Orthohantaviruses Laboratory Detection

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Diagnostic Challenges

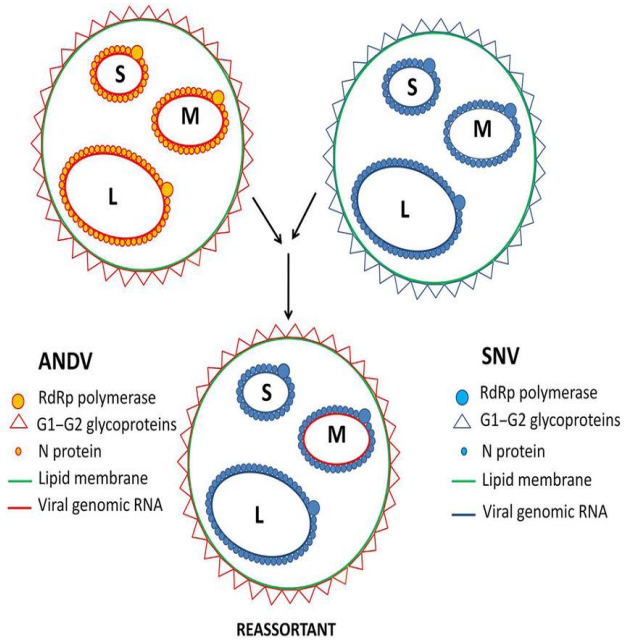
- Nonspecific early symptoms
- Mimics influenza/COVID/leptospirosis/dengue
- Rare disease in non-endemic settings

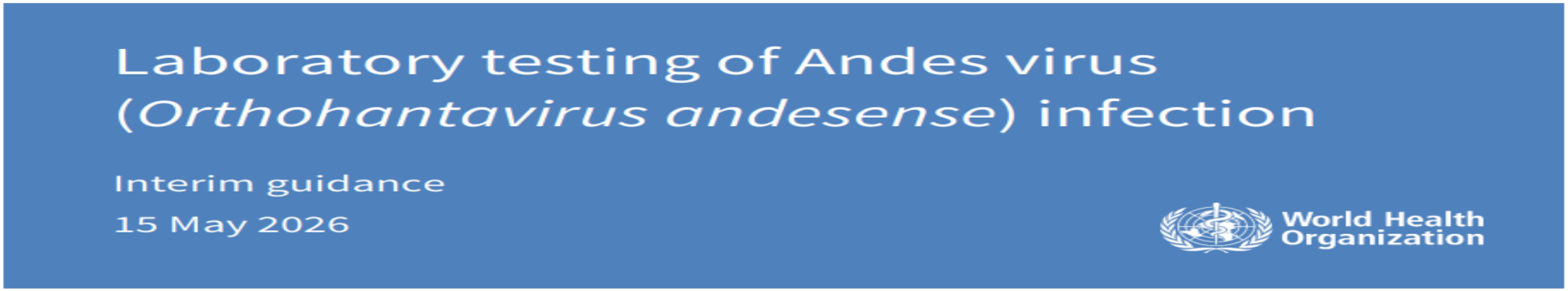
Differential Diagnosis in Real-World Settings

Disease	Overlapping features	Differentiating tests
Leptospirosis	Renal failure, thrombocytopenia, fever	Leptospira PCR, blood culture, IgM
COVID-19	Interstitial pneumonia, fever, shock	SARS-CoV-2 RT-PCR
Sepsis of unknown origin	Shock, coagulopathy	Blood culture, procalcitonin, lactate
Rocky Mountain spotted fever	Fever, petechiae, thrombocytopenia	Rickettsia serology (Weil-Felix)
HCPS (ANDV)	Pulmonary oedema, cardiogenic shock, thrombocytopenia	Hantavirus IgM + RT-PCR

Diagnostic Challenges

- Low viral load in acute phase: often <1000 copies/mL plasma
- Narrow timing windows: 3-7 days after symptom onset
- IgM antibodies appear late (day 5-14)
- Cross-reactive serology
- Genetic variability (reassortment, mutations) → false negatives in specific PCR
- Need for two independent methods (serology + molecular or sequencing)



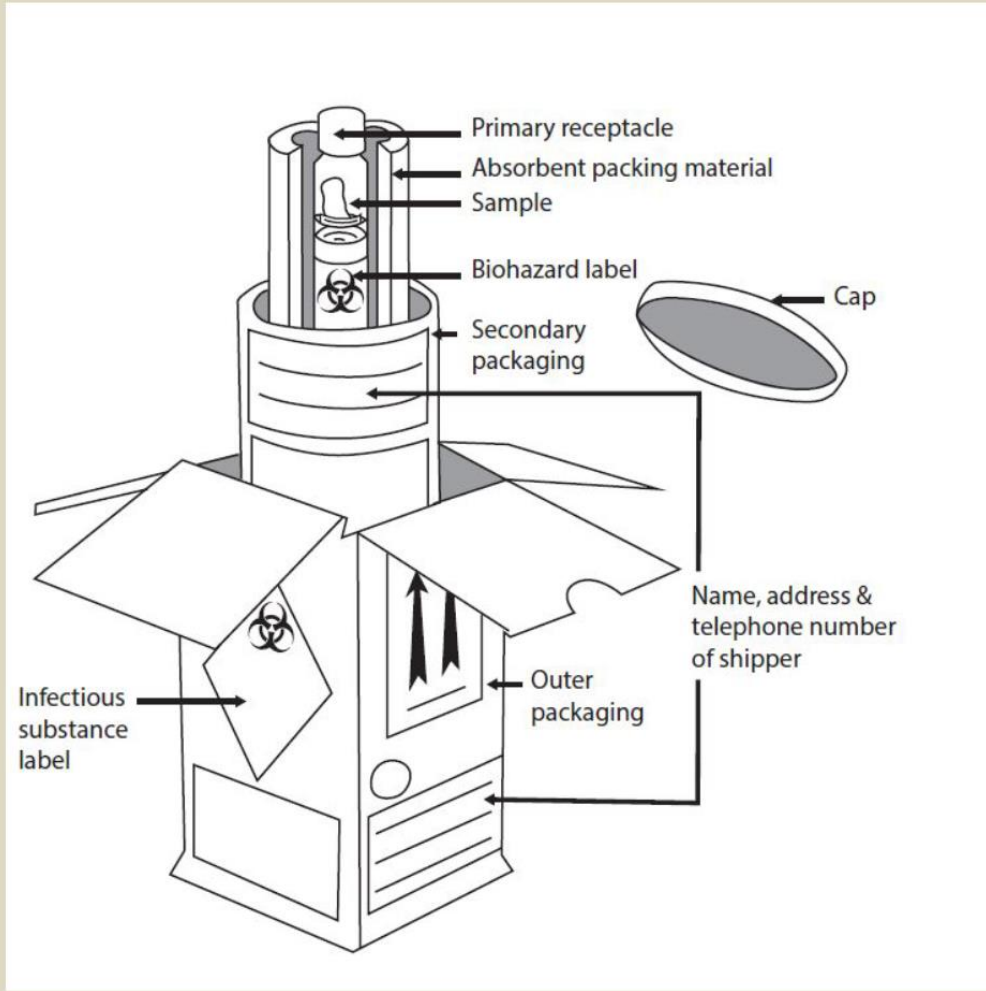


- Preferred sample: **whole blood (EDTA)** – plasma has lower load
- serum, plasma, buffy coats can also be used Other specimens: urine, saliva/gingival swab in VTM, semen, CSF

- For fatal cases: **lung tissue** (fresh frozen or formalin-fixed) – PCR positive days after death

Specimen Handling

Preferred sample	Storage
Whole blood Buffy coat Serum/plasma tissue	2–8°C short-term Delayed shipment −80°C/−20°C



گیرنده:

تهران، میدان پاستور، خیابان ۱۲ فروردین،

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هماهنگی پیش از ارسال نمونه:

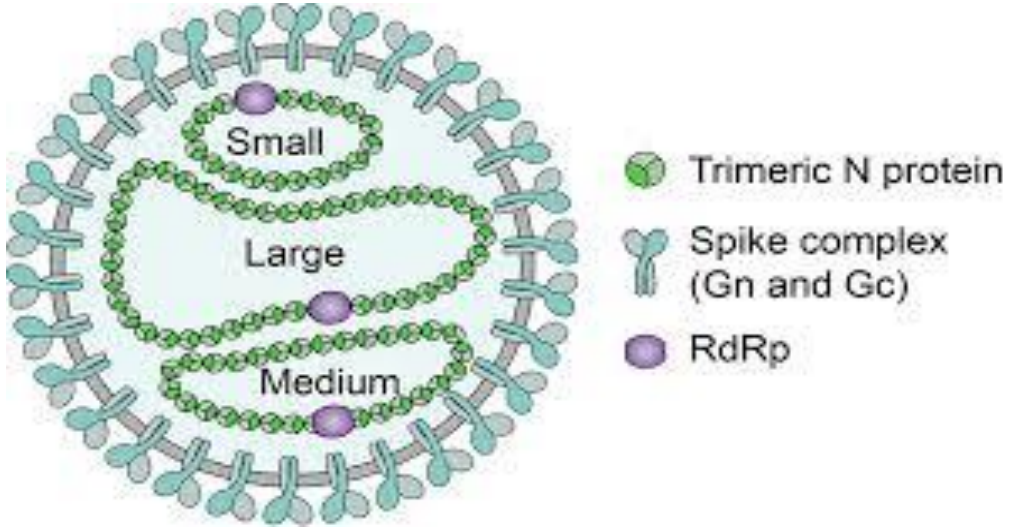
۰۲۱ – ۶۴۱۱۲۸۲۱

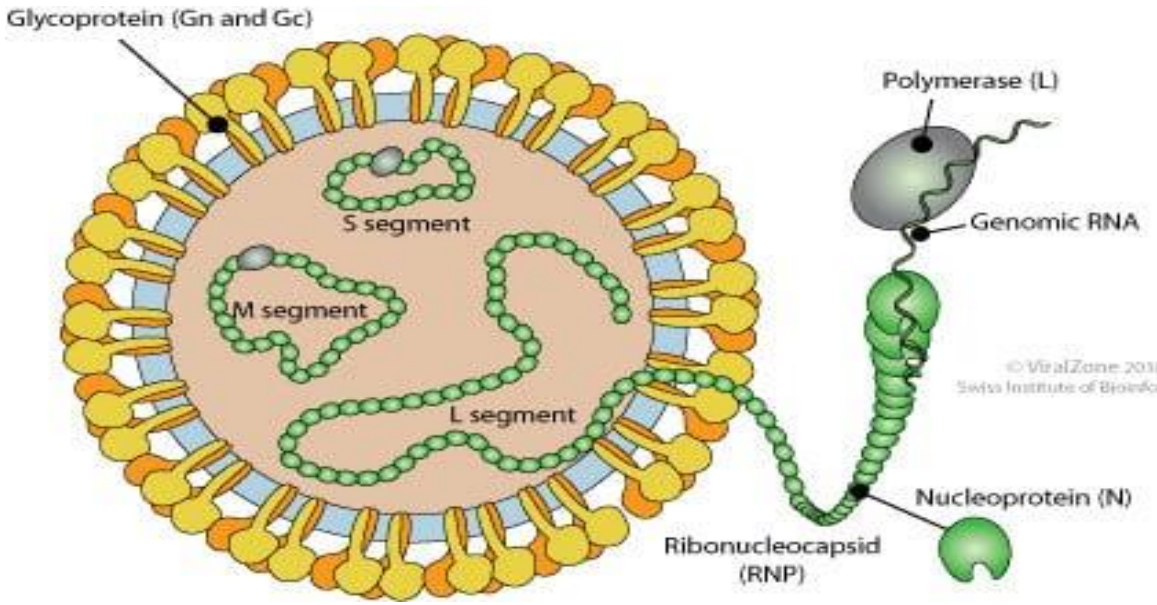
Biosafety Considerations

- BSL2/BSL3 depending on risk assessment
- Risk-based approach
- Aerosol precautions
- BSC required before inactivation
- Inactivation protocols important
  - ANDV is Risk Group 3 (aerosol transmissible)
  - Before working in BSL-2, samples MUST be inactivated.

Chemical	Physical
- Methanol	- UV irradiation (254 n m, >30 min)
- Paraformaldehyde (4%)	- Heat inactivation (56°C for 30 min – may reduce PCR sensitivity
- Acetone/Methanol (1:1)	
- Detergent-based lysis buffer (e.g., AVL buffer)	

Genome Structure and Proteins





Vaheri A. Nature Reviews Microbiology. 2013

Segment	Protein	Key function	Role in diagnosis
S (~1.8 kb)	N	Nucleocapsid – highest expression, immunodominant	Main antigen in ELISA and primer target
S (ORF2)	NSs	Inhibits IRF3 phosphorylation, IFN-β suppression	–
M (~3.6 kb)	Gn + Gc	Receptor binding (αVβ3, αVβ1), cell entry	Serology (less common)
L (~6.5 kb)	RdRp	Transcription/replication, endonuclease domain	Target for pan-hantavirus primers

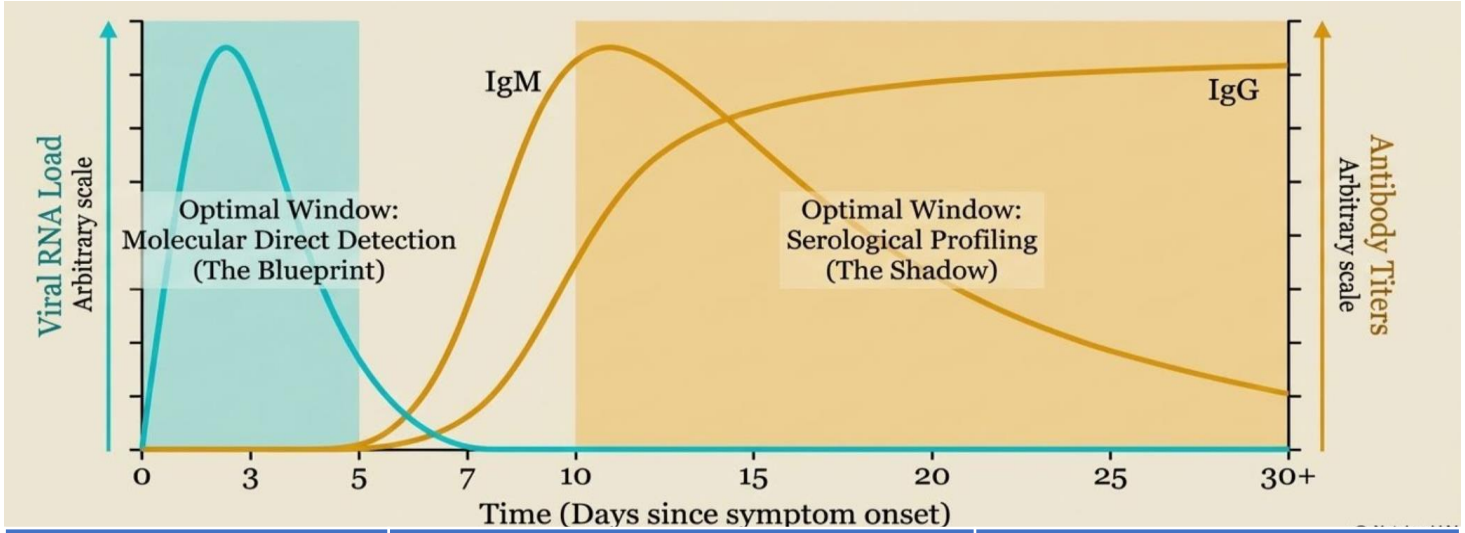
Diagnostic Methods: Molecular (RT-PCR) and Serology (ELISA/IFA)

Molecular (RT-PCR)

Target: Viral RNA (typically conserved S or L segments).

Window: Very early acute phase (prior to robust antibody response).

Advantage: Detects the virus before seroconversion; differentiates specific viral species and identifies early high viral loads linked to severe outcomes.



Stage	Detection Target	Method/Description
Early infection	Viral RNA	Specific primers or pan hanta approach high sensitivity needed
	IgM Antibody	Appears at symptom onset Acute infection marker
Peak protein expression	N protein	ELISA, immunoblot, IFA
Late convalescence	IgG antibodies	ELISA, FRNT / 4 fold rise

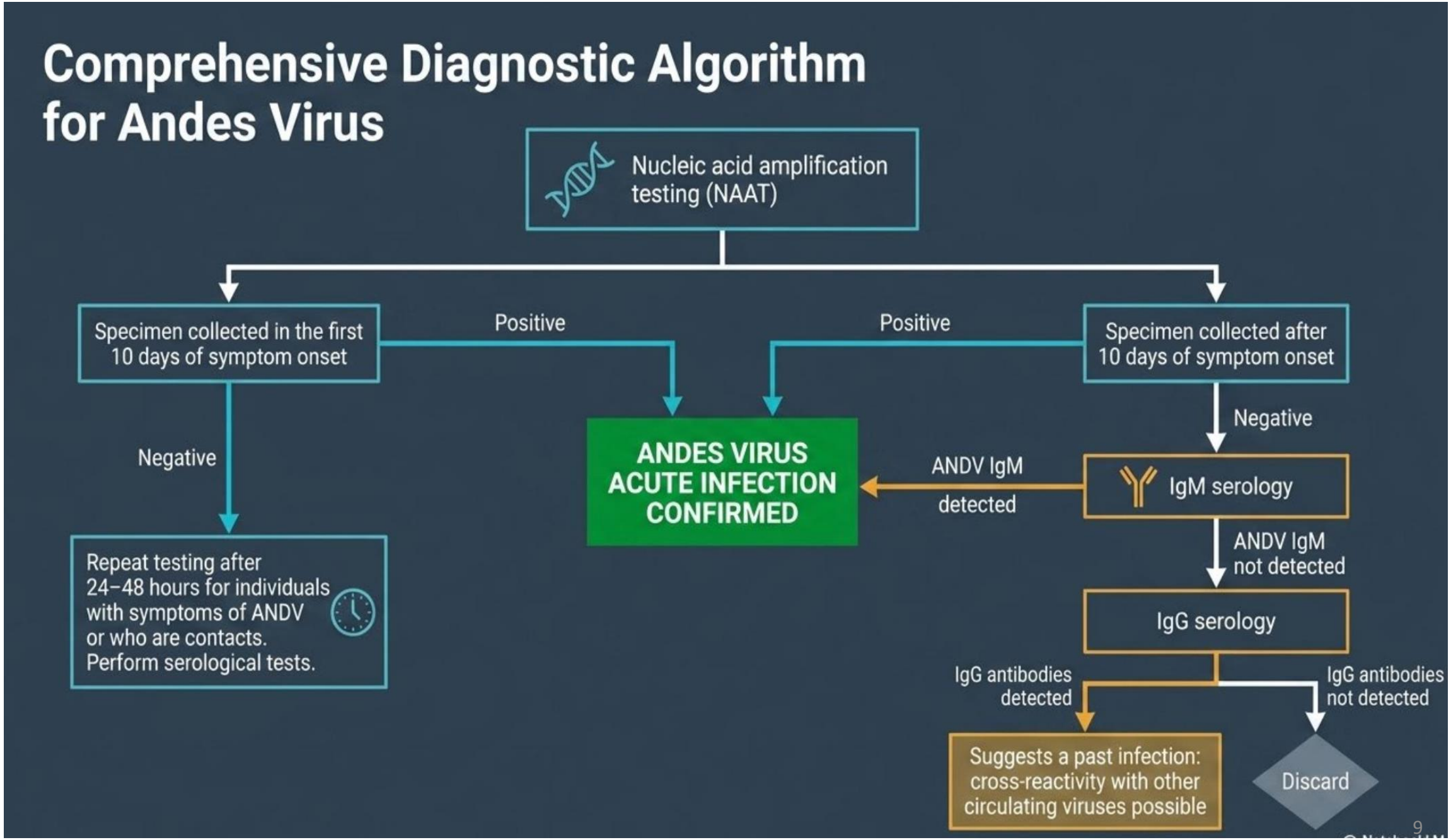
Serology (ELISA/IFA)

Target: N-protein (highly conserved S-segment).

Window: Acute to Convalescent (IgM early, IgG long-term).

Advantage: The global gold standard. Immediate clinical confirmation upon symptom presentation.

Comprehensive Diagnostic Algorithm for Andes Virus



A single negative RT-PCR result is NOT sufficient to rule out ANDV infection.

Possible causes of false negative:

- Specimen collected too early (<3 days) or too late (>7-10days)
- Low viral load (<100 copies/mL plasma)
- Genetic mismatch (reassortment, mutations in primer binding sites)
- PCR inhibition or poor sample quality

What to do?

- If clinical suspicion remains high → Repeat testing after 24–48 hours
- Consider using a different target (e.g., L segment vs. S segment)
- Always run internal controls (positive, negative, inhibition)

Commercial RT-PCR Kits

Kit	Manufacturer	Target	Viruses	Status
RealStar® Hantavirus-HFRS	Altona	RNA	DOBV, SEOV, HTNV, PUUV	RUO
RealStar® Hantavirus-HPS	Altona	RNA	ANDV, SNV	RUO
Sin Nombre Virus Real Time PCR	BioPerfectus	RNA	SNV	CE-IVD
Andes Virus Nucleic Acid Diagnostic Kit	Sansure	RNA	Andes	RUO

Serological Kits: ELISA

Kit	Manufacturer	Antigen	Viruses	Status
Hantavirus (Hantaan) IgG/IgM ELISA	IBL	N protein (broad)	HTNV + cross-reactive	CE-IVD
Puumala IgG/IgM ELISA	Reagena	N protein (PUUV-specific)	PUUV	CE
Hantavirus IgG/IgM ELISA	Euroimmun	N protein	Broad (Old & New World)	CE-IVD

Essential for reliable hantavirus diagnostics.

Common problems in European labs (2023 EQA):

- Detection of 100 copies/mL by PCR: only 68%
- IgM serology agreement: 84%
- Main issues: no internal control, poor melt curves, no replicates

- Requirements:
  - Include positive, negative, and inhibition controls in every PCR run
  - Use RNase control for specimen integrity
  - Participate in EQA schemes (e.g., QCMD – Quality Control for Molecular Diagnostics)
  - Follow ISO 15189:2022 (medical laboratories)

Plaque Reduction Neutralization Test (PRNT)

- Principle: Measures antibodies that neutralize viral infectivity
- Sensitivity & Specificity: 100% (when performed correctly)
- Can distinguish between closely related hantaviruses (ANDV vs. SNV vs. HTNV)

Limitations:

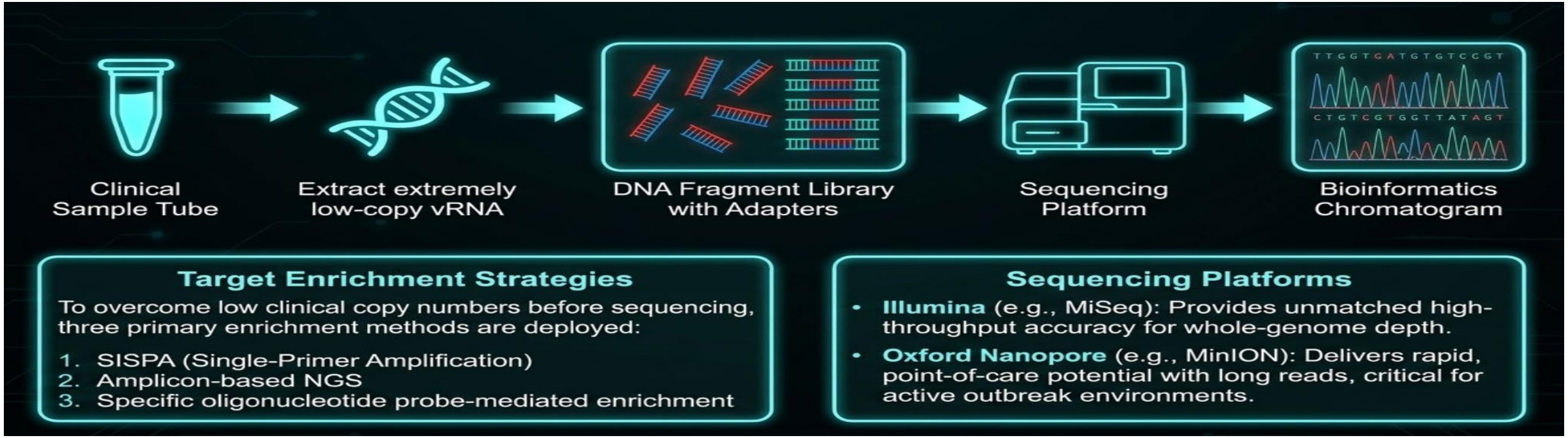
- Requires BSL-3 facility
- Time-consuming (5–7 days)
- Not for routine diagnosis – only reference laboratories

Use case:

- Confirm equivocal ELISA/IFA results
- Discriminate cross-reactive antibodies in co-endemic regions
- Vaccine trials and research

Next-Generation Sequencing (NGS) Methods

- Challenge: low viral load in human samples (often <100 copies/mL)
- Three main enrichment approaches (No et al. 2019; Park et al. 2021)



DNA Microarray for Detection and Typing

- Third-generation **PathogenID v3.0** (Filippone et al. 2019)
- Tiled sequence covering entire S segment (assays 50 genotypes simultaneously)
- Advantage: can discriminate intra-species variants (e.g., ANDV Chile-9717869 vs. Arg-EP)
- Limitation: requires ≥1000 copies, 24 h turnaround
- Application: screening large rodent collections from hot spots

This Will Not Be the Last Emerging Disease

- Next outbreak

- Delayed diagnosis leads to:
  - Missed window for early supportive care
  - Unrecognised transmission chains

- **Prevention:** Equipped laboratories, WHO guidance implementation, global collaboration, trained staff.

Thank you

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