This form should be used for all taxonomic proposals. Please complete all those modules that are applicable.

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.

Part 1: **TITLE, AUTHORS, etc**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code assigned:** | ***2017.013M*** | | | | (to be completed by ICTV officers) |
| **Short title: One** new species (*Gannaruwa bat lyssavirus*)in the genus *Lyssavirus* | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| Denise A. Marston, Thomas Mueller, Conrad Freuling, Ashley C. Banyard and Anthony R Fooks | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| Denise.Marston@apha.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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | | | ***Rhabdoviridae* Study Group** | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
|  | | | | | |
|  | | | | | |
| Date first submitted to ICTV: | | | | 9 June 2017 | |
| Date of this revision (if different to above): | | | |  | |

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| **ICTV-EC comments and response of the proposer:** |
|  |

**Part 2**: **PROPOSED TAXONOMY**

|  |
| --- |
| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet: 2017.013M.N.v1.Lyssavirus\_sp** |

Please display the taxonomic changes you are proposing on the accompanying spreadsheet module 2017\_TP\_Template\_Excel\_module. Submit both this and the spreadsheet to the appropriate ICTV Subcommittee Chair.

**Part 4:** **APPENDIX**: supporting material

| additional material in support of this proposal |
| --- |
| **References:** |
| DIETZGEN, R., CALISHER, CH, KURATH, G, KUZMIN, IV, RODRIGUEZ, LL, STONE, DM, TESH, RB, TORDO, N, WALKER, PJ, WETZEL, T AND WHITﬁELD, A E (2011) Rhabdoviridae. In Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Ed A. KING, ADAMS, MJ, CARSTENS, EB AND LEFKOWITZ, EJ, San Diego: Elsevier. pp 654-681  GUNAWARDENA, P. S., MARSTON, D. A., ELLIS, R. J., WISE, E. L., KARAWITA, A. C., BREED, A. C., MCELHINNEY, L. M., JOHNSON, N., BANYARD, A. C. & FOOKS, A. R. (2016) Lyssavirus in Indian Flying Foxes, Sri Lanka. Emerg Infect Dis 22, 1456-1459  HORTON, D. L., BANYARD, A. C., MARSTON, D. A., WISE, E., SELDEN, D., NUNEZ, A., HICKS, D., LEMBO, T., CLEAVELAND, S., PEEL, A. J., KUZMIN, I. V., RUPPRECHT, C. E. & FOOKS, A. R. (2014) Antigenic and genetic characterization of a divergent African virus, Ikoma lyssavirus. J Gen Virol 95, 1025-1032  SCHNEIDER, L. G., BARNARD, B. J. H., SCHNEIDER, H. P., SCHNEIDER, L. G., ØDEGAARD, Ø. A., MUELLER, J., SELIMOV, M., SCHNEIDER, L. G., COX, J. H., WANDELER, A. I., BLANCOU, J. & MEYER, S. (1985) Application of Monoclonal Antibodies for Epidemiological Investigations and Oral Vaccination Studies. In Rabies in the Tropics. Eds E. KUWERT, C. MÉRIEUX, H. KOPROWSKI, K. BÖGEL. Berlin, Heidelberg, Springer Berlin Heidelberg. pp 47-59 |

|  |
| --- |
| **Annex:**  Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:   * **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing. * **Higher taxa**:   + There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.   + Please indicate the **origin of names** assigned to new taxa at genus level and above.   + For each new genus a **type species** must be designated to represent it. Please explain your choice. * **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance. |

In general, demarcation criteria for species in the genus *Lyssavirus* 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.

**Gannaruwa bat lyssavirus**

Isolated from four Indian flying-foxes (*Pteropus medius*) in Sri Lanka in 2014-15. The bats were collected from the ground, in an area inhabited by a long-established roost of approximately 20,000 Indian flying-foxes ([Gunawardena *et al* 2016](#_ENREF_2)).

1. Genetic distances, with the threshold of 80–81% nucleotide identity for concatenated coding regions of N+P+M+G+L genes to demarcate species.

* The complete genome (Genbank KU244266-9) of GBLV consists of 11,919 nucleotides and includes 5 genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (see Annex Table 1 for comparison of protein coding and non-coding region sizes for all lyssaviruses)
* GBLV demonstrates a range of identity to other lyssaviruses (concatenated genes range from 62.9-77.1% IKOV and ABLV, respectively). The two most closely related lyssaviruses are RABV and ABLV with 76.2-76.6% identity to RABV and 75.8-77.1% identity to ABLV, which is below the threshold of within species identity suggesting that GBLV is a virus belonging to a new lyssavirus species (see Annex Table 2).

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

* Phylogenetic analysis performed on concatenated gene sequences demonstrated limited relatedness between GBLV and all other lyssaviruses using neighbour-joining, and maximum likelihood reconstructions with Kimura-2 parameter and GTR+G+I models respectively. Regardless of the model or reconstruction used, the resulting tree topologies did not alter (Annex Figure 1).

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

* Antigenic patterns in reactions with anti-nucleocapsid monoclonal antibodies clearly distinguish GBLV from other lyssaviruses, most importantly from RABV and ABLV which are the most closely related genetically (Schneider et al., 1985; Annex Table 3)
* Cross-neutralizing assays using a panel of vaccinated human and dog sera with a range of neutralizing antibodies including proven high serum neutralizing antibodies to a laboratory adapted RABV strain (CVS), indicate that vaccination using a RABV vaccine strain cross-neutralises GBLV which is true for all phylogroup I viruses. All sera tested cross-neutralised GBLV as well as or less than RABV (methodology described in Horton et al., 2014). These data indicate GBLV is a member of phylogroup I, distinct from RABV. (Annex Table 4).

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

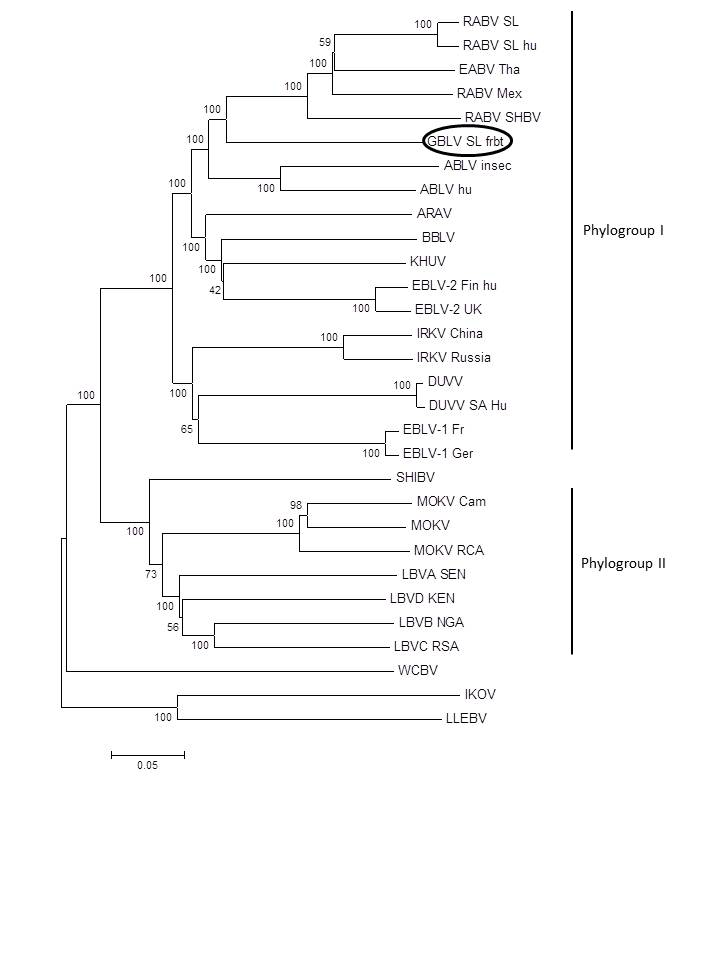
* Isolated from four Indian flying-foxes (*Pteropus medius*) in Sri Lanka in 2014-15. This is the first recorded lyssavirus isolation from *P. medius*.
* RABV has not been identified in a bat species in the ‘old world’ and ABLV has not been detected outside of Australia.
* 3 bats were dead on collection, the 4th, bat AK-42, displayed clinical signs consistent with rabies, including aggressiveness, biting, spontaneous vocalization and inability to fly.
* Pathogenic to laboratory mice via intracranial and peripheral inoculation causing acute progressive fatal encephalitis (rabies).
* During in vitro and in vivo infection GBLV forms intracytoplasmic inclusions, detected by staining with FITC-conjugated anti-nucleocapsid monoclonal antibodies (Fujirebio).

Taken together, these data suggest that GBLV represents a new species in the *Lyssavirus* genus ‘*Gannoruwa bat lyssavirus’*.

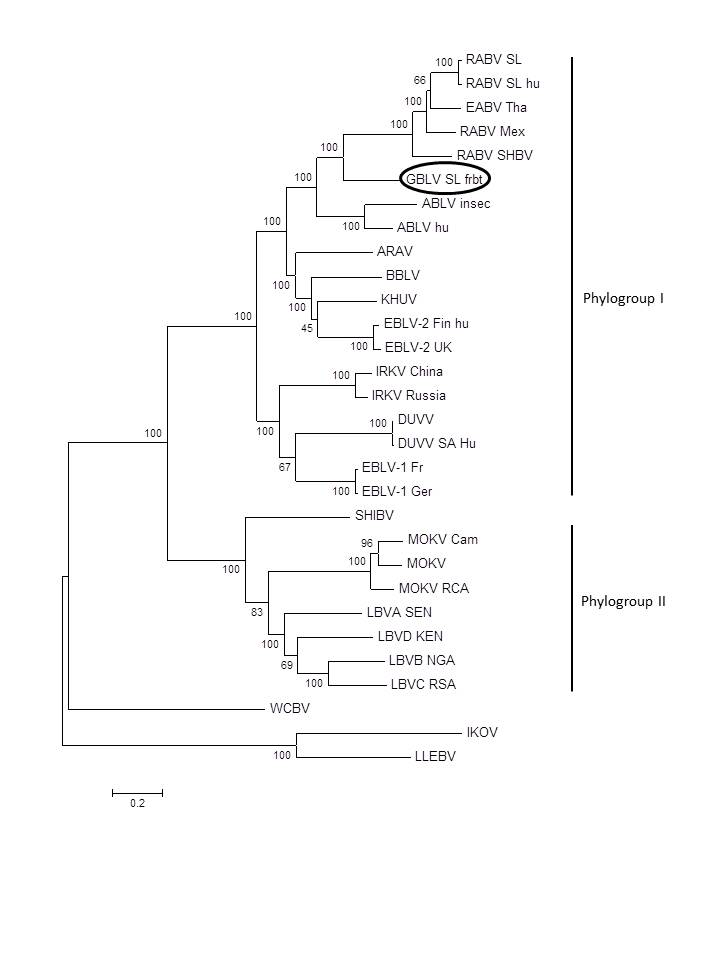
**Annex Figures and Tables:**

**Annex Figure 1:** Phylogenetic reconstructions of members of the *Lyssavirus* genus using concatenated gene sequences including the unclassified GBLV (KY006983) in (A) a neighbour-joining using Kimura-2 parameter implemented in MEGA 6, (B) maximum likelihood using GTR G+I evolutional model implemented in MEGA 6. Significant bootstrap values are shown. Full genomes of BBLV (Bokeloh bat lyssavirus, JF311903), RABV (rabies virus, HQ450386; EU293111; AB635373, AB589299), DUVV (Duvenhage virus, EU293120; EU293119), EBLV-1 (European bat lyssavirus 1, EU293109, EF157976), IKOV (Ikoma virus, JX193798), ABLV (Australian bat lyssavirus, NC\_003243; AF081020), KHUV (Khujand virus, EF614261), IRKV (Irkut virus, FJ905105, JX193798), EBLV-2 (European bat lyssavirus 2, JX129233, KF155004), ARAV (Aravan virus, EF614259), LBV (Lagos bat virus, EU293108; EU293110, GU170202), MOKV (Mokola virus, EU293118, EU293117, NC\_006439), SHIBV (Shimoni bat virus, GU170201), LLEBV and WCBV (West Caucasian bat virus, EF614258) were derived from NCBI Genbank.

(A)



(B)



**Table 1:** Protein coding, non-coding and genome lengths for all lyssaviruses with GBLV highlighted in grey.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Phylogroup I | | | | | | | | | | Phylogroup II | | | | Phylogroup III | | | |
|  | RABV | ABLV | GBLV | EBLV-1 | EBLV-2 | BBLV | DUVV | ARAV | KHUV | IRKV | | SHIBV | LBV | MOKV | WCBV | IKOV | LLEBV |
| 3' UTR\* | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | | 70 | 70 | 70 | 70 | 70 | 70 |
| **N protein** | **1353** | **1353** | **1353** | **1356** | **1356** | **1356** | **1356** | **1356** | **1356** | **1356** | | **1353** | **1353** | **1353** | **1353** | **1353** | **1353** |
| N–P | 90-1 | 94 | 92 | 90 | 101 | 93 | 90 | 85 | 95 | 92 | | 98 | 101-103 | 100-102 | 64 | 66 | 68 |
| **P protein** | **894** | **894** | **894** | **897** | **894** | **894** | **897** | **894** | **894** | **897** | | **918** | **918** | **912** | **894** | **870** | **870** |
| P–M | 87-89 | 89 | 88 | 83 | 88 | 86 | 83 | 85 | 72 | 83 | | 76 | 73-76 | 80-81 | 133 | 74 | 74 |
| **M protein** | **609** | **609** | **609** | **609** | **609** | **609** | **609** | **609** | **609** | **609** | | **609** | **609** | **609** | **609** | **609** | **609** |
| M–G | 211-5 | 207-209 | 212 | 211 | 210 (205) | 210 | 191 | 210 | 208 | 214 | | 205 | 204 | 203-204 | 206 | 209 | 198 |
| **G protein** | **1575** | **1578-81** | **1581** | **1575** | **1575** | **1575** | **1602** | **1581** | **1581** | **1575** | | **1569** | **1569** | **1569** | **1578** | **1575** | **1578** |
| G–L | 516-522 | 508-509 | 505 | 560 | 512 | 496 | 562-563 | 514 | 504 | 569 | | 613 | 574-588 | 546-563 | 862 | 569 | 608 |
| **L protein** | **6384** | **6384** | **6384** | **6384** | **6384** | **6384** | **6384** | **6384** | **6384** | **6384** | | **6384** | **6384** | **6384** | **6384** | **6381** | **6381** |
| 5' UTR | 130-133 | 131 | 131 | 131 | 131 | 129 | 130-131 | 130 | 130 | 131 | | 150 | 143-146 | 112-114 | 125 | 126 | 122 |
| **Genome** | **11,923-8** | **11,918** | **11,919** | **11,966** | **11,930** | **11,902** | **11,975-6** | **11,918** | **11,903** | **11,980** | | **12,045** | **12003-16** | **11,940-57** | **12,278** | **11,902** | **11,931** |
| **Concat** | **10,815** | **10,821** | **10,821** | **10,821** | **10,818** | **10,818** | **10,848** | **10,824** | **10,824** | **10,821** | | **10,833** | **10,833** | **10,827** | **10,818** | **10,788** | **10,791** |

**Table 2:** Nucleotide identity values for concatenated coding regions (N, P, M, G, L genes) of GBLV in comparison with all other identified lyssavirus species. See Annex Figure 1 for details of full genomes used. Concatenated sequences were aligned using ClustalW and a distance matrix was calculated as implemented in MegAlign.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | RABV | LBV | MOKV | DUVV | EBLV-1 | EBLV-2 | ABLV | ARAV | KHUV | IRKV | WCBV | SHIBV | BBLV | **IKOV** | | **LLEBV** | |
| RABV |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| LBV | 67.0-67.7 |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| MOKV | 66.9-67.3 | 73.3-74.1 |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| DUVV | 71.2-71.8 | 67.4-67.6 | 67.0-67.1 |  |  |  |  |  |  |  |  |  |  |  | |  | |
| EBLV-1 | 71.7-72.4 | 68.1-68.5 | 67.3-67.7 | 76.1 |  |  |  |  |  |  |  |  |  |  |  | |
| EBLV-2 | 72.7-73.8 | 67.3-68.1 | 67.8-68.0 | 73.1-73.3 | 74.2-74.4 |  |  |  |  |  |  |  |  |  | |  | |
| 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 | 73.9-74.3 |  |  |  |  |  | |  | |
| 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 |  | |  | |
| LLEBV | 61.3-61.4 | 63.4-64.5 | 63.0-63.6 | 63.1 | 63.5 | 62.7-62.9 | 62.9-63.5 | 63.0 | 63.6 | 63.9 | 64.6 | 64.8 | 63.2 | 71.1 | |  | |
| GBLV | 76.2-76.6 | 68.1-68.3 | 67.6-68.1 | 72.1 | 73.7-73.8 | 75.1-75.2 | 75.8-77.1 | 75.3 | 75.8 | 72.7 | 65.6 | 68.1 | 75.4 | 62.9 | | 63.9 | |

**Table 3:** Reaction pattern of a panel of 10 anti-nucleocapsid monoclonal antibodies with selected lyssaviruses. Note that MSA6.3 was no longer available for testing.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **anti-NC mAb** | **RABV** | **LBV** | **MOKV** | **DUVV** | **EBLV-1** | **EBLV-2** | **ABLV** | **BBLV** | **GBLV** | **LLEBV** |
| W239.17 | **+++** | **+++** | **+++** | **+++** | **+++** | **+++** | **+++** | **+++** | +++ | **-** |
| W187.5 | **+++** | **-** | **-** | **-** | **-** | **-** | **+++** | **-** | - | **-** |
| W187.11.2 | **+++** | **-** | **-** | **-** | **-** | **-** | **+++** | **+++** | +++ | **-** |
| MW187.6.1 | **+++** | **+++** | **+++** | **+++** | **-** | **-** | **+++** | **+++** | +++ | + |
| MSA6.3 | **-** | **-** | **+++** | **-** | **+++** | **+++** | **-** | **+++** | n.a. | n.a. |
| LBV7.36 | **-** | **+++** | **-** | **-** | **-** | **+++** | **-** | **-** | - | **-** |
| DUV6.15.19 | **-** | **-** | **-** | **+++** | **+++** | **-** | **-** | **-** | + | **-** |
| S62.1.2 | **-** | **-** | **-** | **-** | **+++** | **+++** | **-** | **-** | - | **-** |
| P 41 | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | - | **-** |
| Z144.88 | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | - | **-** |

**Table 4:** Cross neutralization of sera from rabies vaccinated humans and animals against GBLV in comparison with RABV. International units (IU) are given for RABV by comparison to a standard control (not applicable to GBLV). Reciprocal titres over 10,000 are considered exceptionally high, and less than 20 would be considered below detectable threshold and therefore effectively negative.

