – i.e. Phylogroup 1, including Rabies lyssavirus (RABV), ABLV, EBLV-1, EBLV-2, IRKV, KHUV, ARAV, BBLV and DUVV *(7,8,9,10)*. Although current studies are limited, available data indicate that rabies vaccines will not produce adequate cross-reactive antibodies against IKOV, LBV, MOKV, SHIBV and WCBV *(5,11)*.

Reports available on genetic classification of the currently-identified 14 lyssavirus species/genotypes suggest that they are divided into at least two phylogroups according to differences in genetic makeup, serological cross-reactivity and comparative pathogenesis. Phylogroup I includes ABLV, ARAV, BBLV, DUVV, EBLV-1, EBLV 2, IRKV, KHUV and RABV. Serological cross-reactivity with Phylogroup 1 has been reported for the viruses ARAV, IRVV and KHUV. Phylogroup 2 includes LBV, MOKV and SHIBV *(6,12,13)*. There is significant serological neutraliziation within each phylogroup, but very limited or no cross-neutralization between the phylogroups. WCBV and IKOV may form an independent phylogroup *(6)*.

[1] See: *https://talk.ictvonline.org/*, accessed 27 October 2017.

Table 1: Genus Lyssavirus[2]

Virus	Abbreviation	Potential vector/host species	Distribution	Neutralizing antibodies produced using currently licensed human rabies vaccines
Aravan lyssavirus	ARAV	Insectivorus bats	Central Asia	Yes
Australian bat lyssavirus	ABLV	Frugivorous/insectivorous(?) bats	Australia	Yes
Bokeloh bat lyssavirus	BBLV	Insectivorous bats	Europe	Yes
Duvenhage lyssavirus	DUVV	Insectivorous bats	S Africa	Yes
European bat lyssavirus 1	EBLV-1	Insectivorous bats	Europe	Yes
European bat lyssavirus 2	EBLV-2	Insectivorous bats	Europe	Yes
Ikoma lyssavirus	IKOV	Isolated from Civet cat (Civettictis civetta)	Africa	No
Khujand lyssavirus	KHUV	Insectivorous bats	Central Asia	Yes
Lagos bat lyssavirus	LBV	Frugivorous bats	Africa	No
Mokola lyssavirus	MOKV	?	Africa	No
Rabies lyssavirus	RABV	Carnivores worldwide, and bats (in the Americas)	Worldwide	Yes
Shimoni bat lyssavirus	SHIBV	Isolated from bat species Hipposiderous commersoni	East Africa	No
West Caucasian bat lyssavirus	WCBV	Insectivorous bats	Caucasian region	No

1.3 Pathology

Human rabies as a disease has the highest case fatality rate ever reported *(14)*. Lyssavirus infection causes an acute progressive encephalitis in a wide variety of mammals that almost invariably results in death of the host *(14,15)*. After an exposure occurs, generally through infiltration of virus-contaminated saliva from a rabid animal into a bite wound or through contact with mucous membrane, these highly neurotropic viruses replicate in muscle tissue and enter peripheral nerves, spread by way of the peripheral nervous system to the spinal cord and ascend to the brain. Neurotropic receptors – such as the nicotinic acetylcholine receptor, the low affinity p75 neurotrophin receptor and the neural cell adhesion molecule receptor – have been implicated in virus entry and transport *(16)*. After dissemination within the central nervous system (CNS), the virus spreads centrifugally from the CNS back along the nerves to various organs, including the salivary glands, where it is emitted into the saliva and passed on to the next victim – again usually through a bite wound or contamination of infected saliva on to a mucous membrane *(17)*. The effect that various lyssavirus proteins have on an infected patient's cellular functions are largely unknown and further research could help to elucidate the pathobiology of virus infection *(18,19)*.

[2] See: *http://www.who-rabies-bulletin.org/site-page/classification*, accessed November 2017.

1.4 Epidemiology

Rabies is an underreported disease that is present on every continent except Antarctica, with an estimated 59 000 human deaths occurring annually *(20,21,22,23)*. Most human deaths occur in Africa and Asia *(20,21)*. Although all mammals are, to varying degrees, susceptible to lyssaviruses, the primary reservoirs of the disease belong to the orders *Carnivora* and *Chiroptera* (i.e. dogs, foxes, jackals, coyotes, raccoon dogs, skunks, raccoons, mongoose, ferret badgers and bats) *(14,15,21)*. Globally, over 98% of all human rabies deaths occur following exposures to infected dogs. Millions of exposures to dogs occur annually, with tens of thousands of human deaths resulting from untreated exposures *(14,15,21)*. Human rabies, especially paralytic rabies (which may represent as much as 30% of total clinical rabies presentations), is often misdiagnosed as other encephalitic diseases such as cerebral malaria or Guillain-Barré Syndrome (GBS), thus masking the true global burden of the disease *(24,25,26).* New approaches for collecting real-time data on the number of human rabies deaths have recently been discussed and implemented in a few regions *(23).* These new strategies for collecting and sharing data may lead to a better understanding of the epidemiology and burden of human rabies globally. WHO and the World Organisation for Animal Health (OIE), in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and supported by the Global Alliance for Rabies Control (GARC) developed a global framework for the elimination of dog-mediated human rabies by 2030 .

2. Immunity

2.1 Preventing clinical disease

Rabies is virtually unique compared to other infections in that the development of clinical disease following exposure to the virus is preventable, even in patients who have not been previously vaccinated, through timely administration of post-exposure prophylaxis (PEP). PEP, as recommended by WHO, has three components: (a) wound treatment with cleansing, flushing, disinfection and debridement; (b) vaccine administration over 7–28 days; and (c) administration of rabies immune globulin (RIG) with, or within the week that follows, administration of the first dose of vaccine in all category III (severe) exposures *(15,27)*. The outcome of a viral exposure depends on many factors, including: the site and severity of the exposure; the dose and variant (genotype or species) of virus inoculated into the wound(s); and the timeliness of administration and adherence to WHO recommendations for PEP *(14,15,42)*. Both the innate immune response (i.e. the basic immune system inducing non-specific resistance to disease) and the adaptive immune responses (i.e. highly specialized, systemic cells and processes) of a patient are involved in securing protection against the development of rabies *(5,15,28,29,30,31,32,33)*.

In addition to wound treatment, the action of which is mechanical and chemical, the primary immunological objective of PEP is to neutralize and destroy virus that was inoculated into a victim's body at the time of exposure. This needs to be achieved as quickly as possible by increasing the amount of VNA available to complete the task. Thus, it is critical for a protective immune response to ensure that VNA directed against the G protein of the virus is produced as soon as possible *(3,28)*. The level of VNA is almost always high enough to be detected between 7 and 14 days after primary vaccination (adaptive or active immunity) *(31,34,35)*. However, because rabies is invariably fatal, the administration of RIG (passive immunity) early in the vaccination regimen aims to provide additional protection, especially for patients with severe and/or multiple wounds *(30,32,33,36,37,38)*.

2.2 Rabies vaccines

Table 2: Past and present rabies vaccines for humans[3]

Vaccine name	Type	Substrate
Nerve tissue		
Pasteur*	Inactivated by drying	Rabbit spinal cord
Fermi*	Phenolized live virus	Sheep, goat or rabbit brains
Semple	Phenol inactivated	Sheep, goat or rabbit brains
Fuenzalida	Inactivated	Suckling mouse brain
Avian		
PDEV	β-Propiolactone inactivated	Duck embryo
DEV*	Inactivated	Duck embryo
Cell culture		
HDCV	β-Propiolactone inactivated	Human cultured fibroblasts
RVA	β-Propiolactone inactivated	Fetal rhesus cell culture
PHKCV	Formalin inactivated	Primary Syrian hamster kidney cell culture
PCECV	β-Propiolactone inactivated	Chick embryo cell culture
PVRV	β-Propiolactone inactivated	Vero cell line

* No longer used. DEV: duck embryo vaccine; HDCV: human diploid cell vaccine; PCECV: primary chick embryo cell vaccine; PDEV: purified DEV; PHKCV: primary hamster kidney cell vaccine; PVRV: purified Vero rabies vaccine; RVA: Rhesus cell rabies vaccine.

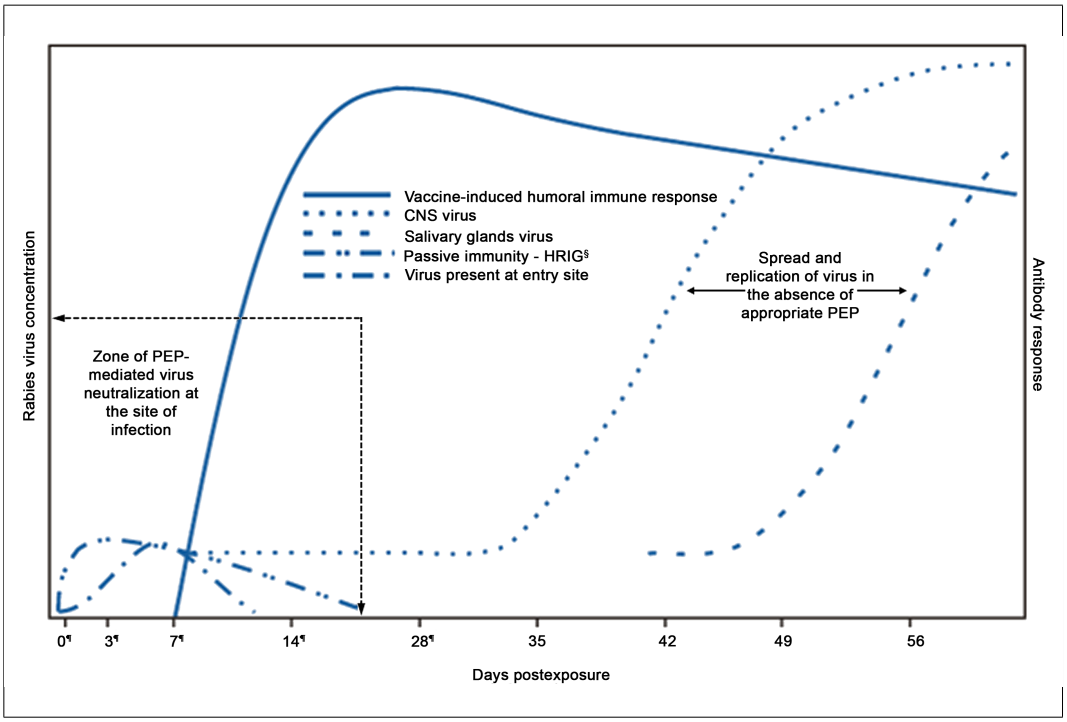
Since their development over four decades ago, cell culture-based and embryonated egg-based rabies vaccines (CCVs) have proved to be highly effective in preventing human rabies, both when administered as pre-exposure vaccination (PrEP) and when used in association with RIG for PEP *(30,39,40,41)*. The production of CCVs represented a significant advance, particularly over the first crude nerve tissue vaccines (NTV) for rabies manufactured over a century ago using the brain material of infected animals *(30)*. All NTVs are reactogenic and WHO has recommended that they be replaced with CCVs. Only a few NTVs are currently being produced for PEP *(15)*. Several different cell substrates have been used for the production of rabies vaccines, including Syrian baby hamster kidney cells (BHK-21), human diploid cells, primary cell lines produced from embryonated chicken and duck eggs, and continuous cell lines produced from Vero cells *(30)*. Rabies vaccines produced in Vero cells and primary cell lines originating from embryonated eggs have expanded the safe use and availability of CCVs throughout the world. CCVs have also allowed a broader use of vaccines for PrEP, thus protecting persons at increased risk of exposure *(15,27)*. Over the past two decades, numerous data have been published demonstrating the effectiveness and safety of CCVs *(9,31,42,43)*.

[3] Adapted with permission from: Rupprecht CE, Nagarajan T, Ertl H. Plotkin's vaccines: cell culture rabies vaccines. Philadelphia (PA): Elsevier; 2018: 927.

The cost of administering a 4-dose or 5-dose intramuscular (IM) PEP regimen using CCVs is beyond the financial capability of many persons living in developing countries *(44)*. Consequently, where budgetary limitations may deter the use of CCVs for PEP, WHO has recommended the administration of intradermal (ID) PEP using CCVs that meet specific potency and immunological criteria *(15)*. Ongoing research specifically aimed at developing new, low-cost and effective vaccines and shorter PEP and PrEP regimens, could eventually reduce the global cost of preventing rabies *(32,39,45,46)*. In order to provide a reliable source of vaccines for countries facing procurement difficulties, there are plans to develop a human rabies vaccine stockpile *(47)*.

2.3 Response to immunization (humoral and cellular)

Figure 2: Schematic of dynamics of rabies virus pathogenesis* in the presence and absence of post-exposure prophylaxis (PEP)-mediated immune responses†4



* Rabies can progress through five stages: incubation period (5 days to >2 years: U.S. median ~35 days), prodrome state (0--10 days), acute neurologic period (2--7 days), coma (5--14 days), and death.

† Once in tissues at the entry site, rabies virus can be neutralized by passively administered rabies immune globulin (RIG). Active immunization (vaccine) stimulates the host immune system and, as a result, virus-neutralizing antibodies (VNAs) are produced approximately 7–10 days after initiation of vaccination. By approximately day 14–28 (after administration of 4 vaccine doses), VNAs peak. In the absence of early and adequate PEP, virus enters host neurons, spreads to the central nervous system (CNS), and causes disease, with inevitably fatal consequence.

§ Human rabies immune globulin.

¶ Day vaccine administered.

4 Reprinted from Rupprecht CE et al., 2010 (Ref. 39). See: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5902a1.htm, accessed November 2017. Reprinted with permission.

Early experiments established the primary role of VNAs in protection against a productive viral infection. Rabies vaccination with inactivated virus stimulates B lymphocytes and, in conjunction with CD4+ T lymphocyte help using major histocompatibility complex class II (MHCII) molecules, induces production of antibody-secreting plasma cells that result in VNAs migrating to the site of the infection and into the nervous system parenchyma *(3,48)*. Neutralizing antibody alone has been shown to clear virus from the central nervous system of mice infected with the virus *(49)*. Cytotoxic (CD8+) T lymphocytes have been proven to be activated by rabies vaccination. To identify the components of the immune system responsible for protection against virus infection, additional experiments investigating the role of cell-medicated immunity in mice have confirmed that cytotoxic T-cells alone do not protect against rabies, as the depletion of CD8+ T cells had no effect on the resistance to disease or on the survival rate of vaccinated animals *(48,50)*. Immunization of victims of exposure plays a major role in protection by the activation of both CD4+ T cell and B cells, ultimately resulting in the production of VNAs that target and destroy the virus before the disease is manifested *(51,52)*.

Inactivated rabies virus, as component of the vaccine, is taken up by antigen-presenting cells (APCs) either in the lymphatic system after IM injection or in the epidermis (which is rich in APC cells) after ID injection for ultimate activation of T cells and B cells responsible for VNA production. The immune characteristics of both the humoral and cellular immune response after rabies vaccination were studied in 17 healthy patients and in five patients suffering from a combined B- and T-cell immunodeficiency *(53)*. In all healthy patients, enzyme-linked immunosorbent assay (ELISA) test results indicated that at one week after primary vaccination there was a significant rise in the level of immunoglobulin (Ig) M. At two weeks after primary vaccination there was a significant rise in the level of IgG (IgG1 and IgG3) and IgA. In the same study, after a booster vaccination was administered, the level of IgG increased significantly faster (measured one week after the booster dose) than after the primary series of doses was administered. Overall, IgG1 is the major IgG subclass present after primary and booster rabies vaccination *(53)*. The highest cellular immune response (as measured by the lymphocyte proliferation stimulation index of 3H thymidine uptake in cell culture) was detected 13 weeks after primary vaccination and 4 weeks after booster vaccination. The five patients with a combined immunodeficiency, vaccinated using the same protocol, showed a number of abnormalities in the humoral and cellular immune responses – such as decreased cellular immune responses and delayed peak IgG responses or limited Ig class humoral responses. Two additional studies investigating the humoral and cellular response to rabies vaccination indicated that both type 1 and type 2 cellular cytokines are produced, with one type or the other prevalent in individuals and a significant correlation between IFNγ and IL-4 with levels of VNA *(54,55)*. In addition, no difference in type 1 or type 2 magnitude of responses was noted between the ID or IM routes for post-vaccination, and booster vaccination resulted in higher cytokine as well as VNA responses, demonstrating that both arms of immunity are boosted *(55)*.

Following experimental inoculation of virulent rabies virus into animals, the virus may either replicate at the site of inoculation (usually muscle tissue) or enter directly into the peripheral nerves innervating the wound site without replication *(56)*. Once virus enters the neurons, neutralization may potentially be possible although, according to earlier research, it seems to be less likely *(57)*. Recent research into the mechanisms of immune cell and antibody crossing the blood brain barrier in infection suggests that a specific sequence of events and timing must unfold in order for efficient immunity to occur in the CNS *(58,59,60)*. However, the pathogenesis of rabies has not yet been completely defined and, because the administration of PEP has been effective several days-to-months after an exposure has occurred, it is possible that VNA can occasionally clear rabies virus from the CNS *(61,59)*.

Antibodies specific for other viral proteins besides G (specifically N) have been detected in the sera of human subjects. Published reports indicate that N-specific antibodies do not neutralize rabies virus, and therefore these specific anti-N antibodies are unlikely to play a major role in protective humoral immunity. At present, the role of non-neutralizing viral antibodies in providing immunity against disease is not fully understood *(29,62)*. There is no specific level of VNA that is recognized as being "protective" against rabies in humans, although WHO recommends an antibody level of 0.5 IU/mL as being evidence of an adequate immune response after vaccination *(15,42,63)*. This is also the level that is accepted as protective in dogs and cats *(64)*. In wildlife, protection is also highly probable at levels near 0.5 IU/mL *(65)*.

2.4 Role of passive immunity (HRIG, ERIG, Mabs)

Because of the critical role that VNA plays in prophylaxis, the level of protection against this disease can be enhanced through the immediate administration of RIG into wounds inflicted by a rabid animal. The administration of RIG delivers VNA specifically targeted against the virus to the anatomical region where it was injected during the trauma of the exposure. Clinical evidence collected during a field study in Iran in 1954 proved convincingly that the administration of anti-rabies antiserum (in conjunction with vaccine) into patients who were severely exposed to rabid animals reduced the risk of rabies *(66)*. In this pioneering early study, different doses of anti-rabies serum and/or vaccine were administered to 29 patients who had received severe bite wounds from a rabid wolf. Of the 29 bite victims, 17 who incurred severe head wounds were treated as follows: five patients received two doses of anti-rabies serum plus vaccine (all five patients survived); seven patients received one dose of anti-rabies serum plus vaccine (one patient subsequently died of rabies); and five patients received only vaccine (three patients subsequently died of rabies). One six-year old patient who had received exceptionally deep head wounds, including a crushed skull, received six doses of serum over a six-day period, plus vaccine, and survived. The other patients involved in the exposure were bitten in the trunk and legs and were administered either vaccine alone, or vaccine and serum. These patients all survived.

The RIG should be infiltrated into and around the wound sites of patients bitten by rabid animals to neutralize virus that may have been deposited in tissues during exposure *(15)*. Human rabies immune globulin (HRIG) produced in human subjects is administered at a dose of 20 IU/Kg of body weight, and equine rabies immune globulin (ERIG) produced in horses is administered at a dose of 40 IU/Kg of body weight. Unfortunately, due to the expense and lack of availability of RIGs, not all patients who should receive passive immunity as part of PEP actually have access to this life-saving product *(15,22,67)*. Although the administration of vaccine alone will save most patients, some patients will need to receive passive immunity immediately to survive *(68)*. Patients with bites into highly innervated regions such as the head or hands, and those with deep or multiple wounds, are the most vulnerable and most in need of RIG *(68,69)*. Specific recommendations for administration of RIG as part of PEP are detailed elsewhere *(15,42)*.[5]

2.5 Routes of active immunization

The first CCVs, initially administered IM, were regarded as the solution to replace early reactogenic NTVs that normally induced a low or moderate immune response *(70)*. However, the high cost of CCVs relative to the cost of NTVs, and the large number of patients who required PEP in countries endemic for canine rabies initially curtailed the widespread use of CCVs. In an effort to alleviate the situation by reducing the cost of CCVs without lowering the efficiency of the vaccine, clinical trials were conducted to investigate the efficacy of ID regimens using a fraction (60–80%) of the IM vaccine dose for PEP *(70,71,72,73)*.

Over the past two decades, results from several clinical trials have confirmed the immunogenicity and efficacy of the ID route for rabies PEP that is now used being effectively in many Asian countries – including India, Pakistan, Philippines, Thailand, and Sri Lanka – and is increasingly being used in African countries, including Madagascar and the United Republic of Tanzania *(40,72,74,75,76,77,78,79)*. The ability of the ID route to induce an immunological response is based on the fact that the skin is an important immune organ and vaccine efficacy is enhanced when antigens are presented into the dermal layer *(80,81,82)*. Furthermore, the administration of antigens into the skin layer facilitates their exposure to the numerous antigen-presenting cells, such as macrophages and dendritic cells that are present in higher numbers in skin than in muscle *(83)*.

[5] See: http://www.who.int/rabies/human/en/index.html, accessed 29 October 2017.

2.6 Immune response in different risk groups

Modern CCVs are among the most immunogenic vaccines in the world, as is evidenced by the very few reported human rabies deaths in patients who received prompt PEP according to WHO's recommendations *(29,38,84)*. A few reports have examined the immune response after rabies vaccination in various populations that are or could be immunosuppressed *(85,86,87,88)*. A recent study from Iran evaluated VNA in 50 patients with various medical conditions who presented for PEP with Category II and III bite wounds. The patients included persons with the following conditions: pregnancy; diabetes type 1; diabetes type 2; chronic infection with hepatitis B virus; different types of cancer; and immunocompromised due to receiving corticosteroids for rheumatoid arthritis and for lupus erythematosus *(89)*. Lower titres were reported in patients with cancer and diabetes II but all patients developed an immune response above 0.5 IU/mL by Day 14.

Immunosuppressed patients

Rabies vaccines are highly immunogenic in almost every population, with perhaps the exception of immunosuppressed patients with very low CD4+ cells. A few published studies have examined the immune response of rabies vaccine in HIV-infected patients *(85,86,87,88,90,91)*. One recent study reported lower VNA titres in patients receiving intermittent sustained antiretroviral therapy (ART) *(91)*. The objective of this study was to examine the affect that non-adherence to ART had in the immune response to a neoantigen in HIV-infected patients. In this study, a cohort of patients with CD4+ counts of 200–350 cells/µl received antiviral suppression treatment for 6 months. A cohort of subjects was then separated into two groups and received a three-dose PrEP rabies vaccination regimen. Group 1 (n = 25) continued to receive uninterrupted treatment with ART for 72 weeks and Group 2 (n = 26) received intermittent treatment with ART for 72 weeks. VNA responses were initially similar in patients from both groups. However, VNA titres decreased at a greater rate in Group 2 and, at week 80, 74% of subjects in Group 1 had VNA titres above 0.5 IU/mL while 24% of subjects in Group 2 had VNA titres above 0.5 IU/mL. Patients from both groups received one booster dose of rabies vaccine at the end of the study regardless of their antibody level. After the booster, similar proportions of subjects in each group – 100% of subjects tested in Group 1 and 95% of subjects tested in Group 2 – had VNA titres above 0.5 IU/mL, demonstrating that intermittent treatment with ART did not impair the ability to mount a recall response.

In another study investigating the immune response of CCV in selected populations of HIV-infected adults, only 57% of symptomatic HIV-infected patients with CD4+ counts below 400 developed a measurable VNA response above 0.5 IU/mL after receiving a 5-dose regimen of PEP intramuscularly on days 0, 3, 7, 14 and 30 *(85)*. In another study, 10 HIV-infected adults with CD4+ counts between 25 and 472 were given a multi-site PEP regimen whereby four doses of CCV were administered ID on days 0, 3 and 7 and two doses of CCV were administered ID on days 28 and 90 ("4-4-4-0-2-2") *(87)*. A lower-than-expected immune response was reported in all 10 patients; two of the patients did not develop VNA titres above 0.5 IU/ml by Day 14, and one of those patients did not develop VNA titres above 0.5 IU/ml by Day 30. In another study, the immune response to a three-dose IM PrEP regimen was examined in 13 HIV-infected children with CD4+ counts that were below normal, and was compared to the immune response in nine uninfected children *(92)*. In this study, children with fewer than 15% of the normal CD4+ cells had significantly

lower VNA titres when compared with the control group, while four of the 13 HIV-infected children failed to develop any measurable VNA. In a more recent study that examined the immune response after vaccination with CCVs in HIV-infected patients receiving highly-active antiretroviral therapy (HAART), slightly lower IgG and IgM titres were reported in older patients infected with HIV *(82).* However, this study also reported that 63% of patients receiving HAART still had measurable antibody titres five years after primary vaccination. In another study evaluating more effective vaccination protocols in immunosuppressed patients, two groups of HIV-positive subjects – one with CD4+ counts below 200 and the other with CD4+ counts above 200 – received a modified multiple eight-site series of PEP consisting of eight intradermal injections on each of days 0, 3, 7, 14 and 30. All subjects responded with titres above 0.5 IU/mL (93). PEP administered to children who were exposed to rabies while receiving immunosuppressive therapy after solid organ transplants was also reporded to be successful in all patients *(94)*.

There have been a few documented cases of rabies occuring in patients who unknowingly received donated organs from infected patients *(95,96,97)*. In all reported cases, the diagnosis of rabies in the original organ donor was not confirmed until several weeks or months after the recipient was diagnosed with rabies *(95,97,98,99)*. Few data are available on the immune response to PEP in patients receiving immunosuppresive drugs before, during or after receiving a solid organ transplantion (SOT). A recent review paper examined the immune response in SOT recipients who had received rabies virus-infected organs *(100)*. In the two studies examined in this report, there was a decrease in the VNA levels 28 days after an adequate level of VNA was reached. VNA titres dropped more quickly than in healthy patients *(100)*. In another paper that examined the VNA titres in immunosuppressed recipients of rabies virus-infected organs, VNA levels after a five-dose PEP regimen were detectable but significantly lower than those reported in healthy persons *(97).*

Infants and the elderly

The immune response to rabies vaccine in older and younger populations, without specific immunosuppressive conditions, is also reported to be adequate, although some reports indicate lower VNA titres in children under 5 years of age and in adults over 60 years of age *(101,102,103)*. In a published report that reviewed two studies examining immune responses in subjects of various ages, a reduction was observed in the level of VNA after vaccination in older individuals *(101)*. In one of the reported studies, the immune response of 260 subjects aged between 11 and 25 years who received a 6-dose PEP regimen was compared to patients above the age of 50 years receiving the same regimen. In this study, 52% of the adults above 50 years of age had significantly lower VNA titres after PEP compared to the younger cohort *(102)*. In another study involving 875 patients aged 2–74 years who received either PEP or PrEP, no significant difference in the production of VNA compared to either age or sex was reported *(104)*. The immune respone to rabies PEP was also reported to be highly immunogenic in children with confirmed malnutrition between Grade I and Grade IV *(34)*.

Patients taking antimalarial treatment

The administration of rabies vaccine by the ID route has been reported to produce reduced titres in patients taking chloroquine for antimalarial treatment and, for this reason, vaccines should be administered to this group of patients *(15,39,105,106,107)*.

Pregnant women

Rabies PEP is not counterindicated for pregnant women and is immunogenic, safe and highly effective in this population *(15)*. Rabies PEP should never be withheld from pregnant women as it is a life-saving vaccine. No risk of abortion or other harm to the fetus has been reported due to administration of PEP with CCV in pregnant women *(108,109,110)*.

3. Duration of immunity after immunization

3.1 Primary immune

After initial vaccination, both antibody-secreting plasma cells and memory B and T cells are produced to maintain a level circulating VNA and the ability to mount a secondary response quickly upon subsequent viral antigen exposure. The development of immunological memory after immunization with CCVs is a critical component in establishing long-lasting immunity against rabies in humans *(3)*. Among the millions of persons who have received CCVs, less than a handful of vaccination failures have been reported – all of which occurred in developing countries, and most of which involved deviations from WHO's recommended PEP protocol *(29,38,84,111)*. The indication for post-exposure vaccination with or without rabies immune globulin depends on the type of contact with the rabid animal.

Table 3: Types of contact with rabid animals[6]

Category	Category of exposure to suspect rabid animal	Post-exposure measures
Category I	Touching or feeding animals, licks on the skin	No treatment required
Category II	Nibbling of uncovered skin, minor scratches or abrasions without bleeding, licks on broken skin	Immediate vaccination
Category III	Single or multiple transdermal bites or scratches, contamination of mucous membrane with saliva from licks; exposure to bat bites or scratches	Immediate vaccination and administration of rabies immune globulin are recommended in addition to immediate washing and flushing of all bite wounds and scratches

Although one human death has been reported in a person who was previously vaccinated ID with a CCV and subsequently exposed to a rabid puppy *(112)*, this patient did not seek, nor was she given, the WHO recommended PEP booster series after the exposure occurred. In addition, she was receiving chloroquine to prevent malaria, which has been associated with reduced RVNA levels *(105)*. A study evaluating vaccine potency levels verified the correlation between antigenic content of an ID dose and the VNA level produced; the results confirm the WHO recommendation for vaccine potency (2.5 IU per IM dose) *(113)*. Several clinical trials and retrospective studies have been published that provide evidence that CCVs produce both adequate initial antibody response and long-lasting immunity to rabies.

[6] Adapted from WHO's *Guide for post-exposure prophylaxis.* See: http://www.who.int/rabies/human/postexp/en/, accessed November 2017.

3.2 Duration of rabies virus-neutralizing antibody

Measurement of VNAs is the most convenient method of confirming an immunological response after rabies PrEP or PEP. The duration of humoral immunity after vaccination against non-replicating viral antigens has been demonstrated to be several years long and very stable *(114)*. The relationship between the number of doses a patient receives during the initial vaccination (PrEP or PEP) and the longevity of circulating VNA has been examined in several studies *(104,115,116,117)*. In one retrospective study, a Kaplan-Meier survival analysis was used to evaluate the longevity of antibody in 875 patients who received either a primary three-dose (IM or ID) PrEP series or a five-dose IM PEP series of human diploid cell vaccine (HDCV) *(104)*. The study reported no significant differences between the number of doses of vaccine a patient received and the length of time after initial vaccination that VNA could be detected. In that study, no booster dose of vaccine was administered after the primary series and blood samples from patients were tested at various time intervals up to nine years after primary vaccination. Circulating VNA was detectable for a longer period in patients who were vaccinated IM as opposed to patients who were vaccinated by the ID route, with approximately 80% of patients who received vaccination IM still having detectable VNA titres nine years after primary vaccination.

The longevity of the humoral immune response was also evaluated in 18 patients who had received their primary series of PrEP or PEP using HDCV or purified chick embryo cell vaccine (PCECV) 2–14 years previously *(118)*. The patients in this study did not receive a booster vaccination between their initial series and the subsequent drawing of blood that was analysed for the presence of VNA. All patients in the study had detectable VNA titres up to 14 years after having received their initial vaccination. In another study, levels of VNA were evaluated in 58 patients who received, more than five years previously, PEP using HDCV, purified Vero cell rabies vaccine (PVRV), purified duck embryo cell vaccine (PDEV) or PCECV by either the Essen IM or Thai Red Cross ID regimen *(119).* All patients had detectable VNA at the time their blood was drawn. In another study examining the longevity of antibody and the effect of booster vaccination in 118 patients aged 16–78 years and vaccinated 5–21 years previously with either HDCV or PVRV, all patients had detectable antibody titres when they were tested prior to being given a booster dose of vaccine *(117)*. Finally, a study conducted in 29 travellers who had received their initial PrEP using HDCV by the ID route reported long-lasting immunity in patients who had received their primary vaccination between two and 10-plus years previously *(115)*.

In addition to published data delineating the extended duration of circulating VNA in patients who received only a primary PrEP or PEP vaccination series without an additional booster vaccination at one-year post-primary vaccination, studies have reported long-lasting VNA in patients who received a primary series of PrEP followed by one booster one year later. In one study, 312 subjects were followed for 10 years after receiving either a two-dose or three-dose PrEP regimen, with either HDCV or PVRV, and one booster dose of vaccine one year later *(120)*. The results indicate that approximately 96% of all subjects who received the three-dose PrEP regimen followed by one dose of vaccine one year later still had measurable VNA 10 years after having received their initial series. Similar results were reported in a study in which 10 subjects who had received their initial PrEP series with PCECV 14 years earlier were administered a booster dose one year later *(121)*. In another study, conducted in 72 Vietnamese children, half of the children received a three-dose series of a combined

diphtheria, tetanus, whole-cell pertussis and inactivated poliomyelitis vaccine (DPT-IPV) along with three doses of PVRV given at two and four months and one year, and the other half of the children received only DPT-IPV *(122)*. Results from this study indicated that rabies vaccines had no effect on the long-term antibody levels of diphtheria and poliomyelitis, and the majority of children continued to have measurable VNA titres throughout the five-year follow-up study. Similarly, a study was conducted in 200 Thai children who were vaccinated with PCECV in either a 2-dose or 3-dose IM or ID PrEP regimen concomitant with Japanese encephalitis vaccine (JEV), followed by a booster dose of PCECV either IM or ID (as per the original route of vaccination) one year later, plus a booster dose of JEV *(123)*. Three years after primary vaccination, all children who received their initial PrEP series by the IM route or who received a 3-dose PrEP ID regimen still had detectable VNA.

3.3 Anamnestic response

Two of the arguments in favour of administering PrEP to persons at risk of contracting rabies are that in the event that a previously vaccinated person is subsequently exposed to rabies:

1) a short series of booster vaccinations will elicit a rapid anamnestic response, thus reducing the number of doses of vaccine and visits required for a full PEP;

2) RIG is not required *(15)*.

Several published clinical trials provide data confirming that a previously vaccinated person will respond to one or more booster doses of rabies vaccine even if the initial series of PrEP or PEP was administered several years previously – regardless of whether the initial vaccination regimen was administered IM or ID, regardless of whether they are boosted using the ID or IM route, and independent of whether the previously vaccinated person has detectable VNA or not *(117,119,124,125,126)*. Long-lived antibody-secreting plasma cells and memory B and T cells may be regulated independently and therefore play different roles in maintenance of immunity *(114)*. This indicates that booster doses activate memory cells while not affecting long-lived antibody production, ensuring immunity coverage in both the short and long term. Booster vaccination of persons with either high or low levels of RVNA results in high levels of RVNA in circulation; the immune system is designed to regulate activation of memory cells in a mechanism dependent on the level of circulating antibody specific for the target antigen *(127)*. A three-year study conducted in 194 subjects who initially received one, two or three doses of HDCV administered by either the ID or IM route, and who were boosted 6–24 months later with one dose of HDCV administered by the ID or IM route, reported the highest titres and longest-lasting antibodies in the subjects who had received an initial 3-dose vaccination series (ID or IM) *(124)*. All subjects in this study, regardless of whether they had received one additional dose of vaccine ID or IM, had an anamnestic response when boosted at 6, 12 or 24 months later.

Another study reported that an anamnestic response occurred in 76 individuals initially vaccinated with HDCV by the ID route and then boosted two years later with one ID dose of HDCV *(125)*. The anamnestic response occurred in all persons regardless as to whether they had a detectable antibody titre just prior to the administration of the booster, or not. Similar results were reported in a study in which 29 travellers were initially vaccinated with a 3-dose ID HDCV regimen and boosted with one IM dose of HDCV 2–14 months later *(126)*. In this study, all persons developed an anamnestic

response even though some did not have detectable titres at the time that they were boosted. In another study, the immune response of 57 patients vaccinated for PEP, either by the 5-dose Essen regimen or by the ID Thai Red Cross regimen, were evaluated for a subsequent anamnestic response after receiving a booster vaccination *(119)*. In this study, patients were vaccinated 5–10 years previously with HDCV, PCECV, PVRV or PDEV, and titres were evaluated after patients were boosted with two ID doses of PDEV. All patients developed an anamnestic response after boosters were administered and there was no significant difference between the antibody levels in patients that had received vaccination 5–10 years earlier and those that had been vaccinated more than 10 years previously. In another study the immunological response was examined in 118 patients who had received primary PEP or PrEP with HDCV or PVRV 5–21 years earlier and were boosted with two ID doses of PVRV to determine if they would mount an anamnestic response *(117)*. In this study, all patients vaccinated up to 21 years previously developed an immunological response with no significant difference between the level of titres in patients who received PrEP or in those who received PEP, nor in the length of time since their initial vaccination was administered.

Table 4: Summary of data on anamnestic response according to vaccine administration

Initial vaccination	Route of administration	Booster schedule	Timinig of booster	Results	Author
Initially received 1, 2 or 3 doses of HDCV	ID or IM	1 dose of HDCV	6–24 months	All subjects in this study, regardless of whether they had received the vaccine ID or IM, had an anamnestic response when boosted	Turner GS et al.
Initially vaccinated with HDCV	ID	1 dose of HDCV	2 years	Anamnestic response occurred in all individuals	Horman JT et al.
Initially vaccinated with a 3-dose ID HDCV regimen	IM	with 1 booster dose	2–14 months	Anamnestic response occurred in all individuals	Gherardin AW et al.
Either by the 5-dose Essen regimen or the Thai Red Cross regimen with HDCV, PCECV, PVRV or PDEV	ID	boosted with 2 doses of PDEV	5–10 years	All patients developed an anamnestic response after boosters were administered No significant difference in the antibody level in patients who had received vaccination 5–10 years earlier and those who had been vaccinated more than 10 years previously	Naraporn N et al.
Received primary PEP or PrEP with HDCV or PVRV	ID	with 2 doses of PVRV	5–21 years	All patients vaccinated developed an immunological response with no significant difference in the level of titres whether patients received PrEP or PEP, nor in the length of time since their initial vaccination was administered	Suwansrinon K et al.

3.4 Timeliness of routine booster vaccination

Because rabies is virtually 100% fatal once clinical symptoms are evident and because until recently no long-term immunity studies were available, the recommendations for timely routine booster doses of rabies vaccine after a primary series has varied from one to five years. However, several clinical trials published recently have shown that persons who have received an initial 3-dose to 5-dose series of rabies CCVs will have long-term immunity that lasts for decades *(115,117,119,126)*. These published data also indicate that persons who received their primary series up to 21 years previously will elicit a good anamnestic response after booster vaccination.

As mentioned above, persons who have been vaccinated with a CCV will respond to a booster vaccination – due to the memory cells generated during initial vaccination – regardless of whether or not the vaccinated person had measurable antibody present at the time when the booster was administered *(125,126,128)*. A recent case of survival in a human patient who had been unknowingly given a transplanted liver from a donor who was later diagnosed as having rabies provides an indication of the efficacy of rabies vaccines *(99)*. The patient who received the infected liver survived whereas the recipients of the two kidneys and pancreas from the infected donor died of rabies encephalitis within three weeks following transplant. Further investigation revealed that the liver recipient had received rabies vaccination as a child.

4. Measuring immune response

Laboratory methods for detecting and quantitating the immune response to infectious rabies virus or inactivated rabies vaccine are numerous and can include a variety of serological and cellular techniques.

4.1 Choosing the test to fit the purpose

Many assays are available to test for the presence of virus in the tissues of infected mammals, and to confirm evidence of a humoral or cellular immune response after exposure to viral antigens *(129,130,131,132,133,134)*. Ultimately, the intended purpose of an assay, including the accuracy and precision requirements of the results produced, should be the determining factors when choosing a testing procedure. For example, confirming herd immunity after oral vaccination in animals generally does not require the same level of accuracy as does evaluating the immunogenicity of a new rabies vaccine for humans, or when serological testing is employed as part of the diagnostic workup for human rabies patients *(135,136)*. Practical considerations, such as ease of use and availability of facilities, cost, supplies and equipment will also play a role in selection of an assay. Because of the consequences associated with a misdiagnosis, the importance of the level of quality assurance associated with conducting any assay for evaluating an immune response for diagnoses of a patient, or after immunization, or for identifying virus antigens in tissue samples, cannot be overstated. In summary, the sensitivity and specificity of an assay, the accuracy and precision required by the investigator or clinician, the laboratory facilities that are available, and the purpose of the data to be collected should all be critically evaluated before testing of a sample's status is initiated *(134)*.

4.2 Virus neutralization assays

Virus neutralization assays are among the most widely-used methods of detecting the presence of antibodies to rabies virus. Virus-neutralizing antibodies (VNAs) are not only responsible for immunity against virus; the presence of VNA in serum is seen as a reliable indicator of active immunization after vaccination *(3,15)*. The rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralization (FAVN) test are both in vitro virus-neutralization assays. Both the RFFIT and the FAVN test are equivalent when conducted under good laboratory practices and both are considered to be the most efficient methods for accurate measurement of VNA *(15,135,137)*. The initial rabies virus neutralization assay, the mouse neutralization test (MNT), is an in vivo method to measure VNA that is still utilized in some laboratories that lack the capacity to conduct in vitro tests *(138)*. This method should be replaced by alternative methods whenever possible. Methodologies for all three virus neutralization assays are published elsewhere *(129,130,138,139)*. The RVNA threshold of 0.5 IU/mL

was established during the 1978 Joint WHO/International Association of Biological Standardization (IABS) symposium as a minimum level to demonstrate seroconversion 4 weeks after completion of the vaccination series, using the MNT and RFFIT methods *(63,140)*. Studies have demonstrated protection at 0.1 IU/mL in cats and 0.2 IU/mL in dogs; consequently, 0.5 IU/mL is a conservative RVNA threshold to account for inherent variability in antibody measurement with virus neutralization methods *(64,136)*. This is also the level recognized by OIE as confirmation of a satisfactory vaccine response for dogs and cats *(131)*. Virus neutralization assays are valuable tools that can confirm the presence of antibodies specifically targeted against the neutralizing epitopes of the rabies virus, but these tests are also highly complex to perform and must be conducted by experienced personnel in a high-containment facility *(131,134)*. It is advisable for diagnostic laboratories performing either one or both of these types of VNA assays to participate in an established quality assurance programme, including regular proficiency-testing *(134).*

4.3 Binding assays

The ELISA is the most frequently used binding assay available, with numerous published protocols and professionally marketed ELISA kits available to detect rabies virus antibodies *(141,142,143,144)*. The specificity of the ELISA depends on the choice of target antigen used in the test – whole virus or purified viral proteins. Antibodies detected in an ELISA do not necessarily have a neutralizing function *(134)*. Published reports indicate that cross-reactivity, potentially leading to false positives, may increase in ELISA assays that employ whole virus rather than purified G proteins as the target antigens *(144,145).* Several studies have been published comparing results from serum samples tested by various ELISA techniques and by the RFFIT or FAVN test, with mixed results. Therefore, applying the 0.5 IU/mL threshold for seroconversion is not appropriate unless the technique has been shown to produce equivalent results compared to serum neutralization *(141,143,146,147)*. ELISA kits with increased specificity, such as those using G protein as the antigen, have been reported to have better correlation with the RFFIT and FAVN. It is important to evaluate fully the correlation of neutralizing antibody levels to binding antibody measured in the ELISA across samples representing the kinetics of the humoral response before assigning cut-off levels and interpretation guidelines. For example, a study comparing RFFIT results to ELISA results from a human clinical trial study highlighted the difficulties of using the same cut-off level for both assays to obtain the same conclusions for vaccine comparison – i.e. the response as measured by ELISA peaked later and was lower in comparison to the RFFIT results *(147)*. Recently, a blocking ELISA kit has been shown to be useful in surveillance of oral baiting programmes; analysis of 359 fox and raccoon dog serum samples were tested with both the ELISA and FAVN tests and a concordance of 95% was observed *(142)*. The blocking ELISA was determined to be more sensitive in comparison to an indirect ELISA and was determined to be effective in measurement of rabies virus antibodies in haemolysed serum. A competitive ELISA assay using a cell line expressing the rabies virus G protein as the antigen and two labelled monoclonal VNA as the competing antibodies was used to measure VNA in 4350 canine samples in a comparative study using the FAVN test where the results indicated that there were no false positives or negatives and that there was a correlation of 96.2% between serological titres *(144)*. The remaining 3.8% of serum samples tested had titres above the level of 8.0 IU/mL when assayed by both testing methods, and the serological titre results from both tests were more divergent at high titre levels. In particular, ELISA kits have the ability to provide simplicity of reagent/material

control, and good repeatability compared to complex manual methods provided that attention to two critical factors is maintained – acceptable lot-to-lot quality control and adherence to the manufacturer's instructions for test performance.

4.4 Measuring cell-mediated immunity

Assays to measure a cell-mediated immune response are usually used for research purposes since they are more complicated to perform on a routine basis than serological assays. Detection of a cell-mediated immune response is commonly achieved by measuring an increase in lymphocyte proliferation using a [H3]thymidine assay. Methodologies for [H3]thymidine assays are published elsewhere *(54,118)*. Newer assays to measure cell-mediated immunity have been developed that utilize cell-tracking dyes in conjunction with flow cytometry and are able to quantify the response of specific types of lymphocytes to rabies virus antigens *(54,148)*. Indication of the cellular types (e.g. Th1, Th2) and magnitude of the cellular response can be obtained by measurement of cytokines produced by activated lymphocytes in cell culture *(55)*. Studies employing these techniques have provided insight into the range of cellular responses and their correlation to humoral immunity after vaccination *(54,55)*. Use of these techniques with antigens specific to other lyssaviruses, as has been the case for investigation of humoral immunity, has the potential to expand knowledge into the breadth and range of cellular immunity induced by current rabies vaccines *(149)*.

5. Innocuity and efficacy of rabies biologicals

The development and widespread use of rabies biologicals prepared on cell culture have dramatically increased the safety and efficacy of PEP *(42)*. Failures of rabies PEP have been reported in some patients in developing countries, but in most of these cases some deviation was reported from the WHO-recommended PEP protocol *(29,38,37,84)*. Generally, the reasons associated with such failures (where the correct PEP protocol was not followed) include: delays in seeking medical care; lack of, or improper, primary wound care; lack of, or improper, administration of RIG; suturing wounds without infiltrating with RIG; and poor-quality rabies vaccines *(29,38,150)*. The number of reported "true" PEP failures (where a patient died despite receiving the correct PEP protocol in a timely manner) is small compared to the millions of doses of CCVs administered globally each year *(29,150)*. Short incubation periods of less than one week have been reported in patients with severe head wounds, such as patients who sustained brachial-plexus injuries from dog bites *(24)*. In one paper that examined case records from 15 human rabies patients reported worldwide, it was concluded that seven patients received PEP in a timely and appropriate manner but nevertheless died of rabies *(38)*. The paper discusses potential reasons for these failures, including the possibility that a small unidentified wound may have been overlooked, that perhaps one or more of the patients may have had an underlying immunosuppressive condition, that the biologicals used to treat these patients were of low potency, or perhaps that the PEP protocols were misrepresented. Whatever the reason, all the failures occurred after dog bites and typing of the virus was not attempted; the paper stresses that, on rare occasions, failures may occur even with CCVs and RIGs.

Local and systemic reactions have been recorded following the administration of CCVs in clinical trials *(31,43,71,105)*. These studies generally reported local reactions, including pain, itchiness, redness and/or swelling at the site of injection in 35–45% of the enrolled subjects. Common systemic reactions, which are usually reported in 10–15% of subjects, include fever, myalgia, malaise, headaches, dizziness, hives and rash *(151)*.

6. Future prospects

The CCVs currently recommended by WHO are among the most efficacious vaccines available for combating disease. Only a few human deaths have been reported in the literature in cases when WHO-recommended PEP protocols were adhered to *(29,38)*. Despite this, rabies continues to kill tens of thousands of people and cost billions of US dollars annually *(20,21)*. Most of the burden of rabies falls on persons living in poor countries that can least afford to provide adequate PEP. Unfortunately, rabies continues to spread to previously rabies-free areas, causing human fatalities – as, for instance, following the introduction of canine rabies to the islands of Bali *(152)* and Flores, Indonesia, and the re-introduction of rabies to parts of Malaysia.

Although clinical rabies is preventable, even after exposure, a lack of educational awareness is one of the major reasons why humans exposed to bites from infected animals do not seek proper PEP after exposure *(153)*. The cost of rabies biologicals, and the frequent necessity to travel long distances over extended periods to receive one of the recommended WHO PEP regimens are also deterrents for persons exposed to rabid animals *(154,155)*. The fact that a plethora of vaccine regimens are recommended by WHO and the Advisory Committee on Immunization Practices (ACIP) can add to confusion, leading to incorrect administration of PEP. Nevertheless, most human deaths from rabies are the result of a lack of accessible PEP biologicals, thus increased availability is critical. Shorter regimens, reduced dosage through expanded use of intradermal administration and increased use of PrEP in the populations most at risk are efforts to reduce the burden of human rabies deaths. CCVs are now produced in China and India, providing products for some of the most affected areas of the world *(156)*. Newer rabies biologicals, such as monoclonal antibodies targeted against rabies virus antigens, are being evaluated in clinical trials and will hopefully provide wider global access to passive immunization at a reduced cost *(5)*. Reduced-dose regimens for PEP and PrEP, which can be completed within one week or for PrEP in one or two visits showed induction of an adequate and timely immune response to prevent disease. These regimens may also provide a solution for reducing the expense for patients who cannot afford to travel to clinics located outside their immediate area to receive multiple doses of rabies vaccine over extended periods of time *(157)*. Molecular techniques are providing new concepts for the development of rabies vaccines – such as subunit vaccines and safe modified live viral vaccines – which could reduce the number of PEP and PrEP doses required and significantly lower the cost of protecting an entire population *(158)*. In addition, diagnostic tests, such as the dRIT and immunochromatographic point-of-care devices that are inexpensive, simple to use and capable of producing accurate data rapidly will help facilitate surveillance in many poor settings *(159,160,161,162)*.

Finally, it is only through the introduction and embodiment of comprehensive rabies control strategies – including animal control programmes and particularly dog mass vaccination campaigns, PEP and PrEP for humans, educational programmes, financial commitment, and ultimately cooperation between public-health professionals, research scientists, laboratory technologists, not-for-profit organizations and government officials – that rabies will be controlled effectively *(163,164)*.

Rabies vaccine research focuses on reducing both the cost and the number of doses needed for PEP and PrEP. The use of selected adjuvants to increase the immune response is being evaluated, but adverse reactions also tend to increase. Evaluation is under way of polyinosinic-polycytidylic acid-based adjuvant (PIKA) and of monophospholipid A which has been utilized in hepatitis B and human papillomavirus (HPV) vaccines *(165,166,167,168,169)*. New vaccines under evaluation include genetic vaccines – DNA, virus vectors, bacterial vectors, protein and peptide vaccines *(170)*. Examples of these are a baculovirus-derived glycoprotein, parainfluenza virus 5 vector expressing G protein, and mRNA encoding the G protein *(171,172,173)*. All of which have been shown to be good immunogens and at least one could prove to be stable in storage and for distribution without a cold chain. In addition, an attenuated replication-deficient RABV is being studied, which would allow for a single-shot vaccination.

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[7] According to WHO's Guidelines for Declaration of Interests (WHO expert), an interest is considered "personal" if it generates financial or nonfinancial gain to the expert, such as consulting income or a patent. "Specificity" indicates whether the declared interest is a subject matter of the meeting or work to be undertaken. An interest has "financial significance" if the honoraria, consultancy fees or other received funding, including those received by expert's organization, from any single vaccine manufacturer or other vaccine-related company exceeds US$ 5 000 in a calendar year. Likewise, a shareholding in any one vaccine manufacturer or other vaccine-related company in excess of US$ 1000 would also constitute a "significant shareholding".

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