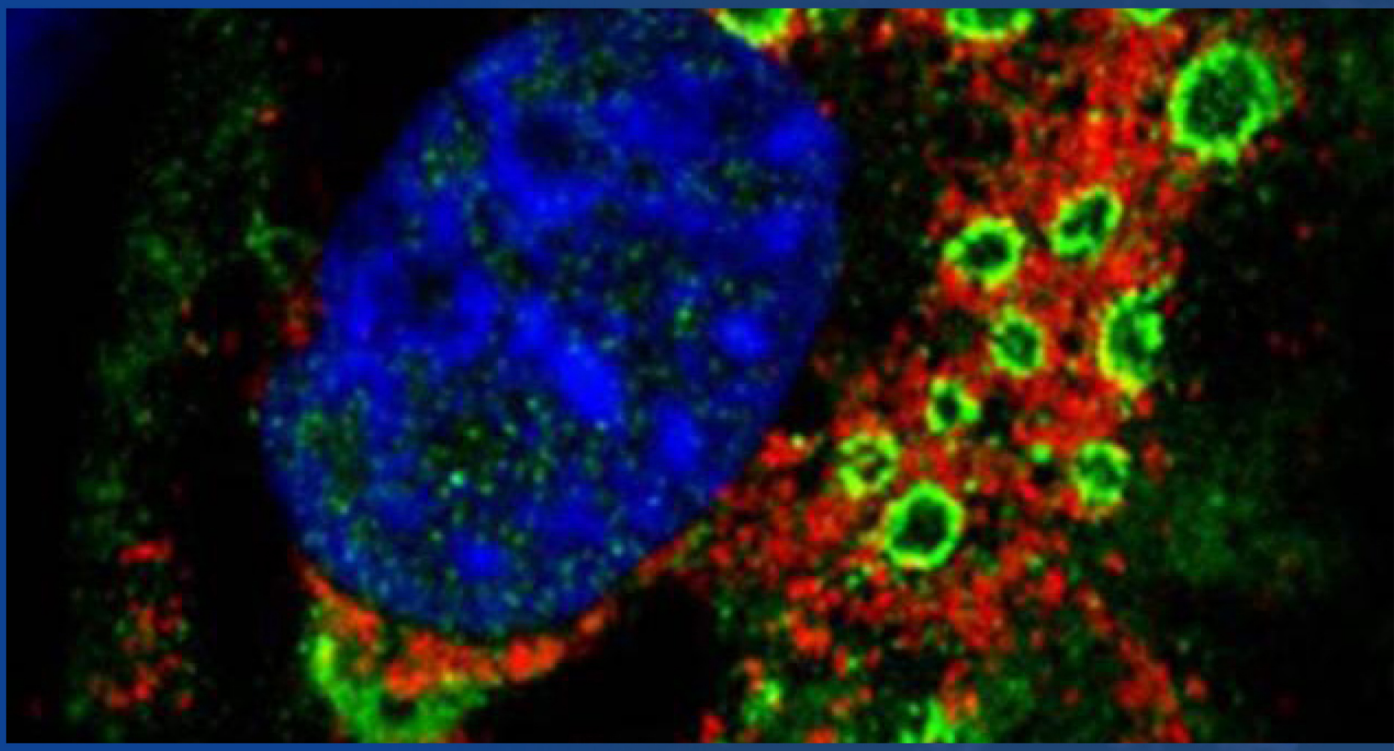
# CLINICAL VIROLOGY MANUAL

## FIFTH EDITION



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Michael J. Loeffelholz

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DEDICATION

We dedicate this edition of the Clinical Virology Manual to our families for their patience and support during this and our other professional endeavors. We are truly blessed to be part of their lives and to receive their unconditional love.

We would also like to thank, and gratefully acknowledge the support and leadership of, our close colleague, mentor, and friend, Dr. Steven Specter, who has worked tirelessly over the years in delivering the first four editions of the Manual, to advance the field of viral diagnostics, and to provide a forum for clinical virologists, academicians, and clinicians to present and discuss the latest scientific discoveries. We will be forever appreciative of his unwavering efforts.

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**Benjamin A. Pinsky** is an Assistant Professor in the Departments of Pathology and Medicine, Division of Infectious Diseases and Geographic Medicine, at the Stanford University School of Medicine and is the Medical Director of the Clinical Virology Laboratory for Stanford Health Care and Stanford Children's Health. Dr. Pinsky is on the editorial boards of several journals including the *Journal of Clinical Microbiology* and *Clinical Microbiology Reviews*. He has also served on a number of committees, including the PASCV, College of American Pathologists Microbiology Resource Committee, and the Association for Molecular Pathology Clinical Practice Committee. Dr. Pinsky is board certified in Clinical Pathology by the American Board of Pathology.

# Preface to the Fifth Edition

The aims of the fifth edition of the *Clinical Virology Manual* remain the same as prior editions and include serving as a reference source to healthcare professionals and laboratorians in providing clinical and technical information regarding viral diseases and the diagnosis of viral infections.

This new edition includes 40 chapters and 3 appendices and, similar to the organization of prior additions, consists of the four sections: general topics, laboratory procedures, viral pathogens, and the appendices. We have modified the content of the appendices to provide basic but practical information on reference virology laboratories at both the national and international levels. The viral pathogen chapters have a consistent organization, with proportionally more content dedicated to diagnostics and testing. Additionally, a new section, with the heading of "Diagnostic Best Practices", has been included in each viral pathogens chapter. The section summarizes recommendations for diagnostic testing and cites evidence-based guidelines when available.

The past several years have been very challenging, as well as exciting, for diagnostic virologists, with outbreaks of enterovirus D68, measles virus, mumps virus, norovirus, Ebola virus, and, most recently, Zika virus. In addition, there is continued emergence of chikungunya, dengue, and influenza viruses, highlighted by the influenza pandemic of 2009. The landscape of hepatitis C virus has changed, and will continue to change dramatically, with the availability of new classes of direct-acting antiviral drugs that provide an excellent probability of cure.

This edition has incorporated these significant events to the extent allowed by the production schedule. We thank the authors for their contributions, particularly during this very busy time for virologists. We also thank the staff of the American Society for Microbiology Press for their support and hard work in bringing this edition to fruition.

The fifth edition of the Manual also brings a major change in editors, as a new editor has been added and a previous editor has cycled off. Also, after successfully leading this series through four editions, Dr. Steven Specter has passed on the reins of Editor-in-Chief. We hope that this edition is a credit to Dr. Specter, as well as to other prior editors, Drs. Lancz and Wiedbrauk.

MICHAEL J. LOEFFELHOLZ
RICHARD L. HODINKA
STEPHEN A. YOUNG
BENJAMIN A. PINSKY

# SECTION I

## General Topics in Clinical Virology

# The Taxonomy, Classification, and Characterization of Medically Important Viruses

STEVEN J. DREWS

# 1

Viruses are a complex and diverse group of organisms that may have incredibly diverse and ancient origins. Their interaction with humans not only involves disease processes, but also evolutionary pressures that shape viral characteristics. Viral taxonomy, classification, and characterization is not a simple academic exercise but practically improves our ability to diagnose, track, and compare viruses of medical importance and develop a better understanding of pathophysiologic processes. Over the last 5 years, there have been significant changes in the proper names of some commonly identified viruses of medical importance, relationships between these medically relevant viruses, technologic tools, as well as websites and bioinformatics tools. Changes, including what constitutes the definition of a viral species, have already had an impact on how viruses are characterized and classified. The expanded utilization of whole genome sequence analysis and metagenomic approaches has increased the amount of biological information available to the scientific community for virus characterization and categorization. With these newer molecular approaches for virus identification and characterization, as well as enhanced bioinformatics approaches, viral classification is as dynamic and challenging as ever, requiring continuous monitoring, reassessment, and updating to achieve a rational taxonomic framework.

## WHAT ARE VIRUSES?

Historically, viruses have been a difficult group of pathogens to describe, and there is continuous and vibrant discussion on whether they should be included in the tree of life, and if so where their places are within that tree (1). The dominant theory, the "escape theory", postulates that viruses evolved recently and arose from genetic elements that escaped from cellular hosts and evolved independent replication processes. In contrast, the "reduction hypothesis" suggests that viruses are the remnants of cellular organisms (2). Finally, the virus "first hypothesis" suggests that viruses have ancient origins and arose before the last universal cellular ancestor (3). Regardless of the theory, it is apparent that mammals evolved in a world with viral threats and that viruses have co-evolved with humans and our cellular ancestors (4). However, the differences in how viruses encode genetic information (DNA versus RNA), or how that information is stored (double stranded versus single stranded) suggests that viruses are polyphyletic; that is, they lack a common origin and are developing along multiple evolutionary pathways. These tensions between polyphyletic and monophyletic characters, although evolutionary focused, also have an impact on viral taxonomy. The key question that arises is, how is it that a group of pathogens that are relatively simply designed so difficult to characterize and categorize?

As living organisms, viruses are also extremely divergent and have great diversity in a variety of other characteristics. In contrast to all other forms of life, viruses can be described as the only organisms that replicate in the form of information (5). The most noticeable difference from other organisms and one of the more variable characters of this group of pathogens is their diversity in how they encode this genetic information (Fig. 1, Tables 1 to 7). In contrast to other forms of life that encode genetic information within double-stranded (ds)DNA, virus genomes may be composed of dsDNA, single-stranded (ss)DNA, dsRNA, and ssRNA. The form of the genome has a direct correlation to factors such as substitution and mutation rate that are associated with viral evolution. In general, mutation rates (mutations/site/replication) are highest in ssRNA viruses, followed by retrotranscribing viruses, ssDNA viruses, and finally dsDNA viruses. In contrast, although substitution rates (substitution/site/year) for ssDNA viruses are greater than dsDNA viruses, the substitution rates of ssDNA, ssRNA, and retrotranscribing viruses may overlap. Retrotranscribing viruses may have wide ranges of substitution rates. Mutation and substitution rate trends in dsRNA viruses are well established (6). Variables impacting substitution rates can include generation time, transmission, and selection, while variables impacting mutation rate can include genomic architecture, replication speed, viral enzymes, host enzymes, and environmental effects (6). However, these trends do not always occur as expected, and mutation rate variation also exists among RNA viruses, which may be due to a variety of factors, particularly of the host (7). Virus genomes are also arranged in a variety of different topologies including linear, circular, single segment, or multiple segments, and this

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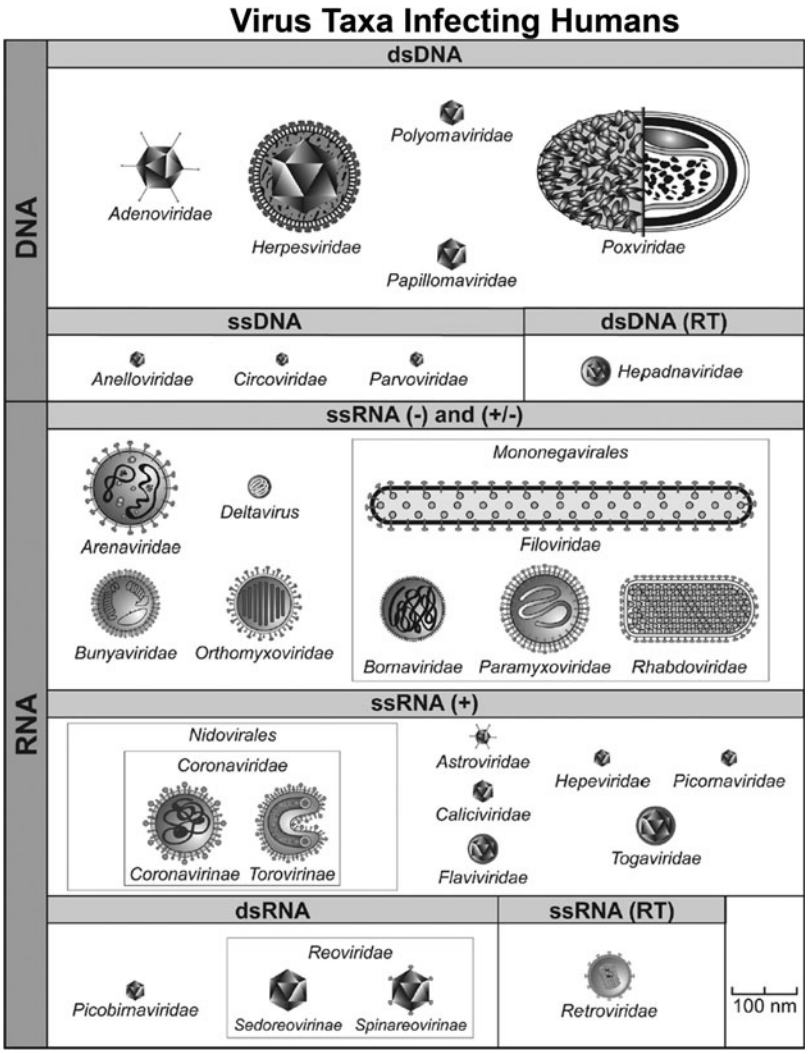


**FIGURE 1** Virus taxa infecting humans. Modified from Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses (Reprinted from Elsevier Books, *Virus Taxonomy*, 2002, with permission from Elsevier.)

organization can have an impact on horizontal transfer of genetic information between individuals of different lineages. Each form of maintenance of the viral genome has its own evolutionary benefits and drawbacks (8, 9). Viruses can also be divided into pathogens that only infect humans, those that infect other mammalian species, and those that infect nonmammalian vectors.

Several factors separate viruses from other forms of life, and these factors are often characterized by vertical but not horizontal gene transfer. Although viruses contain information, their evolution requires host cells (1). They are parasitic agents that infect cells to reproduce virions and disseminate genes (10), and they cannot maintain or replicate themselves without hosts (1). The virally encoded genes that are required for carbon metabolism, energy metabolism, and protein synthesis are postulated to have a cellular origin (1). Multiple differences from other life forms have been presented and include their polyphyletic origins, the lack of a common gene shared by all viruses, the lack of membrane heredity, the cellular origin of translation genes, and a biased one-way direction of horizontal gene transfer (1). However, four factors have been described that viruses share with other living organisms: (i) the ability of genomes and gene products to produce progeny genomes, (ii) the possession of self-regulation, (iii) the ability to adapt and respond to changing environments, and (iv) maintenance of structural organization (11).

Multiple biological pressures drive virus evolution and shape key viral characteristics. Selective processes include positive selection (increases prevalence of adapting traits), negative selection (decreases the prevalence of adapting traits), or neutral selection (random neutral occurrences with no evolutionary advantage). Temporally, evolutionary pressures may not be consistent, and organisms may emerge from long periods of evolutionary stasis and enter periods of heavy selective pressure from factors such as the host immune system (12). Biologic pressure may not be applied equally on all regions of a gene, or genome, with some epitopes under more pressure than others, and the selective pressures that impact one gene may depend on the genetic background of the virus at other gene locations (13). There may also be differences in evolutionary pressure on viruses of the same species, and genotype may be influenced by the impact of climate, vector, and host on the organism, as seen,

**TABLE 1** Taxonomy and characterization of double-stranded DNA viruses of human medical importance (Baltimore classification I)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear | *Herpesvirales* | *Herpesviridae* | *Alphaherpesvirinae* | *Simplexvirus* | *Human herpesvirus 1* | B00 herpesviral (simplex) infection |
| | | | | | (herpes simplex virus 1) | A60 anogenital herpes virus infection |
| | | | | | *Human herpesvirus 2* | P35.2 congenital herpes virus infection |
| | | | | | (herpes simplex virus 2) | |
| | | | | | *Macacine herpesvirus 1* | B00.4+ Herpesviral encephalitis |
| | | | | | (B virus) | |
| | | | | *Varicellovirus* | *Human herpesvirus 3* | B01 Varicella (chickenpox) |
| | | | | | (varicella zoster virus) | B02 Zoster (herpes zoster) |
| | | | *Betaherpesvirinae* | *Cytomegalovirus* | *Human herpesvirus 5* (HHV-5; cytomegalovirus) | B25 Cytomegalovirus disease |
| | | | | | | B27.1 Cytomegaloviral mononucleosis |
| | | | | | | P35.1 Congenital cytomegalovirus infection |
| | | | | *Roseolovirus* | *Human herpes virus 6A* (HHV-6A) | B08.2 Exanthema subitum (sixth disease) |
| | | | | | *Human herpes virus 6B* (HHV-6B) | T86.0 Bone marrow transplant rejection |
| | | | | | *Human herpes virus 7* (HHV-7) | |
| | | | *Gammaherpesvirinae* | *Lymphocryptovirus* | *Human herpes virus 4* (Epstein-Barr virus; HHV-4) | B27.0 Gammaherpesviral mononucleosis |
| | | | | | | C11 Nasopharyngeal carcinoma |
| | | | | | | C83.7 Burkitt lymphoma |
| | | | | | | D82.3 X-linked lymphoproliferative disease |
| | | | | *Rhadinovirus* | *Human herpes virus 8* (HHV-8, Kaposi's sarcoma associated herpes virus [KHSV]) | C46 Kaposi sarcoma |
| | Unassigned | *Adenoviridae* | NA | *Mastadenovirus* | *Human mastadenovirus* A–G | B34.0 Adenovirus infection, unspecified site |
| | | | | | | B30.0+ Keratoconjunctivitis due to adenovirus |
| | | | | | | B30.1+ Conjunctivitis due to adenovirus |
| | | | | | | B97 Adenovirus as the cause of diseases classified to other chapters |
| | | | | | | A08.2 Adenovirus enteritis |
| | | | | | | A85.1+ Adenovirus encephalitis |
| | | | | | | A87.1+ Adenovirus meningitis |
| | | | | | | J12.0 Adenoviral pneumonia |
| | Unassigned | *Poxviridae* | *Chordopoxvirinae* | *Molluscipoxvirus* | *Molluscum contagiosum virus* | B08.1 Molluscum contagiosum |
| | | | | *Orthopoxvirus* | *Cowpox virus* | B08.0 Other orthopox infections |
| | | | | | *Monkeypox virus* | B04 Monkeypox |
| | | | | | *Vaccinia virus* | B08.0 Other orthopox infections |
| | | | | | *Variola virus* | B03 Smallpox (for surveillance purposes only) |

(*Continued on next page*)

**TABLE 1** Taxonomy and characterization of double-stranded DNA viruses of human medical importance (Baltimore classification I) (*Continued*)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| | | | | *Parapoxvirus* | *Orf virus* | B08.0 Other orthopox infections |
| | | | | | *Pseudocowpox virus* | B08.0 Other orthopox infections |
| | | | | *Yatapoxvirus* | *Tanapox virus* | B08.8 Unspecified viral infections characterized by skin and mucous lesions |
| | | | | | *Yaba monkey tumor virus* | B08.8 Unspecified viral infections characterized by skin and mucous lesions |
| Circular | Unassigned | *Papillomaviridae* | NA | *Alphapapillomavirus* | *Alphapapillomavirus 3* (human papillomavirus 6) | B07 Viral warts<br>A63 Anogenital (venereal) warts |
| | | | | | *Alphapapillomavirus 9* (human papillomavirus 16) | D26.0 Papilloma of cervix<br>N87 Dysplasia of cervix uteri<br>D00-09 *In situ* neoplasms, Bowen's disease |
| | | | | | *Alphapapillomavirus 7* (human papillomavirus 18) | D26.0 Papilloma of cervix<br>N87 Dysplasia of cervix uteri<br>D00-09 *In situ* neoplasms, Bowen's disease |
| | | | | | *Alphapapillomavirus 1* (human papillomavirus 32) | D00-09 *In situ* neoplasms, Bowen's disease |
| | | | | *Betapapillomavirus* | *Betapapillomavirus 1* (human papillomavirus 5) | D04 Carcinoma *in situ* of skin; possible association |
| | | | | *Gammapapillomavirus* | *Gammapapillomavirus 1* (human papillomavirus 4) | B07 Viral warts<br>D04 Carcinoma *in situ* of skin; possible association |
| | | | | *Mupapillomavirus* | *Mupapillomavirus 1* (human papillomavirus 1) | B07 Viral warts |
| | | | | *Nupapipillomavirus* | *Nupapillomavirus 1* (human papillomavirus 41) | B07 Viral warts |
| | Unassigned | *Polyomaviridae* | NA | *Polyomavirus* | *BK polyomavirus* | B34.4 Papovavirus infection, unspecified site |
| | | | | | *JC polyomavirus* | |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses; NA, not applicable.

**TABLE 2** Taxonomy and characterization of single-stranded DNA viruses of human medical importance (Baltimore classification II)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear | Unassigned | *Parvoviridae* | *Parvovirinae* | *Bocaparvovirus* | *Primate bocaparvovirus 1–2* | J06 Acute upper respiratory infections of multiple and unspecified sites |
| | | | | *Dependoparvovirus* | *Adeno-associated dependoparvovirus virus A* | — |
| | | | | | *Adeno-associated dependoparvovirus B* | — |
| | | | | *Erythroparvovirus* | *Primate erythroparvovirus 1* | B34.3 Parvovirus unspecified site |
| | | | | *Tetraparvovirus* | *Primate tetraparvovirus 1* | — |
| Circular | Unassigned | *Anelloviridae* | NA | *Alphatorquevirus* | *Torque teno virus 1* | — |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses; NA, not applicable.

for example, in the pressures encountered by temperate and tropical genotypes of *Japanese encephalitis virus* (14). Another key driving pressure behind viral evolution causing human disease includes the immunologic niche or immune-mediated interactions of the human host (15). Differences in pressures on subgroups of viruses may be ameliorated by the differences in numbers of strains or subgroups of a virus below the species level and how often strains are replaced within a specific population or time period (16).

Several definitions, including taxonomy, classification, and characterization, will be used extensively in this chapter. Viral taxonomy has been defined as an approach to arranging viruses into related clusters, defining relatedness within and between clusters, and naming clusters or taxa (17). In contrast, classification can be thought of as an exercise in which one decides to use characters, features, or variables to place a particular virus within a taxonomic system. Characterization can be described as a process in which specific

**TABLE 3** Taxonomy and characterization of double-stranded RNA viruses of human medical importance (Baltimore classification III)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear, segmented | Unassigned | *Reoviridae* | *Sedoreovirinae* | *Orbivirus* | *Changuinola virus* | A93.8 Other specified arthropod-borne viral fevers |
| | | | | | *Lembobo virus* | A93.8 Other specified arthropod-borne viral fevers |
| | | | | | *Orungo virus* | A93.8 Other specified arthropod-borne viral fevers |
| | | | | *Rotavirus* | *Rotavirus A, B, and C* | A08.0 Rotaviral enteritis |
| | | | | *Seadornavirus* | *Banna virus* | A85.2 Arthropod-borne viral encephalitis, unspecified; possible association |
| | | | *Spinareovirinae* | *Coltivirus* | *Colorado tick fever virus* | A93.2 Colorado tick fever |
| | | | | *Orthreovirus* | *Mammalian orthoreovirus* | J06 Acute upper respiratory infections of multiple and unspecified sites; possible association |
| | | | | | | A08.3 Other viral enteritis; possible association |
| | | | | | | A08.4 Viral intestinal infection, unspecified; possible association |
| | Unassigned | *Picobirnaviridae* | NA | *Picobirnavirus* | *Human picorbirnavirus* | A08.3 Other viral enteritis; possible association |
| | | | | | | A08.4 Viral intestinal infection, unspecified; possible association |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses; NA, not applicable.

**TABLE 4** Taxonomy and characterization of positive sense single-stranded RNA viruses of human medical importance (Baltimore classification IV)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear | *Nidovirales* | *Coronaviridae* | *Coronavirinae* | *Alphacoronavirus* | *Human coronavirus 229E* | B34.2 Coronavirus infection, unpsecified site |
| | | | | | *Human coronavirus NL63* | B97.2 Coronavirus as the cause of diseases classified to other chapters |
| | | | | *Betacoronavirus* | *Human coronavirus HKU1* | B34.2 Coronavirus infection, unspecified site |
| | | | | | | B97.2 Coronavirus as the cause of diseases classified to other chapter |
| | | | | | *Severe acute respiratory syndrome-related coronavirus* | U04 Severe acute respiratory syndrome (SARS) |
| | | | | | *Middle Eastern respiratory syndrome coronavirus* | B34.2 Coronavirus infection, unpsecified site |
| | | | | | | B97.2 Coronavirus as the cause of diseases classified to other chapter |
| | | | *Torovirinae* | *Torovirus* | *Human torovirus* | A08.3 Other viral enteritis |
| | *Picornavirales* | *Picornaviridae* | NA | | | |
| | | | | *Cardiovirus* | *Theilovirus* | A88 Other viral infections of central nervous system, not classified elsewhere; possible role |
| | | | | *Cosavirus* | *Cosavirus A* | A08.3 Other viral enteritis; possible association |
| | | | | | | A08.4 Viral intestinal infection, unspecified; possible association |
| | | | | *Enterovirus* | *Enterovirus A* | B08.4 Enteroviral vesicular stomatitis with exanthem |
| | | | | | | B08.5 Enteroviral vesicular pharyngitis |
| | | | | | | B97.1 Enterovirus as the cause of disease classified to other chapters |
| | | | | | | G02.0* Enteroviral meningitis |
| | | | | | | G05.1* Enteroviral encephalomyelitis |
| | | | | | *Enterovirus B* | B08.4 Enteroviral vesicular stomatitis with exanthem |
| | | | | | | B08.5 Enteroviral vesicular pharyngitis |
| | | | | | | B97.1 Enterovirus as the cause of disease classified to other chapters |
| | | | | | | G02.0* Enteroviral meningitis |
| | | | | | | G05.1* Enteroviral encephalomyelitis |
| | | | | | | H13.1* Acute epidemic hemorrhagic conjunctivitis (enteroviral) |
| | | | | | *Enterovirus C* (e.g., CV-A24) | B08.5 Enteroviral vesicular pharyngitis |
| | | | | | | B97.1 Enterovirus as the cause of disease classified to other chapters |
| | | | | | | G02.0* Enteroviral meningitis |
| | | | | | | A80 Acute poliomyelitis |
| | | | | | | G05.1* Enteroviral encephalomyelitis |
| | | | | | | H13.1* Acute epidemic hemorrhagic conjunctivitis (enteroviral) |

(*Continued on next page*)

| | | | | | |
|---|---|---|---|---|---|
| | | | | *Enterovirus D* | B97.1 Enterovirus as the cause of disease classified to other chapters |
| | | | | | G02.0* Enteroviral meningitis |
| | | | | | G05.1* Enteroviral encephalomyelitis |
| | | | | | H13.1* Acute epidemic hemorrhagic conjunctivitis (enteroviral) |
| | | | | *Rhinovirus A,B,C* | J00 Acute nasopharyngitis (common cold) |
| | | | | | J20.6 Acute bronchitis due to rhinovirus |
| | | | *Hepatovirus* | *Hepatitis A virus* | B15 Acute hepatitis A |
| | | | *Kobuvirus* | *Aichivirus A* | - |
| | | | *Parechovirus* | *Human parechovirus* | A88 Other viral infections of the central nervous system, not elsewhere classified |
| | | | | | A41.8 Other specified sepsis |
| | | | *Salivirus* | *Salivirus A* | 08.3 Other viral enteritis; possible association |
| | | | | | A08.4 Viral intestinal infection, unspecified; possible association |
| Unassigned | *Astroviridae* | NA | *Mamastrovirus* | *Mamoastovirus* 1 (human astrovirus) | A08.3 Other viral enteritis |
| Unassigned | *Caliciviridae* | NA | *Norovirus* | *Norwalk virus* | A08.1 Acute gastroenteropathy due to Norwalk virus |
| | | | *Sapovirus* | *Sapporo virus* | A08.3 Other viral enteritis |
| Unassigned | *Flaviviridae* | NA | *Flavivirus* | *Dengue virus* | A90 Dengue fever |
| | | | | | A91 Dengue hemorrhagic fever |
| | | | | *Japanese encephalitis virus* | A83.0 Japanese encephalitis |
| | | | | *Kyasanur Forest disease virus* | A98.2 Kyasanur Forest disease |
| | | | | *Langat virus* | - |
| | | | | *Louping ill virus* | A84.8 Other tick-borne encephalitis |
| | | | | *Murray Valley encephalitis virus* | A83.4 Australian encephalitis |
| | | | | *Omsk hemorrhagic fever virus* | A98.1 Omsk hemorrhagic fever |
| | | | | *Powassan virus* | A84.8 Other tick-borne encephalitis |
| | | | | *St. Louis encephalitis virus* | A83.3 St. Louis encephalitis |
| | | | | *Tick-borne encephalitis virus* | A88 Other viral infections of the central nervous system, not elsewhere classified |
| | | | | *Wesselsbron* | A92 Other mosquito-borne viral fevers |
| | | | | *West Nile virus* | A92.3 West Nile infection |
| | | | | *Yellow fever virus* | A95 Yellow fever |
| | | | | *Zika virus* | A94 Unspecified arthropod-borne viral fever |
| | | | *Hepacivirus* | *Hepatitis C virus* | B17.1 Acute hepatitis C |
| | | | | | B18.2 Chronic hepatitis C |
| Unassigned | *Hepeviridae* | NA | *Hepevirus* | *Hepatitis E virus* | B17.2 Acute hepatitis E |



(*Continued on next page*)

**TABLE 4** Taxonomy and characterization of positive sense single-stranded RNA viruses of human medical importance (Baltimore classification IV) (*Continued*)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| | Unassigned | *Togaviridae* | NA | *Alphavirus* | *Barmah Forest virus* | A92.8 Other specified mosquito-borne viral fevers<br>B33.8 Other specified viral diseases |
| | | | | | *Chikungunya virus* | A92.0 Chikungunya virus disease |
| | | | | | *Eastern equine encephalitis virus* | A83.2 Eastern equine encephalitis |
| | | | | | *Madariaga virus* | A83.2 Eastern equine encephalitis, attenuated |
| | | | | | *Mayaro virus* | A92.8 Other specified mosquito-borne viral fevers |
| | | | | | *O'nyong-nyong virus* | A92.1 O'nyong-nyong fever |
| | | | | | *Ross River virus* | B33.1 Ross River disease |
| | | | | | *Semliki Forest virus* | B33.8 Other specified viral diseases |
| | | | | | *Sinbis virus* | A92.8 Other specified mosquito-borne viral fevers<br>B33.8 Other specified viral disease |
| | | | | | *Venezuelan equine encephalitis virus* | A92.2 Venezuelan equine fever<br>• Encephalitis<br>• Encephalomyelitis virus disease |
| | | | | | *Western equine encephalitis virus* | A83.1 Western equine encephalitis |
| | | | | *Rubivirus* | *Rubella virus* | B06 Rubella (German measles) |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses; NA, not applicable.

**TABLE 5** Taxonomy and characterization of negative sense single-stranded RNA viruses of human medical importance (Baltimore classification V)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear segmented | Unassigned | *Arenaviridae*[a] | NA | *Arenavirus* | *Guanarito virus* | A96.8 Other arenaviral hemorrhagic fevers |
| | | | | | *Junin virus* | A96.0 Junin hemorrhagic fever |
| | | | | | *Lujo virus* | A96.8 Other arenaviral hemorrhagic fevers |
| | | | | | *Lassa virus* | A96.2 Lassa fever |
| | | | | | *Lymphocytic choriomeningitis virus* | A87.2 Lymphocytic choriomeningitis |
| | | | | | *Machupo virus* | A96.1 Machupo hemorrhagic fever |
| | | | | | *Sabiá virus* | A96.8 Other arenaviral hemorrhagic fevers |
| | Unassigned | *Bunyaviridae* | NA | *Hantavirus* | *Andes virus* | B33.4+ Hanta(cardio)-pulmonary syndrome<br>J17.1 Pneumonia in viral diseases classified elsewhere<br>N17.9 Acute renal failure, unspecified |
| | | | | | *Bayou virus* | B33.4+ Hanta(cardio)-pulmonary syndrome<br>J17.1 Pneumonia in viral diseases classified elsewhere<br>N17.9 Acute renal failure, unspecified |
| | | | | | *Black Creek Canal Virus* | B33.4+ Hanta (cardio)-pulmonary syndrome<br>J17.1 Pneumonia in viral diseases classified elsewhere<br>N17.9 Acute renal failure, unspecified |
| | | | | | *Hantaan virus* | A98.5 Hemorrhagic fever with renal syndrome |
| | | | | | *New York virus* | B33.4+ Hantavirus (cardio)-pulmonary syndrome |
| | | | | | *Puumala virus* | A98.5 Hemorrhagic fever with renal syndrome |
| | | | | | *Sin Nombre virus* | B33.4+ Hantavirus (cardio)-pulmonary syndrome |
| | | | | | *Seoul virus* | A98.5 Hemorrhagic fever with renal syndrome |
| | | | | | *Thottapalayam virus* | — |
| | | | | *Nairovirus* | *Crimean-Congo hemorrhagic fever* | A98.0 Other viral hemorrhagic fever not classified elsewhere |
| | | | | | *Dugbe virus* | A93.8 Other specified arthropod-borne viral fevers |
| | | | | *Orthobunyavirus* | *Bwamba virus* | A92.8 Other specified mosquito-borne viral fevers |
| | | | | | *California encephalitis virus* | A83.5 California encephalitis |
| | | | | | *Guama virus* | A92.8 Other specified mosquito-borne viral fevers |
| | | | | | *Madrid virus* | A92.8 Other specified mosquito-borne viral fevers |
| | | | | | *Oropouche virus* | A93.0 Oropouche virus disease |
| | | | | | *Tacaiuma virus* | A92.8 Other specified mosquito-borne viral fevers |
| | | | | *Phlebovirus* | *Rift Valley fever virus* | A92.4 Rift Valley fever |

(*Continued on next page*)

**TABLE 5** Taxonomy and characterization of negative sense single-stranded RNA viruses of human medical importance (Baltimore classification V) (*Continued*)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| | | | | | *Sandfly fever Naples virus* | A93.1 Sandfly fever |
| | | | | | | A93.8 Other specified arthropod-borne viral fevers |
| | | | | | | A87.8 Other viral meningitis |
| Linear, segmented | | *Orthomyxoviridae* | NA | *Influenzavirus A* | *Influenza A virus* | J09 Influenza due to certain identified influenza virus |
| | | | | | | J10 Influenza virus not identified |
| | | | | | *Influenza B virus* | J09 Influenza due to certain identified influenza virus |
| | | | | | | J10 Influenza virus not identified |
| | | | | | *Influenza C virus* | J09 Influenza due to certain identified influenza virus |
| | | | | | | J10 Influenza virus not identified |
| Linear nonsegmented | *Mononegavirales* | *Bornaviridae* | NA | *Bornavirus* | *Borna disease virus* | — |
| | | *Filoviridae* | NA | *Ebolavirus* | *Bundibugyo ebolavirus* | A98.4 Ebola virus disease |
| | | | | | *Reston ebolavirus* | — |
| | | | | | *Sudan ebolavirus* | A98.4 Ebola virus disease |
| | | | | | *Tai forest ebolavirus* | A98.4 Ebola virus disease |
| | | | | | *Zaire ebolavirus* | A98.4 Ebola virus disease |
| | | | | *Marburgvirus* | *Marburg marburgvirus* | A98.3 Marburg virus disease |
| | *Orthomyxoviridae* | *Paramyxoviridae* | *Paramyxovirinae* | *Henipavirus* | *Hendra virus* | B33.8 Other specified viral diseases |
| | | | | | *Nipah virus* | B33.8 Other specified viral diseases |
| | | | | *Morbillivirus* | *Measles virus* | B05 Measles |
| | | | | *Respirovirus* | *Human parainfluenza virus 1* | J00 Acute nasopharyngitis |
| | | | | | | J05.0 Acute obstructive laryngitis (croup) |
| | | | | | | J06 Acute respiratory infections of multiple and unspecified sites |
| | | | | | | J12.2 Parainfluenza virus pneumonia |
| | | | | | | J20.4 Acute bronchitis due to parainfluenza virus |
| | | | | | | J21.8 Acute bronchiolitis due to other specified organism |
| | | | | | *Human parainfluenza virus 3* | J00 Acute nasopharyngitis |
| | | | | | | J05.0 Acute obstructive laryngitis (croup) |
| | | | | | | J06 Acute respiratory infections of multiple and unspecified sites |
| | | | | | | J12.2 Parainfluenza virus pneumonia |
| | | | | | | J20.4 Acute bronchitis due to parainfluenza virus |
| | | | | | | J21.8 Acute bronchiolitis due to other specified organism |

(*Continued on next page*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | *Rubulavirus* | *Human parainfluenza virus 2* | J00 Acute nasopharyngitis |
| | | | | | | J05.0 Acute obstructive laryngitis (croup) |
| | | | | | | J06 Acute respiratory infections of multiple and unspecified sites |
| | | | | | | J12.2 Parainfluenza virus pneumonia |
| | | | | | | J20.4 Acute bronchitis due to parainfluenza virus |
| | | | | | | J21.8 Acute bronchiolitis due to other specified organism |
| | | | | | *Human parainfluenza virus 4* | J00 Acute nasopharyngitis |
| | | | | | | J05.0 Acute obstructive laryngitis (croup) |
| | | | | | | J06 Acute respiratory infections of multiple and unspecified sites |
| | | | | | | J12.2 Parainfluenza virus pneumonia |
| | | | | | | J20.4 Acute bronchitis due to parainfluenza virus |
| | | | | | | J21.8 Acute bronchiolitis due to other specified organism |
| | | | | | *Mumps virus* | B26 Mumps including parotitis: epidemic, infectious |
| | | | *Pneumovirinae* | *Metapneumovirus* | *Human metapneumovirus* | J00 Acute nasopharyngitis |
| | | | | | | J06 Acute respiratory infection of multiple and unspecified sites |
| | | | | | | J12.3 Human metapneumovirus pneumonia |
| | | | | | | J21.1 Acute bronchiolitis due to human metapneumovirus |
| | | | | *Pneumovirus* | *Human respiratory syncytial virus* | J00 Acute nasopharyngitis |
| | | | | | | J05.0 Acute obstructive laryngitis (croup) |
| | | | | | | J12.1 Respiratory syncytial virus pneumonia |
| | | | | | | J20.5 Acute bronchitis due to respiratory syncytial virus |
| | | | | | | J21.0 Acute bronchiolitis due to respiratory syncytial virus |
| | | | | | | B97.4 Respiratory syncytial virus as the cause of disease classified to other chapters |
| | | *Rhabdoviridae* | NA | *Lyssavirus* | *Australian bat lyssavirus* | A82 Rabies |
| | | | | | *Rabies virus* | A82 Rabies |
| | | | | *Vesiculovirus* | *Chandipura virus* | A85.8 Other specified viral encephalitis, possible association |
| | | | | | *Isfahan virus* | — |
| | | | | | *Vesicular stomatitis Indiana virus* | A93.8 Other specified arthropod-borne viral fevers |
| | | | | | *Vesicular stomatitis New Jersey virus* | A93.8 Other specified arthropod-borne viral fevers |
| Circular | Unassigned | Unassigned | NA | *Deltavirus* | *Hepatitis delta virus* | B17.0 Acute delta-(super) infection of hepatitis B carrier |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses; NA, not applicable.
[a]Have been described as ambisense.

**TABLE 6** Taxonomy and characterization of positive sense single-stranded RNA with a DNA replication intermediate viruses of human medical importance (Baltimore classification VI)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Linear | Unassigned | *Retroviridae* | *Orthoretrovirinae* | *Deltaretrovirus* | *Primate T-lymphotropic virus 1* (human T-lymphotropic virus 1 [HTLV-1]) | C84.1 Cezary disease<br>C84.5 Other mature T-cell/NK cell lymphomas<br>C91.4 Hairy-cell leukemia<br>C91.5 Adult T-cell lymphoma/leukemia (HTLV-1 associated)<br>G04.9 Encephalitis, myelitis, encephalomyelitis, unspecified |
| | | | | | *Primate T-lymphotropic virus 2* (human T-lymphotropic virus 2 [HTLV-2]) | — |
| | | | | *Lentivirus* | *Human immunodeficiency virus 1* (HIV-1) | B20-B24 Human immunodeficiency virus (HIV) disease<br>Z21 Asymptomatic human immunodeficiency virus (HIV) infection status<br>O98.7 Complicating pregnancy, childbirth, and puerperium |
| | | | | | *Human immunodeficiency virus 2* (HIV-2) | B20-B24 Human immunodeficiency virus (HIV) disease<br>Z21 Asymptomatic human immunodeficiency virus (HIV) infection status<br>O98.7 Complicating pregnancy, childbirth, and puerperium |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses.

characters (e.g., factors, features, or variables, as described later in this chapter) are attributed to a virus in order to classify it into a structured taxonomy. The practice of a taxonomic approach is not just an academic exercise whereby we develop a better understanding of how viruses are related or just place names to living things. Instead, taxonomy and the exercises described above improve our knowledge of molecular biology, pathogenesis, and epidemiology, as well as our ability to respond to newly emergent viruses with new diagnostics and therapies or preventive approaches (18). Taxonomy creates a common language that aids in how we communicate with colleagues and discuss viral pathogens. We can all quickly understand that we are discussing a specific and definable organism. For example, the Ebolavirus species affecting West Africa in 2014 can be further discussed and characterized as a member of the species *Zaire ebolavirus*, or the enterovirus infecting patients in North America in the summer and fall of 2014 is in fact a member of the species *Enterovirus D*.

The taxonomic grouping of viruses often relies on utilization of a variety of defined characters, and early systems of classification would have utilized characters as seen in Fig. 1. One of the most widely utilized methods for viral classification is the Baltimore classification, a nonhierarchical approach, named after the Nobel Prize winner David Baltimore. This system of categorizing viruses was originally divided into six groups, but with the inclusion of hepatitis B virus, it is now divided into seven groups and is based on the genome present in virions and type of replication (http://viralzone.expasy.org/all_by_species/254.html) (19). As seen in Tables 1 to 7, group I comprises dsDNA viruses, while group II comprises ssDNA viruses. Group III is composed of dsRNA viruses. Group IV is composed of positive sense ssRNA while group V is negative sense ssRNA. Group VI is composed of positive sense ssRNA viruses that replicate by means of a DNA intermediate. Group VII is composed of dsDNA viruses that replicate by means of a ssRNA intermediate (20). However, this method alone does not permit for stratified classification of viruses, and thus does not give a sense of hierarchies of relationships down to species or the subspecies level. An approach like the Baltimore system is also arbitrary in its division of viral characteristics and may miss key attributes such as the ambisense nature of the genomes of arenaviruses or *Rift valley fever virus* within the family *Bunyaviridae* in which an S segment uses an ambisense strategy (Table 5) (21).

There are other historic but less widely used systems of viral classification, and the hierarchical principles seen in some of these earlier systems can be seen as laying the ground work for current hierarchical approaches. The principles identified in these approaches have been utilized for decades but are still used today to help us characterize viruses of medical importance. Early approaches still seen today include elements of the Holmes classification, an early hierarchical classification approach from the 1940s for insect viruses that attempted to classify viruses largely on the basis of their morphology, the physical characteristics of their inclusions (or lack of inclusions), their host insect population, and disease processes (22). Two early approaches that took into account the physical characteristics of viruses were the L.H.T. (Lwoff Horne Tournier) system from the 1960s, a hierarchical classification system focusing on shared physical characteristics (nucleic acid, symmetry, presence/absence of an envelope, diameter of capsid, and number of capsomers) (23) and the Casjens and Kings classification from the 1970s, a nonhierarchical system that classified viruses on the

**TABLE 7** Taxonomy and characterization of DNA reverse-transcribing viruses of human medical importance (Baltimore classification VII)

| Genome composition | Order | Family | Subfamily | Genus | Species (ICTV or other common names) | Related primary ICD-10 codes |
|---|---|---|---|---|---|---|
| Partially double-stranded, circular genome | Unassigned | *Hepadnaviridae* | | *Orthohepadnavirus* | *Hepatitis B virus* | B16 Acute hepatitis B<br>B18 Chronic viral hepatitis |

ICD, International Statistical Classification of Diseases and Related Health Problems; ICTV, International Committee on Taxonomy of Viruses.

basis of nucleic acid, symmetry, presence or absence of an envelope, and site of assembly (24).

Modern taxonomy came into being with the formation of the International Committee on Taxonomy of Viruses (ICTV). The ICTV is a committee of the Virology Division of the International Union of Microbiological Societies with activities governed by statutes. These statutes are intended to (i) develop internationally agreed taxonomy for viruses, (ii) develop internationally agreed names for virus taxa, (iii) communicate taxonomic decisions to the international virology community, and (iv) maintain an index of agreed names for virus taxa (25). The principles of nomenclature identified by the ICTV include (i) essential principles to aim for stability, avoid or reject names that might cause error or confusion, and avoid the unnecessary creation of names; (ii) viral nomenclature that is independent of other biological nomenclature and is a recognized exception; (iii) the primary purpose of a taxon being to supply a means of referring to the taxon rather than to indicate the characters or history of a taxon; and (iv) the name of a taxon having no official status until approved by the ICTV (http://www.ictvonline.org/codeOFVirusClassification.asp). Since 1971, nine reports have been released by the ICTV. Historically, this group decided to use species to classify viruses along with genus and family and set about to create working groups to develop plans to demark these species within a hierarchical structure when possible (e.g., http://www.ictvonline.org/proposals/2005.020-72.04.Herpes.pdf) (26).

The ninth report of the ICTV identified six orders, 87 families, 19 subfamilies, 349 genera, and 2,284 virus and viroid species. Representative viruses of medical importance are outlined in Tables 1 to 7 of this chapter. Within the report, each genus contains a type species and often other species, and some ICTV study groups worked to define "type isolates." Species may or may not be included within a genus, but all species are assigned to a subfamily or family. Genera and families are defined on a phylogenetic basis, and thus most genera are assigned to families, although some are unassigned until they can be further defined in terms of status and relationship. By the ninth report, it became apparent that classification of viruses would need to account for the increasing amount of genetics information available and the strategies used for making decisions about classification (27). In some less common cases, ICTV study groups have also worked on developing standards for naming strains and genetic variants that are becoming more evident with partial and whole genome analysis (28). An extensive and relatively up-to-date species master list is available at the ICTV website (http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx).

Viral taxonomy is a dynamic field, and this is evident by recent updates that have occurred in the ninth report or since that time. In particular, multiple recent changes were ratified by the ICTV in March 2014, some key ones of which are identified in this chapter and described on the ICTV website (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/default.aspx) but may not be yet identified in the master species list (http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx). There are some striking and very important changes within the family *Parvoviridae*, with five new genera, five names expanded, a decrease in the identity required for species determination, new species introduced, and binomial species names used. Most notably, the species *Human parvovirus B19* was removed from the genus *Erythrovirus* in the subfamily *Parvovirinae*, family *Parvoviridae*. The species *Human parvovirus B19* was renamed *Primate erythroparvovirus 1* and placed in the genus Erythroparvovirus. (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/default.aspx) (29).

Other important changes were also included in the 2014 ratification. Within the family *Adenoviridae* multiple changes occurred, including renaming the genus *Adenovirus* to *Mastadenovirus* and renaming the species *Human adenovirus A-G* to *Human mastadenovirus A-G*. These changes were intended to be on the species level and were not intended to impact colloquial virus, strain, or isolate names. To prevent confusion, uppercase letters were proposed to be retained, but in the future, there would be an understanding that the uppercase letters would not be considered sequential, nor would they imply a sense of completeness within a series (30). In the family *Papillomaviridae*, genus *Gammapapillomaviridae*, 10 new species *Gammapapillomavirus 11* to *Gammapapillomavirus 20* were created, and multiple changes were made in this family (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/default.aspx). A new species, *Bokeloh bat lyssavirus*, in the genus *Lyssavirus*, family *Rhabdoviridae* was created (31). This virus has been identified as a potential emerging human pathogen, and a fatal cause of rabies in a Natterer's bat was reported, but a link to human disease has not been identified; this virus is not included in Table 5 at this time (32).

Several recent changes should be noted in the ninth report, or following in the species master list. Within the *Picornaviridae*, the species *Human enterovirus A* to *D* were renamed as *Enterovirus A* to *D*, and the species *Human rhinovirus A* to C were renamed *Rhinovirus A* to C. A new genus *Salivirus* (Stool Aichi-like Virus) was created, with a new type species, *Salivirus A*, created to encompass the previous Salivirus NG-J1. The previous possible species Human cosavirus A was re-assigned with the new species *Cosavirus A* and the Human cosaviruses B to D were left unassigned. Also, the species "Aichi virus" was named *Aichivirus A* within the genus *Kobuvirus*, family *Picornaviridae* (30).

Key taxonomic changes (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/default.aspx) also occurred in a variety of families and are seen in the species master list. Following a proposal in 2010, within the family *Astroviridae*, genus *Mamastrovirus*, the species *Human astrovirus* was changed to *Mamastrovirus 1*. *Lujo virus* was designated as a new species in the genus *Arenavirus*, and it has been described to be associated with viral hemorrhagic fevers in South Africa and Zambia (33). In 2012, a proposal was initiated to create a new species *Madariaga virus* within the genus *Alphavirus*, which comprised strains of the species *Eastern equine encephalitis virus* from Central and South America and the Eastern Caribbean lineages II to IV. Multiple reasons justify this discrimination, including an attenuated illness in *Madariaga virus* disease compared to illness caused by *Eastern equine encephalitis virus* (34). A new species, *Sangassou virus*, was created within the genus *Hantavirus* to describe a murine virus with amino acid sequence similarity to hantaviruses that are possibly associated with fever of unknown origin in patients in Africa (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/default.aspx) (35).

There have been multiple discussions and disagreement about how virologists should be define a species. The sixth report of the ICTV in 1995 defined species as a "polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." This focus on a polythetic origin was a controversial topic even by the time of the ninth ICTV report (27), and in 2011 a proposed species definition that "A virus species should be defined on the basis of a range of criteria to ensure that the viruses assigned to it form a phylogenically distinct lineage" was introduced. Another proposed definition of species was introduced in 2012, which suggested that "A species is a monophyletic group of viruses whose properties can be distinguished by multiple criteria" (36). These multiple criteria could include properties such as natural or experimental host cell range, cell and tissue tropism, pathogenicity, vector specificity, antigenicity, and degree of relatedness in genes and genomes. Further to this, Gibbs commented that a species should consist of viruses that are linked with "a single 'type genomic sequence'" and "should be predominately monophyletic," which would lead to a definition of species that is more informative and acts as a quality assurance measure (37).

Below the level of species, there is no widespread, consistent, generalized, or systemized approach to naming and identifying viruses. However, some well-established approaches do exist, including those that account for variation due to laboratory-originated recombination. For example, for filoviruses, the genetic variant naming takes the approach, <virus name> ("strain>/)<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation> with the proposal to add a "rec" suffix for laboratory-derived recombinants (38). This is a similar approach to the nomenclature for influenza A strains, but use of geographic and temporal variables can be difficult to maintain due to a lack of standardization. In 2011, the World Health Organization (WHO) suggested revising how highly pathogenic influenza H5N1 is named to create a unified system that would allow for interpretation of data from different laboratories, replace geographic labeling with a more representative system, and create a system that accounts for antigenic variation and reassortment in multiple genotypes (http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/). Segmented viruses such as influenza A or rotavirus also have an additional level of characterization based on individual gene segments. The rotavirus working groups have taken a nucleotide-sequencing approach and utilized percentage cutoff values to identify strains. They have also given descriptors to each of the 11 gene segments (Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx) and have proposed that strains are named as "RV group/species of origin/country of identification/common name/year of identification/G- and P-type" (39).

The ICTVdb was a curated virus database initiated following a decision by the ICTV executive in 1991, and it is still accessible (http://ictvdb.bio-mirror.cn/Ictv/ICTVindex.htm). The database used a decimalized numbering system to allow for the easy and unique identification of a virus at the level of species, genus, subfamily, and family. The ICTVdb was integrated with other databases containing genome sequence such as NCBI GenBank and EBI EMBL. Unfortunately, following the retirement of its curator, the ICTVdb became out of date, and by 2011 the ICTV suspended the ICTVdb project. With the suspension of the ICTVdb, other forums have arisen to provide continuity in taxonomic activities (Table 8). Some of these, such as the ExPASY Bioinformatics Resource Portal, are general in nature and provide a quick overview of viral characterization. Others such as the NCBI viral genomes database or the Viral Bioinformatics Resource Centre (University of Victoria), the VIDA 3.0 database, the Icosahedral virus capsid structure database, the RNAs and proteins of dsRNA viruses website and are broadly focused and can be used to study, characterize, and classify a broad variety of viral pathogens. Other websites may focus on one specific virus or smaller clusters of viruses as listed in Table 8. A disease-focused taxonomy involving viruses can also be created using the WHO ICD-10 database for identifying direct and indirect characters associated with human viral pathogens.

The International Statistical Classification of Diseases and Related Health Problems (ICD) is a standardized tool developed by the WHO to organize and code mortality and morbidity data that are then used for statistics, epidemiology, health care management, health care resource planning and allocation, monitoring, evaluation, research, primary care, and treatment. This tool can also be used to characterize the general health of a country or population as well as the impact that viruses have on the morbidity and mortality of individuals and populations (http://www.who.int/classifications/icd/revision/icd11faq/en/). The 10th revision was endorsed by the World Health Assembly in 1990 and is expected to be utilized until work on the current 11th revision is complete around 2017 (http://www.who.int/classifications/icd/en/). The 2010 English version is available online (http://apps.who.int/classifications/icd10/browse/2010/en) and allows for easy searching of viral diseases, syndromes, and viruses themselves and is supported by a user guide (http://www.who.int/classifications/icd/ICD-10_2nd_ed_volume2.pdf).

With the ICD-10, diseases are classified using an alphanumeric system that allows for assigning primary and secondary disease codes. These codes are provided as examples of diseases caused by or associated with specific viruses in Tables 1 to 7. Table 9 outlines how ICD-10 codes focused on a character, in this case viral hemorrhagic fever in humans, could be used to categorize arthropod-borne viral hemorrhagic fevers and create a disease-focused taxonomy (40) separate from one focused on viral order, family, genus, and species. Some codes such as A91 (dengue hemorrhagic

**TABLE 8** Websites for online taxonomy databases

| Focus | Working or other group | Title/topic | Website |
|---|---|---|---|
| Specific viruses | | | |
| Astroviruses | Pirbright Institute | The Astrovirus Pages | http://www.iah-virus.org/astroviridae/ |
| Bat-associated viruses | Institute of Pathogen Biology, Beijing, China | dBatVir/Viral genome database | http://www.mgc.ac.cn/DBatVir/ |
| Coronaviridae | VIPR: Virus Pathogen Resource | CoVDB/Viral genome database | www.viprbrc.org/brc/home.spg?decorator=corona |
| Dengue virus | Broad Institute | Dengue virus portal | http://www.broadinstitute.org/annotation/viral/Dengue/Home.html |
| Group A rotaviruses | Multiple authors | RotaC2.0 automated genotyping tool | http://rotac.regatools.be |
| Hepatitis B | Multiple groups | The Hepatitis B Virus Database (HBVdb) | https://hbvdb.ibcp.fr/HBVdb/ |
| HIV | Los Alamos | HIV resistance mutation database | http://www.hiv.lanl.gov/content/sequence/RESDB/ |
| HIV | Los Alamos | HIV sequence database | http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html |
| HIV | Stanford University | HIV drug resistance database | http://hivdb.stanford.edu/ |
| Human adenovirus | Comparative Virology Team | Adenovirus Genetics and Taxonomy | www.vmri.hu/~harrach/ |
| Influenza | Chinese Academy of Sciences | IVDB/Viral genome database | http://influenza.psych.ac.cn/ |
| Influenza | Swiss Institute of Bioinformatics | Open Flu Database | http://openflu.vital-it.ch/browse.php |
| Picornaviruses | European study group on the molecular biology of Picornaviruses | Europic | http://www.europic.org.uk/ |
| Picornaviruses | ICTV Picornaviridae study group | *Picornaviridiae* Study Group Pages | http://www.picornastudygroup.com/ |
| Picornaviruses | Pirbright institute | The Picornavirus Pages | http://www.picornaviridae.com/ |
| General | | | |
| Bioinformatics | ExPASy Bioinformatics Resource Portal | Viral zone | http://viralzone.expasy.org/all_by_species/677.html |

(*Continued on next page*)

**TABLE 8** Websites for online taxonomy databases (*Continued*)

| Focus | Working or other group | Title/topic | Website |
|---|---|---|---|
| Bioinformatics | University of Victoria | Viral bioinformatics resource centre | http://athena.bioc.uvic.ca/ |
| Poxviruses | | | |
| Disease-focused taxonomy | World Health Organization | ICD-10 Version:2010 | http://apps.who.int/classifications/icd10/browse/2010/en |
| Genomics | NCBI | Viral genomes | http://www.ncbi.nlm.nih.gov/genomes/GenomesHome.cgi?taxid=10239 |
| Icosahedral virus capsid structure database | The Scripps Research Institute | Viperdb: Virus Particle ExploreR[2] | http://viperdb.scripps.edu/ |
| Taxonomy | ICTV | Virus taxonomy:2013 release | http://ictvonline.org/virusTaxonomy.asp |
| Universal protein database | UniProt consortium | UniProt | http://www.uniprot.org/ |
| Multiple | | | |
| *Herpesviridae* | University College London | VIDA 3.0 | http://www.biochem.ucl.ac.uk/bsm/virus_database/VIDA3/VIDA.html |
| *Poxviridae* | | | |
| *Papillomaviridae* | | | |
| *Coronaviridae* | | | |
| *Arteriviridae* | | | |
| Influenza virus | NCBI | Virus variation database | http://www.ncbi.nlm.nih.gov/genomes/VirusVariation/index.html |
| Dengue virus | | | |
| West nile virus | | | |

ICTV, International Committee on Taxonomy of Viruses; NCBI, National Center for Biotechnology Information.

**TABLE 9** Arthropod-borne viral fevers and viral hemorrhagic fevers (A90–A99)

| |
|---|
| **A91 Dengue hemorrhagic fever** |
| **A92 Other mosquito-borne viral fevers** |
| **Excluding:** Ross River disease (B33.1) |
| **A92.0 Chikungunya virus disease** |
| Chikungunya (hemorrhagic) fever |
| **A92.4 Rift Valley fever** |
| **A95 Yellow fever** |
| **A95.0 Sylvatic yellow fever** |
| Jungle yellow fever |
| **A95.1 Urban yellow fever** |
| **A95.9 Yellow fever, unspecified** |
| **A96 Arenaviral hemorrhagic fever** |
| **A96.0 Junin hemorrhagic fever** |
| **Including:** Argentinian hemorrhagic fever |
| **A96.1 Machupo hemorrhagic fever** |
| Bolivian hemorrhagic fever |
| **A96.2 Lassa fever** |
| **A96.8 Other arenaviral hemorrhagic fevers** |
| **A96.9 Arenaviral hemorrhagic fever, unspecified** |
| **A98 Other viral hemorrhagic fevers, not elsewhere classified** |
| **Excluding:** Chikungunya hemorrhagic fever (A92.0), dengue hemorrhagic fever (A91) |
| **A98.0 Crimean-Congo hemorrhagic fever** |
| Central Asian hemorrhagic fever |
| **A98.1 Omsk hemorrhagic fever** |
| **A98.2 Kyasanur Forest disease** |
| **A98.3 Marburg virus disease** |
| **A98.4 Ebola virus disease** |
| **A98.5 Hemorrhagic fever with renal syndrome** |
| Hemorrhagic fever: |
| Epidemic |
| Korean |
| Russian |
| Hantaan virus disease |
| Hantaan virus disease with renal manifestations |
| Nephropathia epidemica |
| **Excluding:** Hantavirus (cardio-)pulmonary syndrome (B33.4+, J17.1*) |
| **A98.8 Other specified viral hemorrhagic fevers** |

fever) and A95 (yellow fever) are tightly linked to an easily identifiable viral species. Other codes, such as A96 arenaviral hemorrhagic fever identify a genus associated with disease but may not identify all species such as *Sabia virus* (Brazilian hemorrhagic fever) or *Guanarito virus* (Venezuelan hemorrhagic fever). Yet codes, such as A92 (other mosquito-borne viral fevers, excluding Ross River disease), may be vector associated and include different genera such as alphaviruses and phleboviruses. Other genera and species not characterized elsewhere would be lumped into A98 other viral hemorrhagic fevers, not classified elsewhere.

ICD-10 codes are considered administrative health data, and there are concerns about how well these data can characterize illness as well as their accuracy. Administrative health data have value in helping us understand clinical outcomes associated with viral diseases at a population level as well as risk factors for disease. The current version is thought to provide both a better description of clinical situations as well as more specificity in describing health care problems than ICD-9 (41). However, using chart reviews, it was found that ICD-9 and ICD-10 had roughly equal sensitivity for coding conditions in general (42). ICD codes, in this case ICD-9 codes, have been shown to be highly predictive of determining pneumonia, herpes simplex virus infections, cirrhosis with hepatitis C virus, and HIV or hepatitis B co-infections with hepatitis C virus when administrative databases were analyzed (43). However, validations need to be undertaken to ensure each code is accurately describing a viral disease process.

Character-based description allows for the use of descriptors, variables, or characters to classify and compare viruses. The ICTV uses an extensive and comprehensive listing of different characters, and these generally include isolation details, historic ICTVdb virus codes, classification at taxonomic level, virion properties, morphology, physiochemical and physical properties, nucleic acid, proteins, genome organization and replication, antigenicity, and biological properties including natural host range and pathology. The ICD-10 codes described earlier could also be considered pathology-focused characters. As an example, the following species demarcation criteria would be used within the genus *Flavivirus*: nucleotide and deduced amino acid sequence data, antigenic characteristics, geographic association, vector association, host association, disease association, and ecological characteristics (http://ictvdb.biomirror.cn/Ictv/fs_flavi.htm). Use of these multiple and diverse characters allows for the systematic understanding of how viruses compare to each other, and it could be argued that they are a natural progression of other historical methods while still ensuring that a hierarchical classification based on a modern multidisciplinary approach can be undertaken. One of the issues with using a character-based system and character-based descriptors is that their demarcation criteria can vary greatly within and between families and as such they lack a single unifying property. This variability is required to ensure that each virus is classified (44). However, as described earlier, there now seems to be a greater role for a genetics-based approach in defining virus taxonomy.

Molecular phylogenetics is an approach that allows for the comparison of nucleic acid and/or protein sequences to investigate evolutionary relationships. The multiple issues with non-sequence-based viral taxonomy, including the subjective nature of other characters, poor clinical characterization, or more practical factors, such as the lack of adequate tissue culture propagation systems or animal infection models for certain viruses, suggests that nucleic acid or protein sequence should be the primary driver of taxonomic decisions (45). The most common method used is a pairwise analysis of a particular gene, amino acid sequence, or subgenomic marker and the creation of a "tree" that allows for an estimation of genetic relatedness; this has traditionally been a method for comparing sequences to determine phylogeny at the subgenomic level (46). Much of this work will be described in chapter 15 and several previously reviewed approaches to genome tree formation include (i) alignment-free trees, (ii) gene content trees, (iii) chromosomal gene order trees, (iv) average sequence similarity trees, and (v) meta-analysis trees (47, 48). As described later in this chapter

there are some examples in which classification systems are based largely, or even purely, on sequence homology including human papillomaviruses.

Different approaches in terms of target, such as amino acids versus nucleotides, as well as genes sequenced and whether to include hypervariable regions in the analysis, can impact taxonomic classifications. One important choice is whether to use nucleotide or amino acid sequences within the analysis. It has been argued that phylogenetic relationships based on nucleotide sequences alone may be misleading since they analyze sites with saturated substitutions, and it has been suggested that these biases should be compensated for by using Bayesian methods or maximum likelihood methods or by analyzing aligned amino acid sequences (49). However, amino acid analysis alone may not be sufficient because some taxonomic or phylogenetic approaches may take into account noncoding regions. Another key choice is whether to include partial or full genome sequences. For obvious reasons, including earlier technologic issues with sequencing long regions of nucleic acid and the management of sequencing information, earlier classification approaches were often based on partial genome sequences of viruses. For example, the RNA-dependent RNA-polymerase (RdRp) protein sequence was used as one tool to understand relatedness of families within the order *Picornavirales* and could be used to distinguish members of different genera within the family *Reoviridae* (27). Subgenomic analysis of one or multiple genes will not reveal the nature of all genetic changes within a virus and may not confidently classify a virus that is being studied within an appropriate taxonomic framework. The increased use of whole genome sequencing rather than sequencing only subgenomic regions has led to instances in which greater diversity or variants are identified from previously studied viral populations (49). Whole genome approaches have also uncovered previously undescribed evolutionary relationships, including evidence of interspecies transmission and related recombination events (50), that can then assist in how viruses are classified. When these approaches are applied, they can be used to generate more consistent nomenclature (39). This new information identified by analysis of a complete genome is important because it increases our awareness of relatedness between individual viruses being studied and improves our knowledge of viral epidemiology and pathophysiology.

The impact of the viral metagenome on understanding the virome and characterizing virus components within primary specimens or natural samples should also be noted. High-throughput deep-sequencing approaches have played important roles in the discovery of viruses and viral communities, or the virome, within primary specimens and biological samples (51). However, one of the issues with this approach is the incredibly large amount of information produced and how to manage this information as it significantly increases on a yearly basis (52). Other key problems include concerns in the bioinformatics community about how to account for factors such as their small genomes, fast mutation rates, and low conservation (53), and how to assign taxonomy to very short reads of nucleic acid sequence (54).

Once phylogenetic approaches are used, questions then arise as to how comparisons between viruses will be made, and whether these approaches will be consistent or inconsistent with the previously defined taxonomy (55). These questions have not only been faced by virologists but are universal when phylogenetic approaches are taken to classify organisms. Multiple factors will impact phylogenetic analysis, including how trees are established and how they change as new sequences are added (56). In some cases a tree model may not be used, and phylogenetic networks may instead be used for investigating evolutionary relationships to establish relationships; however, these often require extensive full genome sequences (57). Other methods such as the calculation of genetic distances between nucleotide sequences of full genome sequences can be used without construction of trees and can correlate well to subgenomic regions, without the requirement of extensive full genome sequences being available (57). Regardless of the approaches to determine phylogenetic relationships, the conclusions may still be biased if they do not account for recombination and convergence (58).

Descriptions of viral taxonomy and categorization can easily diverge from clinically relevant viruses unless a strong effort is made to link the virological information to information describing disease processes. Furthermore, viral infections may not actually be linked to any disease processes, or infections may be associated with disease processes but may not be confirmed with Koch's postulates. Part of this problem may be that until recently we had very limited tools for diagnosing viral diseases and the age of viral discovery is now outstripping our ability to show causality with exercises such as the use of Koch's postulates. Tables 1 to 7 show a summary of viruses of medical importance and use ICD-10 codes to indicate the associated disease processes attributed to these viruses. These codes act as the disease- or pathology-focused character associated with viral infection. A framework of these relationships can also be seen in Table 9, which uses a viral hemorrhagic disease as an example. However, it should be noted that the disease-focused taxonomy provided in Tables 1 to 7 is not intended to be an exhaustive description of the diseases caused by each pathogen but is shown to indicate medical relevance and to identify specific disease-focused characters.

As seen in the Tables 1 to 7, if the virus is not directly listed with an ICD-10 code then the correlation becomes more complicated. For example, the pathophysiology linked to *Human torovirus* could be linked to A08.3 other viral enteritis. Other disease processes may not be related to all species of a genus, and the diseased-focused taxonomy may not be entirely specific. In the case of code B30.0 + keratoconjunctivitis due to adenoviruses, it would be simplistic to link this disease to all types of adenovirus because types 8, 19, and 37 are usually involved, while type 5 can be involved with severe disease. In contrast, B30.1 + conjunctivitis is mostly caused by types 3, 4, and 7, but most types can cause this disease. Similarly, enterovirus categorization is complex and examples given use a previous review on enterovirus infection (59). ICD-10 coding to describe a viral infection may primarily link a virus to a specific disease process, while other secondary disease processes may be described later, sometimes as footnotes. For examples, *Venezuelan equine encephalitis virus* disease is described in ICD-10 as a viral fever, but in a minority of cases they lead to viral encephalitis as described in a footnote in ICD-10 coding.

Other infectious processes may be hard to define in terms of an ICD-10 code or another disease- or pathology-focused character and may not currently fulfill Koch's postulates. *Betapapillomavirus 1* may play a role in carcinoma *in situ* of the skin and in actinic keratitis, *Mupapillomavirus 1* is sometimes found in warts and other times on normal skin (60), while the role for gamma papilloma viruses in human disease is even less obvious (61). *Banna virus* has been identified in patients with viral encephalitis, and there may

be a possible association with illness (62). Mammalian orthoreoviruses have been identified in humans with multiple illnesses (63); however, evidence on causation is not strong, and these are listed in the table as associations. The role of *Borna disease virus* in human disease, including viral encephalitis and neurologic or psychiatric disorders, is still controversial (64). There is also a possible association of *Cosavirus* A with diarrhea in immune-compromised and pediatric patients (65). *Aichivirus* A, *Salivirus* A, and *Theilovirus* or "Saffold virus" have been shown to circulate in children with diarrhea, but their roles are not well understood. *Theilovirus* virus may also be an emerging viral cause of central nervous system disease (66). *Human picobirnavirus* also has an associated role in diarrheal illness (67). Although *Torquetenovirus 1* has been identified in human specimens, its role in human disease is unclear (68), as is the role for *Thottapalayam virus* (69).

The following scenarios describe the issues faced by the scientific community in determining taxonomy. Some are relatively straightforward, while others have required significant discussion or are still points of discussion. Examples are described for the papillomaviruses, picornaviruses, adenoviruses, and noroviruses. A common theme that appears in all examples, and one that has been described previously, is the impact of whole genome sequence analysis on categorization of viruses within a taxonomic framework. Primarily, much of these discussions focuses on what criteria should be used to classify these viruses, with the understanding that these criteria are key because typing needs to be consistent across methods to ensure continuity in understanding the epidemiology and clinical presentation of these viruses and to allow for the effective identification of new strains or types that may cause severe illness.

## Papillomaviruses

Multiple characteristics can be used to develop a taxonomic approach; however, in some cases the taxonomic approach is restricted to genetic approaches, and the question still arises about which genetic approach to use. With human papillomaviruses, genetic approaches were required because of a lack of reliable cell culture systems and animal models of infection for these particular viruses (27). As a result of these pressures, taxonomy developed on two basic themes: host specificity and the use of phylogenetic analysis. Also, some categorization focused on whether the HPV type could be grouped as cutaneous or mucosal, but this approach was not maintained following more extensive phylogenetic analysis (45). Coordination within the scientific community studying human papillomaviruses emerged early, and in the 1980s, the community established a reference center in Germany. Basic rules established that identifying a new type required storing the full-length cloned genome at this reference center. Even with this strong coordination, there was no consensus on which gene targets to utilize for taxonomic classification, and for a considerable period of time, there was significant discussion on the gene targets or sequences (e.g., L1, and E6 and E7), whether open reading frames (ORFs) and partial gene sequences or full gene sequences should be used, and what level of similarity should be used for each target to classify a new species (70). As new technologies increased the output of sequence available to be analyzed, there was an increased need to standardize approaches to classification (71). Currently, human papillomaviruses are classified by phylogenetic analysis of the L1 gene ORF with variations in the percentage of difference used to determine if a newly identified sequence belongs to a new species, a new subtype, or a new variant (71). Following this approach, new discussions have now moved onto whether to accept new types that are sequenced and identified by metagenomic approaches (45).

## Picornaviruses

The following illustrative example describes the issues the scientific community may need to deal with when transitioning from a taxonomic approach involving multiple potentially variable characteristics to one using potentially more objective characteristics. Current picornavirus taxonomic classification is carried out by the Picornavirus Study Group on behalf of the ICTV. Classification of picornaviruses involves a number of rules that take into account several different characteristics, including polyprotein sequence homology, genome organization, genome base composition, host range, host cell receptor variety, and replicative processes. Multiple molecular markers may also be used to create a picornavirus taxonomy (27, 72). At the species level the use of VP1 pairwise sequencing can often be used to determine relationships between viruses (73). However, for the purposes of developing hierarchal categorizations, it is argued that this approach lacks a gold standard, and a growing number of picornaviruses are not assigned to any taxonomic grouping or are in provisional groupings (72). Also, the identification of clades and relationships between strains at the subspecies level, such as those within human enterovirus 68 (EV-D68), requires the analysis of several other non-VP1 targets including the 5′-untranscribed region and VP4 (74). The inability to assign specific viruses to a particular taxonomic grouping is problematic because there is a need to link clinical disease with specific types, as well as a need to develop and define the characteristics of new tests that may need to account for the absence of current assay targets. As described previously, the increased utilization of whole genome sequencing has allowed for the characterization of viruses to identify new relationships within the picornaviruses (75). New bioinformatics approaches for comparing whole genome sequences, including quantitative procedures to hierarchically classify picornaviruses based on intervirus genetic divergence, are now being attempted by some scientists (76). A side-by-side comparison with ICTV classification has already been undertaken using this approach, with the authors proposing that the genome contains enough information to act as the sole demarcation criterion for the picornaviruses (72).

## Adenoviruses

As stated earlier, the lowest level of taxonomic classification that the ICTV undertakes is the species, and multiple criteria are used to determine a species within the genus *Mastadenovirus* (http://www.vmri.hu/~harrach/AdVtaxlong.htm). Below the level of species, serotype has been used to understand the clinical epidemiology and pathophysiology of these viruses in humans; however, in the case of adenoviruses, serotype/type has played a key role in linking a species to a disease process. Traditional adenovirus typing involved the isolation and propagation of the virus followed by serotyping, which in the case of a suspect novel type would require an extremely large number of virus neutralization assays. However, for almost a decade, the amplification of the hexon gene provided a reasonable surrogate to the traditional approaches (77). Recently, major points of discussion include how to define type, how to deal with recombination events (including intertypic recombination), the extent of sequences required for comparison, and how

to manage and identify new strains as well as storage of sequence information, and it is clear that a typing method focusing on one gene target will not be operationally viable going into the future (78). There are already significant criteria being introduced at the Human Adenovirus Working Group to address the use of sequencing information, link species to type in a new nomenclature system, require the use of complete genome sequencing and phylogenetics in the creation of a new type identifier, provide a rule for naming priorities, and deal with the issue of recombination (79). Some researchers have already proposed that whole genome analysis should be used to identify new lineages of adenoviruses and provide the evidence for either a new species or a new type number (80), and this approach has also been used to speculate on viral evolution and search for potentially emerging types and subtypes (81). Regardless, these issues will definitely create changes in how adenoviruses are characterized over the next 5 to 10 years and will push consensus groups further into the realm of subspecies classification.

## Norovirus

Norovirus genogroups I, II, and IV are clinically important for humans, with recent novel strains emerging and data suggesting that strain variation can be driven by positive selection during chronic infection within immunocompromised hosts (82). Currently, real-time PCRs to determine genogroups I and II are in broad use and the ability to genotype has also been widely established, but multiple approaches exist and there is a need for consistency for genotyping as well as identification of new strains (83). These genogroups are further divided into genotypes (84). Since the mid-1990s, norovirus genotypes have been based on the complete VP1 gene sequence (ORF2; open reading frame 2), encoding the 60 kDa capsid protein, with new genotypes being designated when more than 20% of VP-1 amino acids differed using pairwise analysis. In 2011, researchers who were part of the Food-Borne Viruses in Europe Network proposed a molecular epidemiologic approach focused on the analysis of ORF2 (85). This focus on ORF2 and its epitopes B, C, and D is still used to characterize new strain variants (86). However, the primary focus on ORF2 as a sole target for genotyping has begun to shift within the last 5 years. During the 4th International Conference on Caliciviruses in 2010, a need for common classification of noroviruses was identified and a norovirus working group was established. This group was influenced by the *Picornaviridae* and *Flaviviridae* working groups described earlier in this chapter that had created practical standards for universal nomenclature and typing systems. By 2013, members of this working group proposed a phylogenetic analysis of the full VP1 sequence as well as the partial 3′ ORF1 sequence being utilized to generate new genotypes (84). The ORF1 encodes for a nonstructural polyprotein that undergoes proteolytic cleavage to release six nonstructural proteins (87). An expanded approach has been shown to allow for identification of recombination events at the ORF1/ORF2 overlap (88, 89), recombinations within VP1 (ORF2) (90), and possibly within the ORF2/ORF3 boundary (90) in emerging variants, which would not be identified if only ORF1 or ORF2 sequences were analyzed (91). As seen previously, other groups have gone to full genome analysis to characterize the emergence of new strains within their jurisdictions (92, 93). These whole genome approaches have already been used in outbreak settings to identify minor genetic variations that could suggest transmission events and might be utilized to suggest a direction of transmission (94).

In conclusion, in spite of their simplicity, viruses are a complex and diverse group of organisms that may have equally diverse origins and evolutionary pressures. Their interaction with their human hosts may cause disease but also impacts viral evolution and shapes key viral characteristics. Viral taxonomy, classification, and characterization can be thought of as important tools that improve our ability to diagnose and compare viruses of medical importance. This framework also allows us to place newly identified viruses within the tree of life and may provide clues to pathophysiology when they may not yet be completely evident. Linkage to well-understood disease processes also allows for the characterization and classification of viruses into disease-focused frameworks that may not be completely driven by the biology of the organisms. Over the last 5 years, there have been significant changes in the field of viral taxonomy, and this includes changes in the proper name of some commonly identified viruses as well as realignment of relationships between these medically relevant viruses. Some resources, such as databases, have ceased to exist as up-to-date tools, while new databases have emerged or been strengthened to support viral taxonomy. Some of these changes, such as with the nature of what constitutes a viral species, have led to vibrant discussion and are critical for the development of taxonomy in the future. Related to this discussion with the nature of species, the changes in taxonomic approaches and even our understanding of viral evolution have also changed and are now being driven by significant increases in genetic information created by whole genome analysis and metagenomic approaches as well as the bioinformatics tools to support this information. These molecular approaches now allow for the classification of viruses in new ways, which will in turn also impact how currently known and yet to be discovered viral pathogens are characterized and classified. No doubt, we will continue to see a greater role for phylogenetics in the placement of viruses within a structured framework, while other more subjective or historic characters of these viruses will have a lesser impact on viral taxonomy.

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# Quality Assurance and Quality Control in Clinical and Molecular Virology

MATTHEW J. BANKOWSKI

# 2

The clinical virology laboratory provides important and often critical information to the health care provider in order to support the diagnosis or monitoring of viral disease for the patient. Testing results will often serve as a guide for optimal treatment of the disease, contribute to infection control and prevention of a hospitalized patient or offer insight into the prognosis for the disease. Therefore, the quality of the virology laboratory testing has to be highly accurate and offered in a timely fashion in order to achieve optimal patient management. A well-structured and ongoing quality assurance (QA) program will provide the framework for maintaining accuracy in all phases of the testing process. These phases include the preanalytical, analytical, and postanalytical stages of the testing. However, no process is perfect, and every QA program should include a surveillance component that continuously identifies and corrects any weakness in the system. This corrective action should also be followed by preventative action in order to eliminate weaknesses and improve the entire QA program.

## REGULATORY REQUIREMENTS

In the United States all clinical laboratories have to be certified under the Clinical Laboratory Improvement Amendments (CLIA) (1, 2). This amended USA federal law governing clinical laboratory testing is listed in Section 353 of the Public Health Service Act (42 U.S.C. 263a) as published in the Federal Register on 28 February 1992 as a final rule. The CLIA regulations established three levels of complexity corresponding to minimal quality standards for the type of laboratory. These categories consist of waived, moderate-complexity, and high-complexity. CLIA was established to ensure the quality of laboratory services based on these complexity levels. The CLIA regulations incorporate provisions for clinical laboratory personnel, facilities, quality assurance, quality control, proficiency testing, record keeping, and record retention.

Subsequently, the Department of Health and Human Services (HHS) published a revised final rule in the Federal Register on 24 January 2003. This revised final rule contained clarifications and reorganization to make the document more concise. Importantly, this revised final rule incorporated the quality system concept into clinical laboratory testing. All clinical laboratories must be certified under CLIA. However, depending upon the state in which the clinical laboratory is located, other agencies, such as the Centers for Medicare and Medicaid Services (CMS), may approve the laboratory licensure. CLIA-certified laboratories are also subject to biennial inspections, which are intended to be educational and aid in improving testing and optimizing patient care. Clinical laboratories may also meet the CLIA requirements through being inspected by CMS-approved nonprofit organizations (e.g., College of American Pathologists [CAP] or The Joint Commission).

## VIROLOGY QUALITY ASSURANCE

Quality assurance in the clinical laboratory is a multifaceted process. QA includes quality control, proficiency testing, technical staff training and competency, instrument calibration, and clinical correlation. It is an ongoing process that maintains optimum test performance that is controlled at every stage of the testing process. This includes testing personnel from preanalytical to analytical and postanalytical test procedures. Quality control reagents are included in the day-to-day testing process, and frequent challenging of the process is also carried out by proficiency testing. A troubleshooting process is instituted when tests fail, which is followed up by investigation, corrective action, and preventive action. Useful documents that serve as guidelines for maintaining quality assurance in the clinical virology laboratory can be obtained from the Clinical and Laboratory Standards Institute (CLSI) (Table 1) and the American Society for Microbiology (ASM) (Table 2). General documents from these two reference sources include CLSI QMS02A6 and Cumitech 3B.

## CLINICAL LABORATORY PERSONNEL

All laboratory staff involved in any part of the testing process need to be qualified according to CLIA and applicable state licensure requirements. The laboratory director is responsible for defining the qualifications and responsibilities in written form for all of the staff involved in this process.

Virology testing is considered as moderate or high complexity according to CLIA-88. Therefore, any staff involved

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**TABLE 1** Guideline documents from the Clinical and Laboratory Standards Institute (CLSI)[a]

| Document no. | Date | Document title and description |
|---|---|---|
| **General laboratory** | | |
| GP17A3 | 06/29/12 | Clinical Laboratory Safety; Approved Guideline—Third Edition |
| GP27A2 | 02/22/07 | Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline—Second Edition |
| GP29A2 | 08/29/08 | Assessment of Laboratory Tests When Proficiency Testing Is Not Available; Approved Guideline—Second Edition |
| GP31A | 08/22/12 | Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline |
| QMS02A6 | 02/28/13 | Quality Management System: Development and Management of Laboratory Documents; Approved Guideline. Sixth Edition. |
| QMS03A3 | 05/02/09 | Training and Competence Assessment; Approved Guideline Third Edition. |
| QMS04A2 | 02/22/07 | Laboratory Design; Approved Guideline—Second Edition |
| QMS05A2 | 09/28/12 | Quality Management System: Qualifying, Selecting, and Evaluating a Referral Laboratory; Approved Guideline—Second Edition |
| QMS12A | 12/29/10 | Development and Use of Quality Indicators for Process Improvement and Monitoring of Laboratory Quality; Approved Guideline |
| **Method evaluation** | | |
| EP12A2 | 01/25/08 | User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition. |
| EP15A3 | 09/11/14 | User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition |
| EP23A | 10/25/11 | Laboratory Quality Control Based on Risk Management; Approved Guideline |
| EP25A | 09/23/09 | Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline |
| EP26A | 09/30/13 | User Evaluation of Between-Reagent Lot Variation; Approved Guideline |
| **Microbiology** | | |
| M41A | 11/30/06 | Viral Culture; Approved Guideline |
| M53A | 06/30/11 | Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline |
| **Molecular methods** | | |
| MM03A2 | 02/17/06 | Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition |
| MM06A2 | 11/30/10 | Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition |
| MM09A2 | 02/28/14 | Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition |
| MM13A | 01/06/06 | Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline |
| MM14A2 | 05/23/13 | Design of Molecular Proficiency Testing/External Quality Assessment; Approved Guideline—Second Edition |
| MM17A | 03/21/08 | Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline |

[a]Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, http://clsi.org

in the actual testing of specimens are required to be qualified under these categories. Staff involved in the analytical phase need to be adequately trained on a test in order to ensure that there is a complete understanding of the test procedure. In order to ensure fulfillment of this step, an evaluation by actual observation of the technologist performing the test on a recurrent basis (i.e., operator competency assessment) is instituted. This approach to testing is to be unaltered, and strict adherence to the procedure manual, biosafety training and awareness, patient confidentiality, result interpretation, reporting, and quality control are to be maintained at all times. Competency assessment is instituted to identify employee performance issues. Documentation of problems, especially a pattern of performance issues, is to be addressed using remediation. Testing personnel also need to be knowledgeable enough to recognize unusual results and to be proficient in troubleshooting of failed runs. In addition, laboratory personnel are to have documented evidence of continuing education and active licensure. Refer to Table 1 (CLSI) and Table 2 (Cumitech) for further information and guidance from documents QMS03A3, Cumitech 39 and 41.

## PROCEDURE MANUAL

The procedure manual is one of the most important documents in the laboratory. It is customized to the individual laboratory but is standardized to contain procedures with sections that are required as described in the CLIA document QMS02A6 (Table 1). It is required that the procedure manual contain directions and guidance for all three stages of the testing process: preanalytical, analytical, and postanalytical. The procedure is not just a rewritten form of the package insert but a highly organized, concise, step-by-step document customized to the individual laboratory.