This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2012.009aV***	(to be completed by ICTV officers)

Short title: create 1 new species in the genus *Lyssavirus*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)					
	1 ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Tony Fooks (Tony.Fooks@ahvla.gsi.gov.uk) Denise Marston (Denise.Marston@ahvla.gsi.gov.uk),

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Rhabdoviridae Study Group

ICTV-EC or Study Group comments and response of the proposer: Supported with some modifications, which have all been completed.

Date first submitted to ICTV: 21/06/2013
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2012.009aV***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Lyssavirus***
Subfamily:	
Family:	***Rhabdoviridae***
Order:	***Mononegavirales***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Ikoma lyssavirus	JX193798

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

In general, demarcation criteria for lyssavirus species include (Dietzgen et al., 2011):

1. Genetic distances, with the threshold of 80–82% nucleotide identity for the complete N gene, that provides a better quantitative resolution compared to other genes, or 80–81% nucleotide identity for concatenated coding regions of N+P+M+G+L genes. Globally, all isolates belonging to the same species have higher identity values than the threshold, except the viruses currently included into the LBV species. For that reason some authors suggested that LBV be subdivided into several genotypes. However, as these LBV representatives are segregated into a monophyletic cluster in the majority of phylogenetic reconstructions, in the absence of other sufficient demarcation characters there is currently no possibility to subdivide LBV into several viral species.

2. Topology and consistency of phylogenetic trees, obtained with various evolutionary models.

3. Antigenic patterns in reactions with anti-nucleocapsid monoclonal antibodies (preceded by serologic cross-reactivity and definition of lyssavirus serotypes, using polyclonal antisera).

4. Whenever available, additional characters, such as ecological properties, host and geographic range, pathological features are recruited.

Phylogenetic and serological relationships correlate, which has enabled the delineation of two major phylogroups within the genus *Lyssavirus* (Badrane et al., 2001). Phylogroup I, includes rabies virus (RABV), Duvenhage (DUVV), European bat lyssaviruses type 1 and 2 (EBLV-1 and -2), Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV), and Irkut virus (IRKV). Phylogroup II, includes Lagos bat virus (LBV), Mokola virus (MOKV), and Shimoni bat virus (SHIBV). West Caucasian bat virus (WCBV) does not cluster in either phylogroup and has been suggested to be a representative of Phylogroup III. There is significant cross-neutralization within phylogroups, but very limited, to no cross-neutralization detected between phylogroups.

Based on the criteria above, Ikoma lyssavirus belongs to the *Lyssavirus* genus but cannot be included in any of the existing species. A new species should be created for this virus. The evidence is documented (Marston et al., 2012a and b).

Ikoma Lyssavirus (IKOV)

- Isolated from an African Civet (*Civetticus civetta*) in Tanzania in 2009. The African civet was displaying clinical signs consistent with rabies and had attacked a child without provocation.
- Pathogenic to laboratory mice via intracranial and peripheral inoculation, causing acute progressive fatal encephalitis (rabies).
- During infection, IKOV forms typical to lyssavirus, intracytoplasmic inclusions, detected by staining with FITC-conjugated anti-nucleocapsid monoclonal antibodies.
- The complete genome (Genbank JX193798) of IKOV consists of 11,902 nucleotides and includes 5 genes: Nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (see Annex Table 2 for comparison of gene and intergenic region sizes for all lyssaviruses).
- IKOV demonstrates 63.1-63.5% identity to other lyssaviruses using concatenated 5 gene sequences, much lower than the threshold of 80-81% (Annex Table 1)
- Phylogenetically IKOV belongs to the *Lyssavirus* genus (Annex Figure 1). Within the genus it is placed ancestrally to all other lyssaviruses and is the most divergent lyssavirus described to date. It groups with WCBV outside of both phylogroup I and II using Neighbour-joining, Bayesian and Maximum likelihood models (Annex Figure 1).
- Cross-neutralizing assays using a panel of human and dog sera with proven high serum neutralizing antibodies to RABV (CVS) clearly show little/no neutralization of the vaccinated sera to IKOV (Table 3)
- IKOV does not readily cross-react with WCBV (most closely phylogenetically related lyssavirus). Miniopterus bat sera neutralizing WCBV did not neutralize IKOV.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Bourhy H, Kissi B, Tordo N (1993) Molecular diversity of the Lyssavirus genus. Virology 194: 70-81.

Badrane H, Bahloul C, Perrin P, Tordo N (2001) Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. J Virol 75: 3268-3276.

Delmas O, Holmes EC, Talbi C, Larrous F, Dacheux L, et al. (2008) Genomic diversity and evolution of the lyssaviruses. PLoS One 3: e2057.

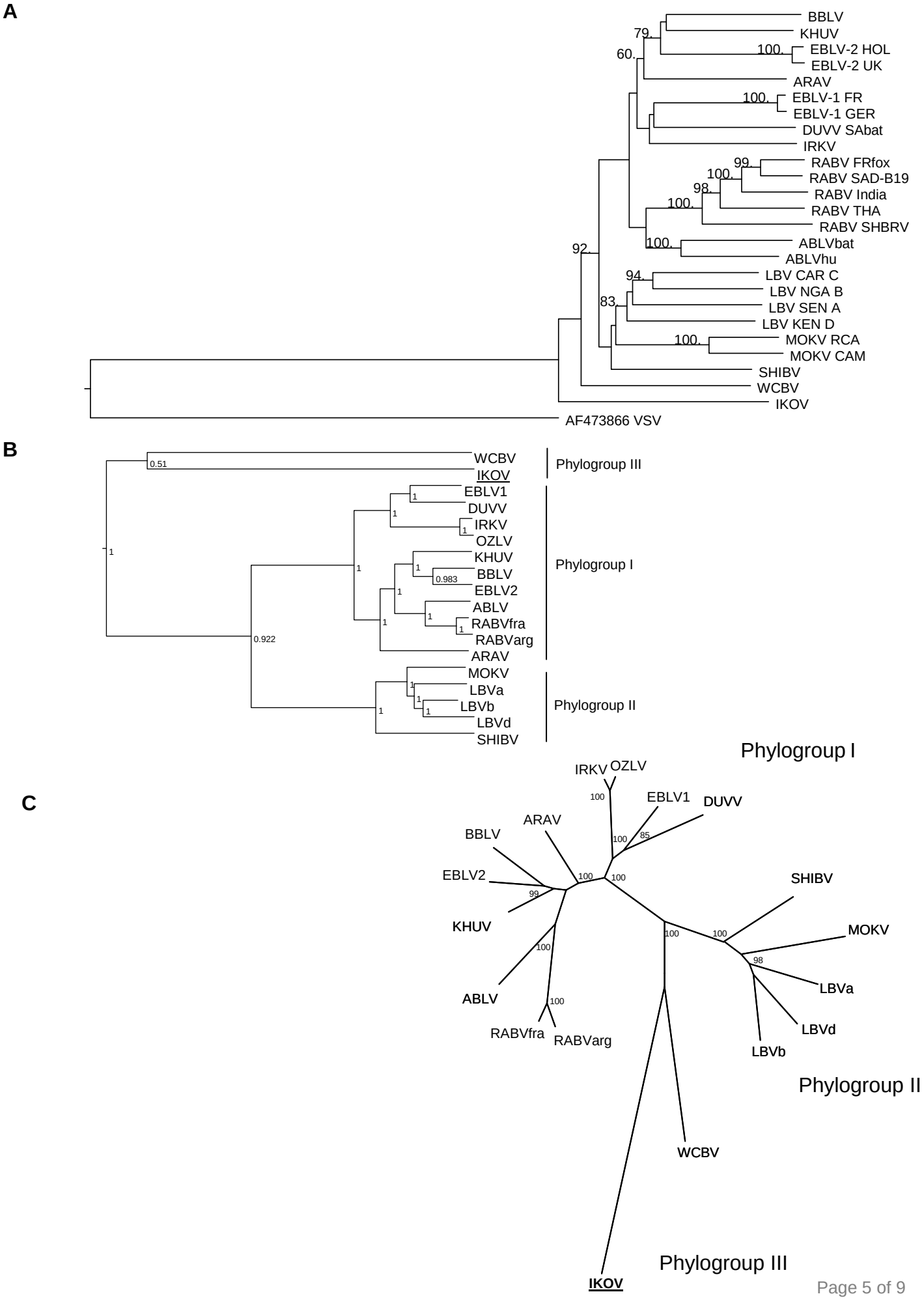
Dietzgen, R. G., Calisher, C. H., Kurath, G., Kuzmin, I. V., Rodriguez, L. L., Stone, D. M., Tesh, R. B., Tordo, N., Walker, P. J., Wetzel, T. and Whitfield, A. E. (2011). Rhabdoviridae. In Andrew M. Q. King, Michael J. Adams, Eric B. Carstens and Elliot J. Lefkowitz (Ed.), *Virus taxonomy: Ninth report of the International Committee on Taxonomy of Viruses* (pp. 654-681) Oxford, United Kingdom: Elsevier.

Marston DA, Horton DL, Ngeleja C, Hampson K, McElhinney LM, et al. (2012) Ikoma lyssavirus, highly divergent novel lyssavirus in an african civet. Emerg Infect Dis 18: 664-667.

Marston DA et al., (2012). Complete genomic sequence of Ikoma lyssavirus. J. Virol. 86 (18): 10242-10243.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.


A
BBLV
KHUV
EBLV-2 HOL
EBLV-2 UK
ARAV
EBLV-1 FR
EBLV-1 GER
DUVV SAbat
IRKV
RABV FRfox
RABV SAD-B19
RABV India
RABV THA
RABV SHBRV
ABLVbat
ABLVhu
LBV CAR C
LBV NGA B
LBV SEN A
LBV KEN D
MOKV RCA
MOKV CAM
SHIBV
WCBV
IKOV
AF473866 VSV
79.
60.
100.
100.
99.
100.
98.
100.
92.
100.
94.
83.
100.
B
WCBV
IKOV
EBLV1
DUVV
IRKV
OZLV
KHUV
BBLV
EBLV2
ABLV
RABVfra
RABVarg
ARAV
MOKV
LBVa
LBVb
LBVd
SHIBV
Phylogroup III
Phylogroup I
Phylogroup II
0.51
0.983
0.922
C
IRKV
OZLV
EBLV1
DUVV
ARAV
BBLV
EBLV2
KHUV
ABLV
RABVfra
RABVarg
SHIBV
MOKV
LBVa
LBVd
LBVb
WCBV
IKOV
Phylogroup I
Phylogroup II
Phylogroup III
0.2

Annex Figure 1. Phylogenetic reconstructions of the *Lyssavirus* genus (A), neighbour-joining using Megalign and (B), Bayesian, using Mr Bayes based on full N-gene sequences, and (C) full genome sequence with maximum likelihood evolutionary models using MEGA 5. Significant bootstrap values or posterior probabilities are shown for key nodes. Sequences used: RABV frafox (Rabies Virus French fox strain, EU293115), RABVarg (Rabies virus Argentina bat strain, EU293116), RABV SAD B19 (Rabies Virus SAD B19 vaccine strain, EU877069), RABV India (Rabies Virus India strain, EF437215), RABV THA (Rabies Virus Thailand strain, EU293111), LBV SEN A (Lagos Bat Virus, Strain A, Senegal, EU293108), LBV NGA B, (Lagos Bat Virus, Strain B, Nigeria, EU293110), LBV CAR C (Lagos Bat Virus, Central African Republic, Strain C, EF547449), LBV KEN D (Lagos Bat Virus, Strain D, Kenya, EU259198), MOKV RCA (Mokola Virus, Central African Republic, EU293118), MOKV CAM (Mokola Virus, Cameroon, EU293117), DUVV SAbat (Duvenhage Virus, South African bat, EU293120), EBLV-1 FR (European Bat Lyssavirus Type-1, France bat , EU293109), EBLV-1 GER (European Bat Lyssavirus Type-1, Germany, EF157976), EBLV-2 UK (European Bat Lyssavirus Type-1, United Kingdon, EF157977), EBLV-2 Hol (European Bat Lyssavirus Type-1, Netherlands, EU293114), ABLVbat (Australian Bat Lyssavirus, NC_003243), ABLVhu (Australian Bat Lyssavirus, AF418014), ARAV (Aravan Virus, EF614259), KHUV (Khujand Virus, EF614261), IRKV (Irkut Virus, EF614260), OZLV (Ozernoe Lyssavirus, FJ905105), WCBV (West Caucasian Bat Virus, EF614258), SHIBV (Shimoni Bat Virus, GU170201), BBLV (Bokeloh Bat Lyssavirus, JF311903), IKOV, Ikoma Lyssairus, JX193798).

Species	RABV	LBV	MOKV	DUVV	EBLV-1	EBLV-2	ABLV	ARAV	KHUV	IRKV	WCBV	SHIBV	BBLV	**IKOV**
RABV	81.6-92.3													
LBV	67.0-67.7	76.2												
MOKV	66.9-67.3	73.3-74.1	86.6											
DUVV	71.2-71.8	67.4-67.6	67.0-67.1	98.9										
EBVL-1	71.7-72.4	68.1-68.5	67.3-67.7	76.1	95.6-98.1									
EBLV-2	72.7-73.8	67.3-68.1	67.8-68.0	73.1-73.3	74.2-74.4	98.2								
ABLV	73.2-73.8	67.0-67.4	66.4-66.8	71.2	72.2-72.3	73.9								
ARAV	72.9-73.2	68.2-68.3	67.7-68.1	73.4-73.5	75.4-75.5	76.9	73.6							
KHUV	72.9-73.4	67.5-68.0	67.1-67.3	73.5-73.6	74.7	78.7-78.9	74.5	77.5						
IRKV	71.5-72.3	67.9-68.5	67.7-68.3	74.3-74.4	76.3-76.5	73.9	71.6	73.6-74.2	73.6-74.3	91.9				
WCBV	64.8-65.5	65.8-66.0	65.3-65.5	65.8	65.5-65.7	65.5	65.2	65.7	65.4	65.2				
SHIBV	67.2-67.7	73.8-75.1	71.9-72.0	67.7-67.8	68.2-68.3	68.1	67.2	68.1	67.9	68.7	66.4			
BBLV	72.7-73.6	67.5-67.9	67.6-68.1	73.1	74.2-74.3	78.2	74.3	76.3	78.4	73.6	65.1	68.7		
IKOV	61.9-62.5	62.7-62.9	62.3-62.5	62.5-62.6	62.6-62.7	62.8	62.3	62.6	62.4	62.4	63.2	63.5	62.5	

Table 1. Nucleotide identity values for concatenated coding regions (N, P, M, G and L genes) of IKOV in comparison with lyssaviruses from all identified species. Full genomes of BBLV (Bokeloh Bat Lyssavirus, JF311903), RABV (Rabies Virus, M31046; EU293111; EU293115; EU293113; EU293116), DUVV (Duvenhage Virus, EU293120; EU293119), EBLV-1 (European Bat Lyssavirus type-1, EU293109, EU293112, EF157976), IKOV (Ikoma Lyssavirus, JX193798), ABLV (Australian Bat lyssavirus, NC_003243; AF081020), KHUV (Khujand Virus, EF614261), IRKV (Irkut Virus, FJ905105, EF614260), EBLV-2 (European Bat Lyssavirus type-2, EF157977, EU293114), ARAV (Aravan Virus, EF614259), LBV (Lagos Bat Virus, EU293108; EU293110), MOKV (Mokola Virus, EU293118, EU293117), SHIBV (Shimoni Bat Lyssavirus, GU170201) and WCBV (West Caucasian Bat Virus, EF614258) were derived from NCBI Genbank. Concatenated sequences were aligned using ClustalW and a distance matrix was calculated as implemented in BioEdit.

Table 2: The lengths of coding and non-coding regions of lyssavirus genomes. See Table 1 legend for abbreviations.

	RABV	LBV	MOKV	DUVV	EBLV-1	EBLV-2	ABLV	ARAV	KHUV	IRKV	WCBV	SHIBV	**BBLV**	**IKOV**
3' UTR*	70	70	70	70	70	70	70	70	70	70	70	70	70	70
N protein	**1353**	**1353**	**1353**	**1356**	**1356**	**1356**	**1353**	**1356**	**1356**	**1356**	**1353**	**1353**	**1356**	**1353**
N–P	90-1	101	100-102	90	90	101	94	85	95	93	64	98	91	66
P protein	**894**	**918**	**912**	**897**	**897**	**894**	**894**	**894**	**894**	**897**	**894**	**918**	**894**	**870**
P–M	88	75	80	83	83	88	89	85	72	82	133	76	86	74
M protein	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**
M–G	211-5	204	203-204	191	211	210 (205)	207-209	210	208	214	206	205	210	209
G protein	**1575**	**1569**	**1569**	**1602**	**1575**	**1575**	**1578-1581**	**1581**	**1581**	**1575**	**1578**	**1569**	**1575**	**1575**
G–L	522	578-588	546-563	562-563	560	512	508-509	514	504	569	862	613	496	569
L protein	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6381**
5' UTR	131	145	112-114	130-131	131	131	131	130	130	131	125	150	129	126
Genome	**11 923-8**	**12006-16**	**11 940-57**	**11 975-6**	**11 966**	**11 930**	**11 918**	**11918**	**11903**	**11980**	**12278**	**12045**	**11900**	**11902**

Table 3. Cross neutralization of sera from rabies vaccinated humans and animals against IKOV in comparison with RABV. International units (IU) are given for RABV by comparison to a standard control (not applicable to IKOV). Reciprocal titres of over 10,000 are considered exceptionally high, and less than 20 would be considered below the detectable threshold and therefore effectively negative. HRIG (Human Rabies Immunoglobulin)

	VIRUS		
		CVS	**IKOV**
Sample ID	IU/ml	Reciprocal titre	Reciprocal titre
HUMAN - 1	23	959	<8
HUMAN - 2	31	1263	<8
HUMAN - 3	1093	34092	<8
HUMAN - 4	1093	34092	<8
DOG 1	53	1263	<8
DOG 2	41	960	<8
DOG 3	1094	25904	8
DOG 4	122	2878	<8
DOG 5	365	8635	<8
DOG 6	1	16	<8
DOG 7	1094	34109	<8
DOG 8	364	11364	<8
DOG 9	14	421	<8
DOG 10	41	1263	<8
HRIG	270	nd	<16