Diagnostic and Molecular Challenges in Hantavirus Identification

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Hantavirus identification is difficult because clinical disease can begin with nonspecific symptoms, viremia may be transient, related viruses can cross-react in serological assays, and genetically diverse viruses circulate in different reservoir hosts and regions (Afzal et al., 2023). The early phase of infection may resemble influenza, leptospirosis, dengue, sepsis, or other febrile illnesses. By the time hantavirus pulmonary syndrome (HPS), also called hantavirus cardiopulmonary syndrome, progresses to severe pulmonary edema and shock, deterioration can be rapid. Hemorrhagic fever with renal syndrome (HFRS) presents differently, with renal injury and hemorrhagic manifestations more prominent, but the early diagnostic uncertainty is similar.

Modern taxonomy also requires precision. Human-pathogenic hantaviruses are classified within the genus *Orthohantavirus*, subfamily Mammantavirinae, family Hantaviridae. The International Committee on Taxonomy of Viruses describes Hantaviridae as a family of segmented negative-sense RNA viruses whose genomes are divided into small, medium, and large segments (International Committee on Taxonomy of Viruses [ICTV], 2024). These encode the nucleoprotein, glycoprotein precursor, and RNA-dependent RNA polymerase, respectively. Many orthohantaviruses have long-standing associations with specific rodent or small-mammal reservoirs, helping explain their geographic distribution (ICTV, 2024).

Most human infection occurs after inhalation of aerosols contaminated with infected rodent urine, feces, or saliva. Direct contact and bites can also occur. Most orthohantaviruses are not known for sustained human-to-human transmission, although Andes virus is a recognized exception. These ecological and epidemiological differences matter diagnostically because the likely virus depends partly on geography, exposure, and clinical syndrome. A laboratory result should therefore be interpreted together with exposure history rather than as an isolated number.

Clinical Suspicion

Clinical recognition begins with the combination of symptoms and exposure. The Centers for Disease Control and Prevention (CDC) current Clinical Overview of Hantavirus advises clinicians to consider testing when compatible illness occurs in a person with possible rodent exposure (CDC, 2026a). A patient may report cleaning an infested shed, sleeping in a rodent-contaminated cabin, agricultural work, pest-control activity, handling rodents, or travel in an endemic area. Absence of a remembered

exposure does not exclude infection because contact can occur without being noticed.

HPS commonly begins with fever, fatigue, headache, myalgia, nausea, or abdominal symptoms before progressing to cough, dyspnea, pulmonary edema, hypoxemia, and shock. CDC's Clinician Brief: Hantavirus Pulmonary Syndrome emphasizes the potential for rapid cardiopulmonary deterioration (CDC, 2026b). HFRS more commonly involves acute kidney injury, thrombocytopenia, vascular leak, and varying degrees of hemorrhage. These syndromes are not completely separate: renal findings can occur in HPS, and pulmonary manifestations can occur in HFRS.

Routine laboratory findings can strengthen suspicion but are not diagnostic. Thrombocytopenia, leukocytosis, hemoconcentration, atypical or immunoblastic lymphocytes, elevated creatinine, proteinuria, or other abnormalities may appear depending on the syndrome and phase of illness. Imaging may reveal pulmonary edema in HPS, but imaging cannot determine the causative virus. A diagnostic workflow should therefore avoid treating one clinical pattern as pathognomonic.

The timing of specimen collection is critical. Viral RNA is most detectable during the early viremic phase, whereas antibodies become increasingly useful as the immune response develops. Romeo et al. (2025) emphasize that serological and molecular methods are complementary because each has a different diagnostic window. The practical question is not which method is universally "best," but which method is most informative at the stage when the patient is tested.

Laboratory Diagnosis

Serology remains central to hantavirus diagnosis. IgM antibodies against viral antigens, especially nucleoprotein, can often be detected around the time symptomatic patients seek care. IgG antibodies develop and can persist much longer, so an isolated IgG result may indicate previous exposure rather than a new illness. Paired serum samples demonstrating seroconversion or a substantial rise in IgG can help clarify uncertain cases.

Cross-reactivity is a major limitation. Conserved regions of the nucleoprotein can cause antibodies to react with related orthohantaviruses. A positive IgM assay may therefore confirm recent hantavirus infection without reliably identifying the exact virus. The result must be interpreted according to assay design, circulating viruses, exposure location, and validation data. False-positive IgM reactions are also possible, which is why unusual or discordant results may require confirmation at a reference laboratory.

Reverse-transcription polymerase chain reaction detects viral RNA and is especially useful during the early phase of illness. RT-PCR can provide stronger evidence of active infection and can also support virus identification when sufficient sequence information is obtained. Sensitivity depends on viral load, specimen type, timing, RNA integrity, and whether primers and probes match the circulating virus.

Targeted assays work efficiently when the expected virus is known, but diversity creates a design challenge. Primers optimized for one lineage may perform poorly against a newly emerging or genetically divergent virus. Broad-range or pan-hantavirus assays can increase coverage but may sacrifice sensitivity or require subsequent sequencing. Romeo et al. (2025) therefore describe diagnostics as a balance among speed, breadth, sensitivity, specificity, and access to specialized laboratories.

Neutralization testing can provide greater specificity for species-level serological attribution. Plaque-reduction or focus-reduction neutralization methods assess whether patient antibodies block infection by particular viruses. These tests are useful for epidemiology and confirmation but are slower, technically demanding, and may require live virus and high-containment facilities. They are generally less suitable than rapid serology or RT-PCR for immediate bedside decisions.

Immunohistochemistry can detect viral antigen in tissue and may be useful in fatal cases. Pathology can support the diagnosis, but tissue appearance alone is not sufficiently specific to distinguish hantavirus from every other cause of acute pulmonary or renal failure. Serology, PCR, exposure history, and clinical course should be integrated whenever possible.

Molecular Identification

Molecular identification becomes especially important for outbreak investigation, surveillance, and newly recognized lineages. Sequencing of RT-PCR products can determine how a patient virus relates to reference strains. Phylogenetic analysis should use appropriate reference sequences and, where possible, information from more than one genome segment because the hantavirus genome is segmented.

Next-generation sequencing can extend this approach by generating larger portions of the genome or detecting divergent viruses when routine targeted assays fail. Metagenomic sequencing can be particularly useful when the suspected pathogen is unknown. However, clinical samples often contain far more host RNA than viral RNA, and results can be distorted by contamination, incomplete genome coverage, index misassignment, or bioinformatic errors. Sequence detection therefore requires independent confirmation and should not be treated automatically as proof of causation.

Species attribution may also be limited by serology. A patient may have a convincing antibody response to several related antigens because of cross-reactivity. In such cases, geography, reservoir ecology, PCR, sequencing, and neutralization results may be needed to reach a more specific conclusion. Clinical management often depends more on recognizing severe hantavirus disease than on naming the exact virus, whereas precise identification becomes more important for surveillance and public-health investigation.

Reservoir testing adds another layer. Finding viral RNA in a local rodent population can support an epidemiological hypothesis, but an environmental or animal sequence does not prove that the same

virus caused a particular human infection. Strong attribution requires a coherent relationship among patient sequence, reservoir sequence, exposure location, and timing.

The virus itself also complicates molecular research. The segmented genome permits reassortment when compatible viruses coinfect the same cell, while mutation and selection contribute to diversity over time. Claims of recombination or reassortment require careful analysis because contamination or assembly artifacts can mimic true evolutionary events. The current Family: Hantaviridae taxonomy should be used when reporting formal names because classification changes as sequence and ecological knowledge expands.

Research Challenges

Biosafety limits what many laboratories can do. Routine clinical specimens can be handled using established procedures, but propagation and neutralization of live pathogenic viruses can require specialized containment and experienced personnel. Laboratories should coordinate early with public-health or reference laboratories when hantavirus is strongly suspected so that specimen type, packaging, timing, and testing authorization are appropriate.

Another challenge is that laboratory confirmation and functional research answer different questions. A sequence difference found in severe cases may correlate with disease without causing that severity. Demonstrating a virulence mechanism requires experimental systems capable of manipulating the viral genome, appropriate cell or animal models, and careful control of host factors. Reverse-genetics systems have improved for some orthohantaviruses, but they remain more technically demanding than systems available for many better-studied RNA viruses.

Recent structural and immunological research is also expanding knowledge of the viral glycoproteins Gn and Gc, which mediate entry and are targets for neutralizing antibodies. These studies may improve future vaccines and therapeutics, but diagnostic practice still relies mainly on established serological and molecular methods. Tscherne et al. (2025) note that no broadly available licensed vaccine or specific antiviral treatment exists for most medically important orthohantaviruses, making prevention, rapid recognition, and supportive care especially important.

The strongest diagnostic strategy therefore combines clinical context with more than one laboratory method when necessary. In an acutely ill patient, IgM serology can provide rapid evidence of recent infection while RT-PCR may confirm active viremia and permit sequencing. Paired IgG testing can resolve uncertain timing, and neutralization can clarify cross-reactive species attribution. Reference-laboratory consultation is particularly valuable when the clinical pattern is severe but routine results are discordant.

Hantavirus identification remains challenging not because laboratory medicine lacks tests, but because each test observes a different part of the infection. Serology observes the host response; RT-PCR observes viral RNA; sequencing observes genetic identity; neutralization tests functional antibody

specificity; pathology observes tissue effects. Accurate diagnosis emerges when these forms of evidence agree with the patient's exposure and clinical course.

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