VIRUS NOMENCLATURE BELOW THE SPECIES LEVEL: A STANDARDIZED NOMENCLATURE FOR LABORATORY ANIMAL-ADAPTED STRAINS AND VARIANTS OF VIRUSES ASSIGNED TO THE FAMILY FILOVIRIDAE

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#Members of the International Committee on Taxonomy of Viruses (ICTV) Filoviridae Study Group

Filoviruses – Animal-adapted Variants/Isolates 3
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ABSTRACT

The International Committee on Taxonomy of Viruses (ICTV) organizes the classification of viruses into taxa, but is not responsible for the nomenclature for taxa members. International experts groups, such as the ICTV Study Groups, recommend the classification and naming of viruses and their strains, variants, and isolates. The ICTV Filoviridae Study Group has recently introduced an updated classification and nomenclature for filoviruses. Subsequently, and together with numerous other filovirus experts, a consistent nomenclature for their natural genetic variants and isolates was developed that aims at simplifying the retrieval of sequence data from electronic databases. This is a first important step toward a viral genome annotation standard as sought by the US National Center for Biotechnology Information (NCBI). Here, this work is extended to include filoviruses obtained in the laboratory by artificial selection through passage in laboratory hosts. The previously developed template for natural filovirus genetic variant naming (<virus name> <isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>) is retained, but it is proposed to adapt the type of information added to each field for laboratory animal-adapted variants. For instance, the full-length designation of an Ebola virus Mayinga variant adapted at the State Research Center for Virology and Biotechnology “Vector” to cause disease in guinea pigs after seven passages would be akin to “Ebola virus VECTOR/C.porcellus-lab/COD/1976/Mayinga-GPA-P7”. As was proposed for the names of natural filovirus variants, we suggest using the full-length designation in databases, as well as in the method section of publications. Shortened designations (such as “EBOV VECTOR/C.por/COD/76/May-GPA-P7”) and abbreviations (such as “EBOV/May-GPA-P7”) could be used in the remainder of the text depending on how critical
it is to convey information contained in the full-length name. “EBOV” would suffice if only one EBOV strain/variant/isolate is addressed.

INTRODUCTION

Modern molecular virology, medical countermeasure development, and epidemiology are increasingly dependent on electronic databases that make exponentially increasing datasets, such as genomic sequence information, accessible and easy to interpret. Efforts to improve databases such as GenBank or efforts to develop novel databases (which in turn often are dependent on GenBank information) are often hindered due to lack of standardized nomenclature and classification systems for particular datasets. This is especially true for virology. The International Committee on Taxonomy of Viruses (ICTV, http://www.ictvonline.org) was tasked by the International Union of Microbiological Societies (IUMS) to make decisions on matters of virus classification and nomenclature for increased efficiency and consistency in the assignment of individual viruses to taxa (orders, families, subfamilies, genera, and species). However, the ICTV is currently not responsible for the nomenclature of viruses and their strains, variants, and isolates. This task is usually designated to the ICTV Study Groups, which serve as advisory committees. Fauquet observed correctly that it “is de facto accepted by the virologists that there is no homogeneity in the demarcation criteria, nomenclature and classification below the species level, and each specialty group is establishing an appropriate system for its respective family” [9]. Unfortunately, this also means that the naming of viruses and of their strains, variants, and isolates is more or less arbitrary and differs for viruses of one family to those of another. Recently, several ICTV Study Groups and other experts, including those of the US National
Center for Biotechnology Information (NCBI), have begun to develop more consistent naming schemes for virus strains, variants, and isolates in anticipation of increased submissions of population genomic sequences of viruses to databases resulting from increased availability of deep-sequencing technologies. The most notable naming scheme was developed by the Rotavirus Classification Working Group (RCWG) for rotaviruses in conjunction with the development of a new electronic database [30]. Members of the ICTV *Filoviridae* Study Group and many other filovirus experts have adopted most of the RCWG’s suggestions and have recently published a similar scheme for natural (aka, wild-type or naturally-occurring) filoviruses [25]. Here, it is proposed to expand this naming scheme to filoviruses generated by artificial selection through serial passages in laboratory hosts.

**SUMMARY OF NOMENCLATURE BELOW THE SPECIES LEVEL FOR NATURAL FILOVIRUSES**

The current, ICTV-accepted, taxonomy for filoviruses is summarized in Table 1 [1, 23, 24]. In terms of natural filoviruses, it was agreed that the term “strain” is currently not applicable [25]. Natural filovirus variants were defined as follows:

“A natural genetic filovirus variant is a natural filovirus that differs in its genomic consensus sequence from that of a reference filovirus (the type virus of a particular filovirus species) by \( \leq 10\% \) but is not identical to the reference filovirus and does not cause an observable different phenotype of disease (filovirus strains would be genetic filovirus variants, but most genetic filovirus variants would not be filovirus strains if a strain definition would be brought forward)” [25].
The definition for natural filovirus isolates is:

“A natural filovirus isolate is an instance of a particular natural filovirus or of a particular genetic variant. Isolates can be identical or slightly different in consensus or individual sequence from each other” [25].

Templates were proposed for naming individual natural filovirus variants and isolates for a) Materials and Methods sections of manuscripts (full-length designations); b) alignment and phylogram figures (shortened designations); and c) flow-text (abbreviations) [25]. These templates are generally organized in the order <virus name> <isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>. Suffixes were proposed to be used for natural filoviruses sequenced directly from the matrix of the initially infected organism in the absence of in vitro propagation (“-wt”), for filoviruses sequenced from cell or tissue cultures (“-tc”), for filoviruses sequenced only partially (“-frag”), or for unsequenced filoviruses not available for study anymore due to loss or destruction (“-hist”).

NOMENCLATURE BELOW THE SPECIES LEVEL FOR LABORATORY FILOVIRUSES

There are several non-natural filoviruses. For instance, Marburg virus (MARV), Ravn virus (RAVV), and Ebola virus (EBOV) have been passaged through adult rodents, such as laboratory mice and guinea pigs (Cavia porcellus Linnaeus, 1758), which do not develop disease upon
exposure to natural (wild-type) filoviruses and which are not known to be infected by filoviruses in nature. Serial passaging in these rodents, however, culminated in filoviruses that cause disease and death (for studies on adaptation see, for instance, [2, 5-8, 11-18, 20-22, 26-29, 31-39, 41, 42]), and recent studies correlated this evolution with specific changes in the genomes of these viruses [3, 28, 29, 39, 40]. Artificial selection results in filovirus laboratory variants that need to be distinguished from naturally occurring variants. Since natural filoviruses do not cause disease in standard adult laboratory rodents, rodent-adapted virus variants that do cause disease are clearly phenotypically different and therefore warrant designation as laboratory strains (for a more thorough discussion on the term “strain” see [25]), whereas those that do not cause disease but are characterized by genomic mutations brought on by artificial selection should be classified as laboratory variants.

Definition of “filovirus laboratory strain”:

A filovirus laboratory strain is a genetically stable filovirus laboratory variant that evolved via artificial selection through serial passaging of a natural filovirus and causes disease in an animal that does not develop disease upon infection with the natural (wild-type) virus. The extent of genomic sequence variation is irrelevant for the classification of a variant as a strain.

“Genetically stable” means that a genomic area associated with strain characteristics needs to be maintained by the virus over several rounds of replication in the laboratory host, rather than being a random mutation that occurs and disappears over time.

The definitions for filovirus laboratory variants and isolates follow those proposed for natural filoviruses:
Definition of “filovirus laboratory variant”:

A filovirus laboratory variant is a mutant natural genetic filovirus variant that

a) evolved through serial passaging of a reference filovirus in a laboratory host;
b) is ≤10% different but is not identical in sequence with the natural reference filovirus; and
c) does not necessarily differ from the natural reference filovirus in infection phenotype.

Definition of “filovirus laboratory isolate”:

A filovirus laboratory isolate is an instance of a particular filovirus laboratory strain or variant. Isolates can be identical or slightly different in consensus or individual sequence from each other.

We propose to designate full-length and shortened designations and abbreviations for filovirus laboratory strains/variants/isolates according to the templates published for natural filovirus variants/isolates [25]. The suffix field should be “-lab” (for “laboratory-adapted”) or a combination of “-lab” and other prefixes established in [25] if necessary (for instance, “-lab_hist”, “-lab_seq”):

**Full-length designation**

<virus name> <strain>/<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>

- the virus name should be given in full, as outlined recently [23, 24]. For instance: “Marburg virus,” “Ebola virus,” “Sudan virus”
• the **strain** field should contain the abbreviation of the institute at which the strain was developed (Table 2)

• the **isolation host** should be provided in one word in the format “First letter of genus name.full name of species descriptor” of the laboratory host, but remain unitalicized to denote the fact that the virus was isolated from an entity and not from a taxon [4]. For instance: “C.porcellus” (member of the species *Cavia porcellus*). Laboratory mice and some other laboratory animals cannot be assigned to a species. Consequently, this field should be filled with the official strain designation of the animal used for the experiments – in the case of laboratory mice and laboratory rats in accordance with the most recent “Guidelines for Nomenclature of Mouse and Rat Strains”, e.g. “C57BL/6” or “BALB/c” [19]

• the **country of sampling** field should contain the same information provided as in the field for the natural (wild-type) virus

• the **year of sampling** field should contain the same information provided as in the field for the natural (wild-type) virus

• the **genetic variant designation-isolate designation** field should contain the same information provided in the same field for the natural (wild-type) virus connected by a hyphen to a laboratory isolate descriptor. For instance: “Mayinga-GPA-P7”

Example for the full-length designation of an isolate in the method section of a manuscript:

“Ebola virus VECTOR/C.porcellus-lab/COD/1976/Mayinga-GPA-P7”.

[REFERENCES: 4, 19]
Shortened designation

<virus name abbreviation> <strain>/<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>

- the **virus name abbreviation** should be accepted by the ICTV *Filoviridae* Study Group, as outlined recently [23, 24]. For instance: “MARV,” “EBOV,” “SUDV”

- the **strain** field should contain the abbreviation of the institute at which the strain was developed (Table 2)

- the **isolation host** should be provided in a four-letter format “First letter of genus name.first three letters of species descriptor” of the laboratory host. For instance: “C.por” (member of the species *Cavia porcellus*). Laboratory mice and some other laboratory animals cannot be assigned to a species. Consequently, this field should be filled with the official strain designation abbreviation of the animal used for the experiments – in the case of laboratory mice and laboratory rats in accordance with the most recent “Guidelines for Nomenclature of Mouse and Rat Strains”. For instance, “B6” for C57BL/6 mouse strains or “C” for “BALB/c” mouse strains [19]

- the **country of sampling** field should contain the same information provided as in the field for the natural (wild-type) virus

- the **year of sampling** field should contain the same information provided as in the field for the natural (wild-type) virus

- the **genetic variant designation-isolate designation** should contain the same information as provided in the field for the natural (wild-type) virus connected by a hyphen to an isolate abbreviation, e.g. “May-GPA-P7”
Example for the shortened designation of an isolate in figures (alignments, phylograms) of a manuscript: “EBOV VECTOR/C.por/COD/76/May-GPA-P7”.

**Abbreviation**

 `<virus abbreviation>/<genetic variant designation-isolate designation>`

- the **virus abbreviation** should be one accepted by the ICTV *Filoviridae* Study Group, as outlined recently [23, 24]. For instance: “MARV,” “EBOV,” “SUDV”

- the **genetic variant designation-isolate designation** should contain the same information as provided in the field for the natural (wild-type) virus connected by a hyphen to an isolate abbreviation, e.g. “May-GPA-P7”

Example for abbreviation in the text of a manuscript: “EBOV/May-GPA-P7” (if other isolates of the same genetic strain/variant/isolate are addressed in the same article); or simply EBOV (if the article only addresses work with one particular genetic strain/variant/isolate).

**USAGE OF DESIGNATIONS**

As outlined in [25], we recommend that the full-length isolate designations always be used once in the Materials and Methods section of manuscripts. For example:

“HeLa cells in 96-well plates were infected for 1 h with Ebola virus VECTOR/C.porcellus-lab/COD/1976/Mayinga-GPA-P7 (derived from an Ebola virus, family *Filoviridae*, species *Zaire ebolavirus*, GenBank accession No. EU224440) at Filoviruses – Animal-adapted Variants/Isolates 13
MOIs of 0.5, 1, or 5. Virus was obtained from the State Research Center for Virology and Biotechnology “Vector”, Koltsovo, Russia, and had been passaged twice through grivet (species Chlorocebus aethiops) kidney epithelial (Vero E6) cells before use.”

or

“Ebola virus VECTOR/C.porcellus-lab/COD/1976/Mayinga-GPA-P7 was obtained after i.m. serial passaging of Ebola virus H.sapiens-tc/COD/1976/Mayinga-ME718 in guinea pigs (Cavia porcellus), a laboratory host that is susceptible to fatal infection only after adaptation.”

As for natural filoviruses, we recommend using only the virus abbreviation in the remainder of the manuscript text (in the example above: “EBOV”) after proper introduction. Abbreviated designations should be used if several variants or isolates of one filovirus are addressed. For instance:

“Here we demonstrate that infection of guinea pigs with EBOV/May-GPA-P7 protects from subsequent infection with EBOV/May-8ms-N4”.

**CREATING NEW DESIGNATIONS**

Ideally, it is up to the investigators who developed a novel laboratory filovirus to create an appropriate isolate designation according to the scheme proposed here. A framework for creating such designations is presented in [25].
Table 1. Summary of the current filovirus taxonomy as endorsed by the ICTV *Filoviridae* Study Group and accepted by the ICTV

<table>
<thead>
<tr>
<th>Current taxonomy and nomenclature (Ninth ICTV Report and updates) [1, 23, 24]</th>
<th>Previous taxonomy and nomenclature (Eighth ICTV Report) [10]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Order Mononegavirales</strong></td>
<td><strong>Order Mononegavirales</strong></td>
</tr>
<tr>
<td>Family <em>Filoviridae</em></td>
<td>Family <em>Filoviridae</em></td>
</tr>
<tr>
<td>Genus <em>Marburgvirus</em></td>
<td>Genus <em>Marburgvirus</em></td>
</tr>
<tr>
<td>Species <em>Marburg marburgvirus</em></td>
<td>Species <em>Lake Victoria marburgvirus</em></td>
</tr>
<tr>
<td>Virus 1: Marburg virus (MARV)</td>
<td>Virus: Lake Victoria marburgvirus (MARV)</td>
</tr>
<tr>
<td>Virus 2: Ravn virus (RAVV)</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Ebolavirus</em></td>
<td>Genus <em>Ebolavirus</em></td>
</tr>
<tr>
<td>Species <em>Taï Forest ebolavirus</em></td>
<td>Species <em>Cote d’Ivoire ebolavirus</em> [sic]</td>
</tr>
<tr>
<td>Virus: Taï Forest virus (TAFV)</td>
<td>Virus: Cote d’Ivoire ebolavirus [sic] (CIEBOV)</td>
</tr>
<tr>
<td>Species <em>Reston ebolavirus</em></td>
<td>Species <em>Reston ebolavirus</em></td>
</tr>
<tr>
<td>Virus: Reston virus (RESTV)</td>
<td>Virus: Reston ebolavirus (REBOV)</td>
</tr>
<tr>
<td>Species</td>
<td>Virus:</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><em>Sudan ebolavirus</em></td>
<td>Sudan virus (SUDV)</td>
</tr>
<tr>
<td><em>Zaire ebolavirus</em></td>
<td>Ebola virus (EBOV)</td>
</tr>
<tr>
<td><em>Bundibugyo ebolavirus</em></td>
<td>Bundibugyo virus (BDBV)</td>
</tr>
<tr>
<td><em>Lloviu cuevavirus</em></td>
<td>Lloviu virus (LLOV)</td>
</tr>
</tbody>
</table>

*Taxa proposed to and provisionally approved by the ICTV Executive Committee.
Table 2. Proposed abbreviations for BSL-4 institutes with filovirus research programs for the <strain> field in names of laboratory animal-adapted filovirus strains*

<table>
<thead>
<tr>
<th>Institute</th>
<th>Proposed &lt;strain&gt; field abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian Animal Health Laboratory (AAHL), Geelong, Victoria, Australia</td>
<td>AAHL</td>
</tr>
<tr>
<td>Bernhard Nocht Institute for Tropical Medicine (BNI), Hamburg, Germany</td>
<td>BNI</td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA</td>
<td>CDC</td>
</tr>
<tr>
<td>Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon</td>
<td>CIRMF</td>
</tr>
<tr>
<td>Defence Science and Technology Laboratory (DSTL), Porton Down, Salisbury, UK</td>
<td>DSTL(^1)</td>
</tr>
<tr>
<td>Galveston National Laboratory (GNL), National Biocontainment Facility and Robert E. Shope Laboratory, University of Texas Medical Branch (UTMB), Galveston, Texas, USA</td>
<td>UTMB</td>
</tr>
<tr>
<td>Health Protection Agency (HPA) Centre</td>
<td>CEPR(^1)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Acronym</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>for Emergency Preparedness and Response, Porton Down, Salisbury, UK</td>
<td></td>
</tr>
<tr>
<td>Integrated Research Facility at Fort Detrick (IRF-Frederick), Fort Detrick, Frederick, Maryland, USA</td>
<td>IRF-F</td>
</tr>
<tr>
<td>Laboratoire P4 Jean Mérieux INSERM</td>
<td>INSERM</td>
</tr>
<tr>
<td>National Biodefense Analysis and Countermeasures Center (NBACC), Fort Detrick, Frederick, Maryland, USA</td>
<td>NBACC</td>
</tr>
<tr>
<td>National Emerging Infectious Diseases Laboratory (NEIDL), Boston University, Boston, Massachusetts, USA</td>
<td>NEIDL</td>
</tr>
<tr>
<td>National Institute for Communicable Diseases of the National Health Laboratory Service (NICD), Sandringham-Johannesburg, Gauteng, South Africa</td>
<td>NICD¹</td>
</tr>
<tr>
<td>National Microbiology Laboratory – Public Health Agency of Canada (NML), Winnipeg, Manitoba, Canada</td>
<td>NML</td>
</tr>
<tr>
<td>Institut für Virologie - Philipps-Universität Marburg, Marburg, Hesse, Germany</td>
<td>UMR</td>
</tr>
<tr>
<td>Institution</td>
<td>Abbreviation</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Republican Research and Practical Center for Epidemiology and Microbiology (RRPCEM), Minsk, Republic of Belarus</td>
<td>RRPCEM¹</td>
</tr>
<tr>
<td>Rocky Mountain Laboratory Integrated Research Facility (RML-IRF), Hamilton, Montana, USA</td>
<td>RML-IRF</td>
</tr>
<tr>
<td>State Research Center for Virology and Biotechnology “Vector” (SRCVB “Vector”), Koltsovo, Novosibirsk Oblast, Russia</td>
<td>VECTOR</td>
</tr>
<tr>
<td>Texas Biomedical Research Institute, San Antonio, Texas, USA</td>
<td>TBRI¹</td>
</tr>
<tr>
<td>United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland, USA</td>
<td>USAMRIID</td>
</tr>
<tr>
<td>Virological Center of the Research Institute of Microbiology, Sergiev Posad, Moscow Oblast, Russia</td>
<td>VC</td>
</tr>
</tbody>
</table>
* Only institutes that have been, are, or will be majorly involved with filovirus research are listed here. Abbreviations for other institutes can be suggested by their investigators when a name for a filovirus strain needs to be created.

1 These institutes have undergone name changes over the years. We recommend that researchers use the abbreviations in use at a particular time for filovirus strain/variant/isolate names created at that time. For instance, MRE and DERA were pervious abbreviations for the laboratories now referred to as DSTL; CAMR preceded CEPR; SRIEM preceded RRPCEM etc.

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18. Institut Pasteur de Dakar (1967) Résultats des investigations sur la matériel reçu de Frankfurt et Marburg. Dakar, Senegal (November) [French]


