Hantavirus

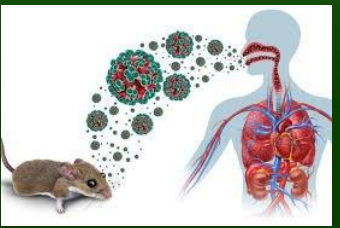
Diagnosis and Laboratory Methods

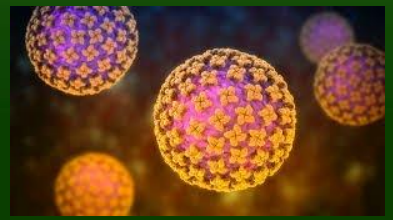
Dr. Fatemeh Sameni

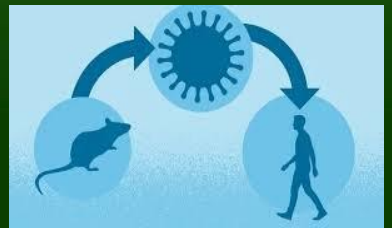
Infectious Diseases and Tropical Medicine Research Center

Shahid Beheshti University of Medical Sciences

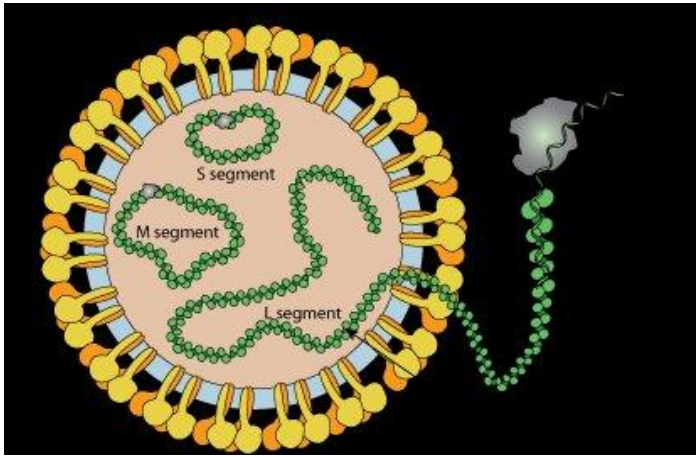
Sameni.f@yahoo.com







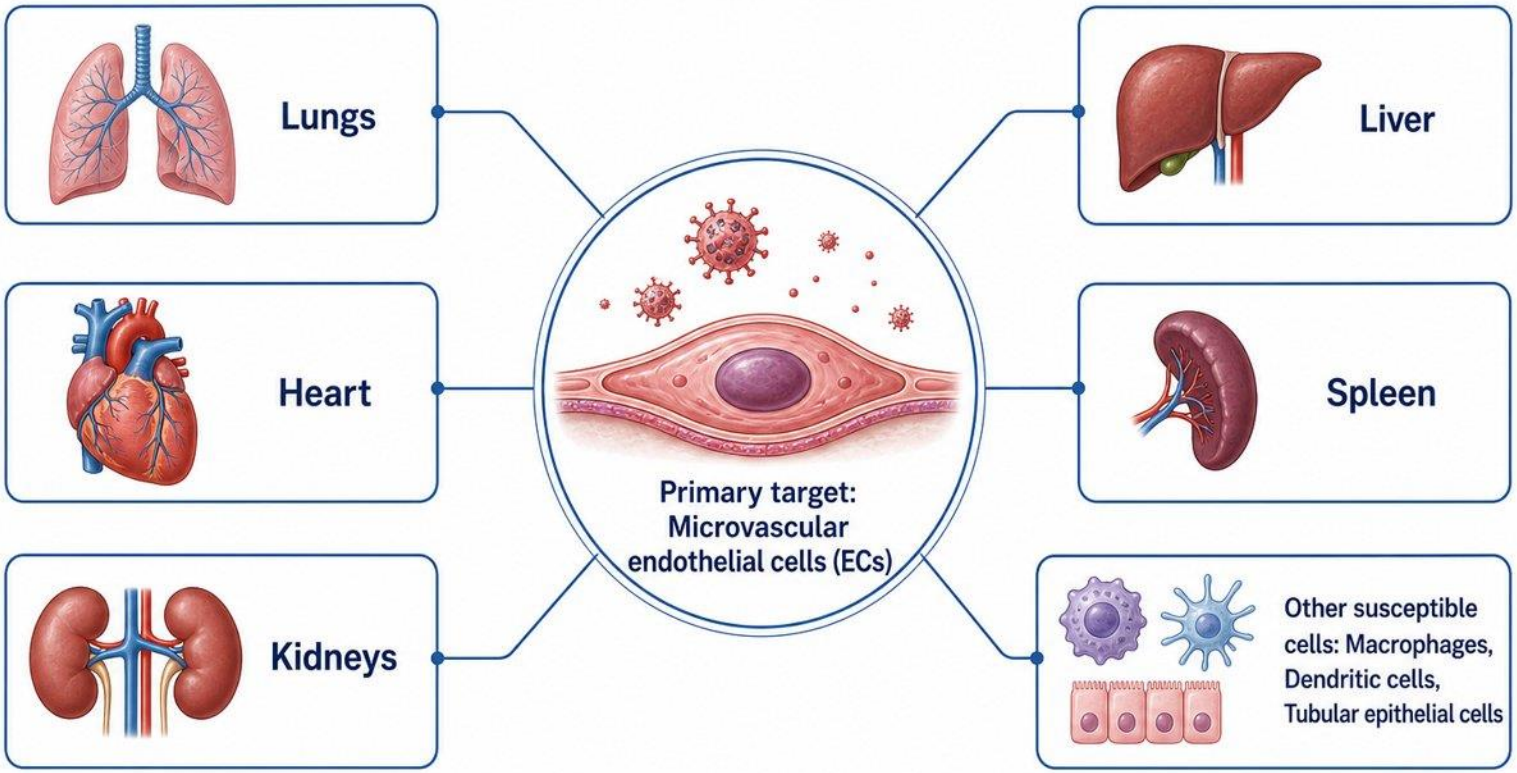
Introduction to Hantaviruses



- Enveloped, spherical RNA viruses (80–120 nm)
- Negative-sense, single-stranded RNA virus
- Genome composed of 3 segments:
  - **S** → Nucleocapsid (N protein)
  - **M** → Glycoproteins (Gn, Gc)
  - **L** → RNA-dependent RNA polymerase (RdRp)

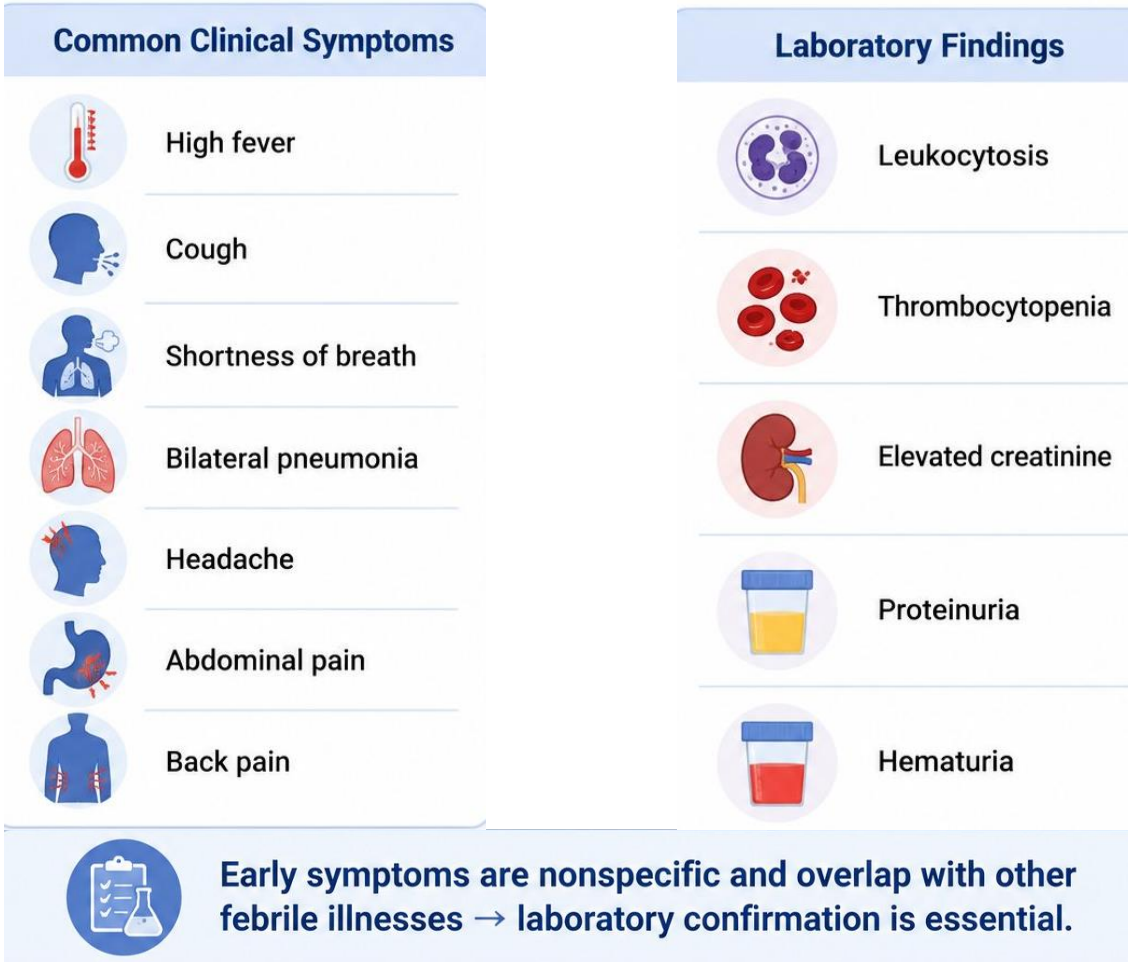
Taxonomy and Classification

Orthohantavirus Cell Tropism

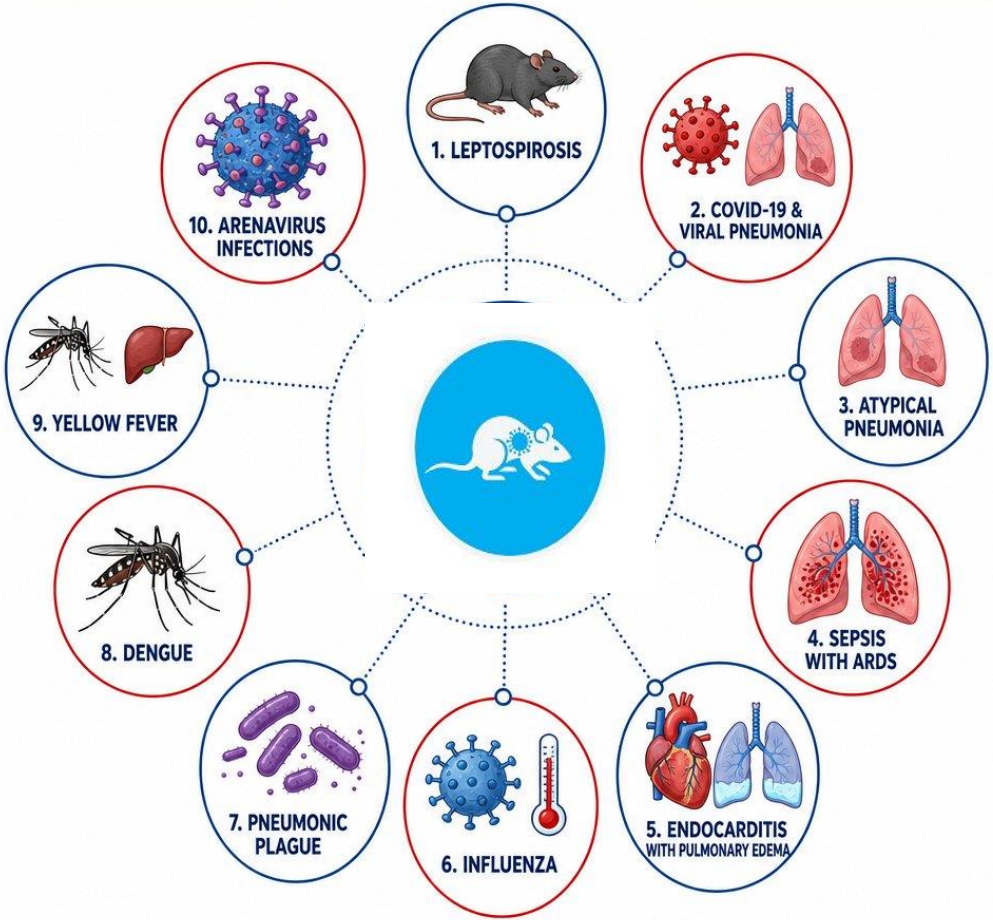


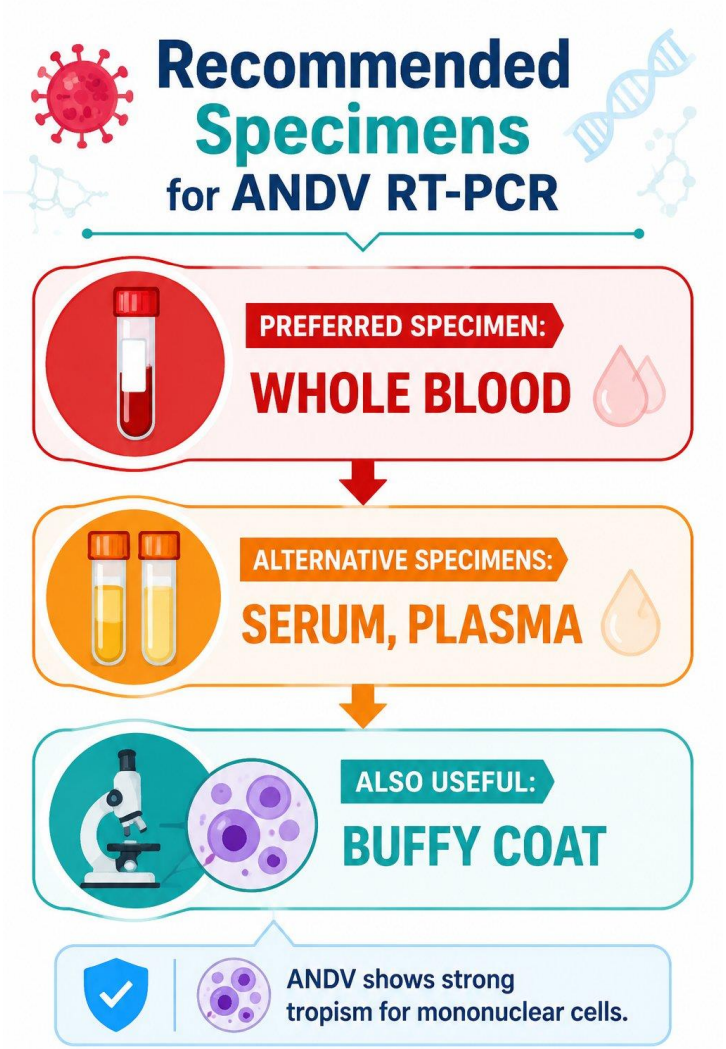
These cells may contribute to the early stages of pathogenesis.

Common clinical features of HPS caused by ANDV



Differential Diagnoses (Prodromal Phase)




Recommended
Specimens
for ANDV RT-PCR
PREFERRED SPECIMEN:
WHOLE BLOOD
ALTERNATIVE SPECIMENS:
SERUM, PLASMA
ALSO USEFUL:
BUFFY COAT
ANDV shows strong
tropism for mononuclear cells.

Recommended Specimens for ANDV Serological Testing

1 SUITABLE SPECIMENS

- Serum

- Plasma

2 ADDITIONAL SPECIMENS

- Urine

- Saliva / Gingival Swabs (VTM)

- Semen

- Cerebrospinal Fluid (CSF)

- Lung Biopsy

- Spleen Biopsy

- Liver Biopsy

SPECIMEN STORAGE GUIDELINES

1 Short-term Storage (≤24 h)

Whole Blood | Clotted Blood | Serum | Plasma

Use ice packs during transport

2 Delayed Transport (>72 h)

CSF

Serum

-80°C
ultra-low freezer

Ship on Dry Ice

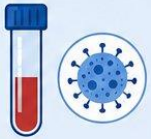

Specimen Shipping – Important Warnings
Do NOT ship clotted blood or EDTA / heparin / citrated whole blood on dry ice
CLOTTED BLOOD
EDTA
HEPARIN
CITRATED
DRY ICE
Avoid repeated freeze–thaw cycles – they degrade specimen quality
BIOHAZARD
SPECIMEN INSIDE
Proper Packaging
Correct Labeling
Follow Shipping Regulations

ANDV Infection Dynamics

1 Clinical presentation & exposure history

2 Incubation period

Range 1–6 weeks, median ~18 days



3 Virological & serological markers at symptom onset

High viremia, IgM detectable, IgG appears shortly after

4 Antibody kinetics

IgM declines within 1–3 months, IgG persists for years

5 Diagnostic approach

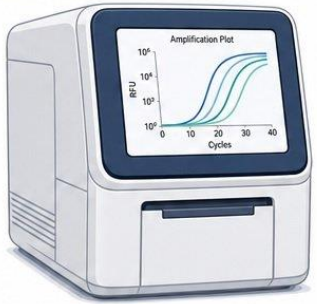
RT-PCR

Serological assays

Nucleic Acid Amplification Testing (RT-PCR) for Andes Virus (ANDV)

1 WHO Recommendation

- Confirmation of ANDV infection using Nucleic Acid Amplification Testing (NAAT)
- Preferred method: laboratory-based RT-PCR
- Detects ANDV RNA-specific sequences.

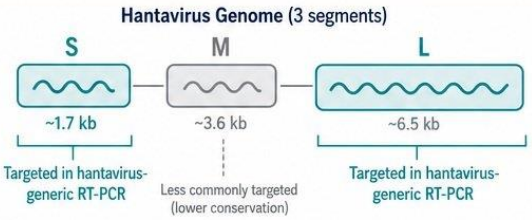


2 Recommended Specimens

Whole blood (preferred) | Serum | Plasma | Buffy coat

3 Alternative Testing Strategy

If ANDV-specific RT-PCR unavailable: use hantavirus-generic RT-PCR targeting conserved genomic regions: S segment and L segment; confirm ANDV by sequencing; M segment less commonly targeted due to lower conservation.



Serological Testing for Hantavirus (ANDV / HPS)

Standard methods

ELISA

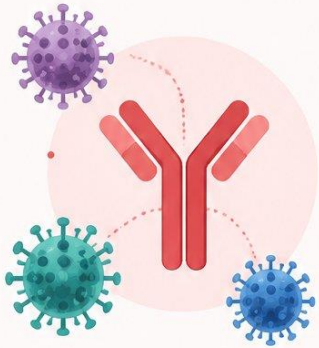
(Enzyme-Linked Immunosorbent Assay)

IFA

(Immunofluorescence Assay)

LIMITATIONS

Cross-reactivity issues:



- New World hantaviruses (e.g., Sin Nombre virus)
- Other viruses (e.g., Epstein–Barr virus)
- Interference factors (e.g., rheumatoid factors)

Serology must be interpreted cautiously due to:

- Global hantavirus exposure evidence
- Presence of endemic regions
- Circulating cross-reactive orthohantaviruses
- Some hantavirus strains remain not fully taxonomically classified, complicating interpretation

IgM ELISA / IFA

- **Appears at symptom onset**
- **Rises within ~1 week**
- **Disappears after 1–3 months**
- **Important in early infection diagnosis**
- **Possible false positives due to:**
  - EBV and other cross-reacting viruses
  - Rheumatoid factors
  - Non-validated assays

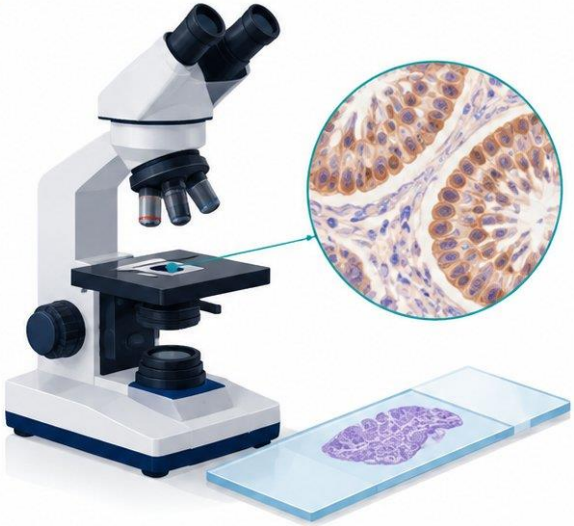
IgG ELISA / IFA

- **Appears shortly after IgM**
- **Persists for years**

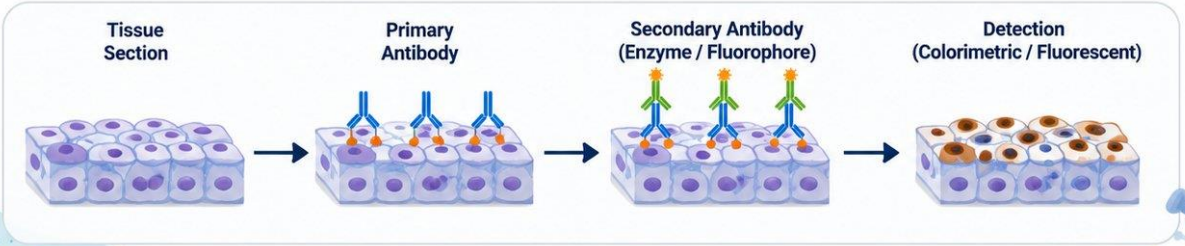
- **Useful for:**
  - Past exposure detection

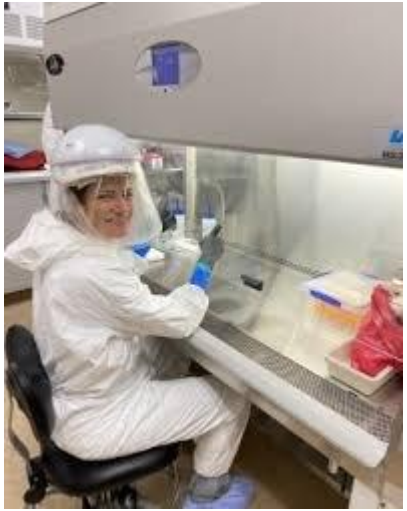
- **Limitations:**
  - Not useful in early disease phase
  - Possible false positivity due to cross-reactivity in endemic regions

Immunohistochemistry



- Detection in infected tissues
- Based on ELISA / immunofluorescence
- Mostly post-mortem use
- Requires complex preparation





Viral Culture

Not recommended for routine diagnosis

Virus isolation is:

1. Technically complex
2. Requires specialized expertise
3. Requires BSL-3 (Biosafety Level 3) laboratories

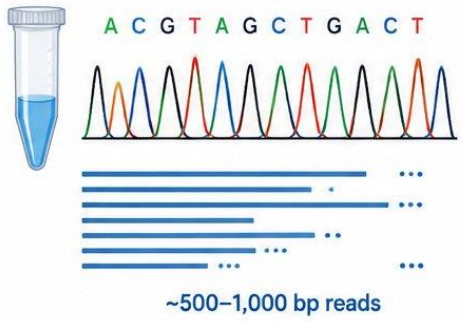
Due to these limitations, detailed protocols are not routinely used in clinical settings

Genomic Sequencing

Genomic sequencing methods vary widely

Range:

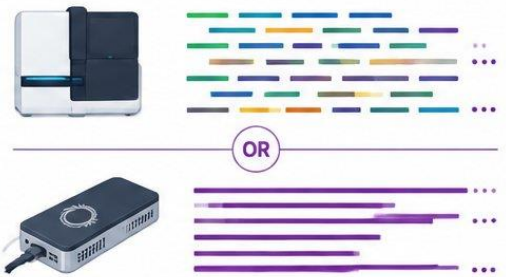
Sanger sequencing (partial genome fragments)



~500–1,000 bp reads

Good for small regions, validation, and targeted studies

Whole genome sequencing (Illumina / Oxford Nanopore)



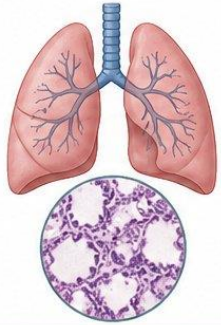
Short reads (Illumina) or long reads (Nanopore)

Comprehensive coverage, variant discovery, and *de novo* assembly

A G T C T G A C C T G A T G C T A A G C T T G A C C T G A T G C T A A G C T T G A C C T G A T G C T A A G C T

Relative Difficulty of Direct Sequencing from Different Human Samples

Easier — Sequencing Challenge Level — More Challenging



Lung Tissue
(Fresh or FFPE)

Lower technical challenges

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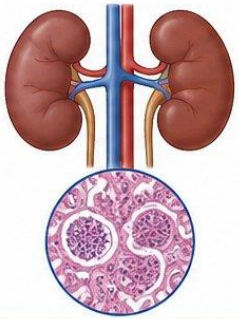

Kidney Tissue
(Fresh or FFPE)

Moderate technical challenges

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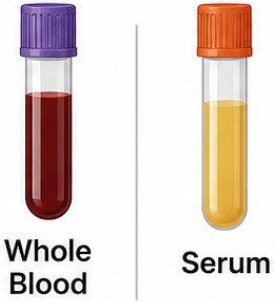


Whole Blood or Serum

Higher technical challenges

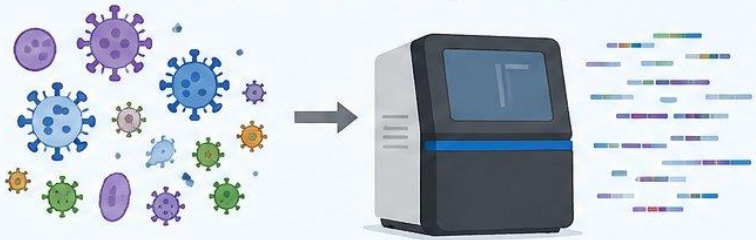
Limited anecdotal data suggests that sequencing directly from human whole blood or sera is more challenging than sequencing total RNA extracted from lung or kidney tissues (fresh or formalin-fixed paraffin-embedded).

Choosing the Right Sequencing Method

It depends on expected strain diversity and viral load

Unbiased Next Generation Sequencing
(Metagenomic / Shotgun NGS)

Detects known and unknown viral strains (or hantavirus-like organisms)



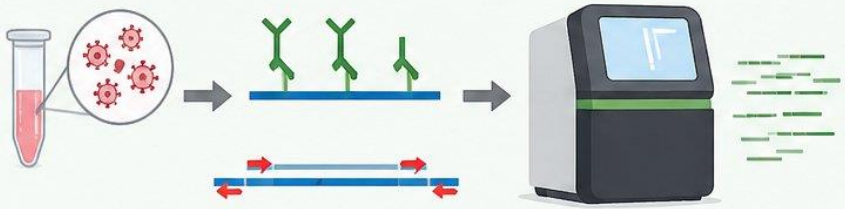
Advantages: Unbiased, broad detection, can discover novel viruses

Limitation: Lower sensitivity, especially in low viral load samples

VS.

Targeted Sequencing
(Probe-based Enrichment / Amplicon-based)

Designed to detect known viral targets with high sensitivity



Advantages: Higher sensitivity, better performance in low viral load samples

Best for: Specimens with low viral loads when target viruses are expected


Thanks for
Your Attention