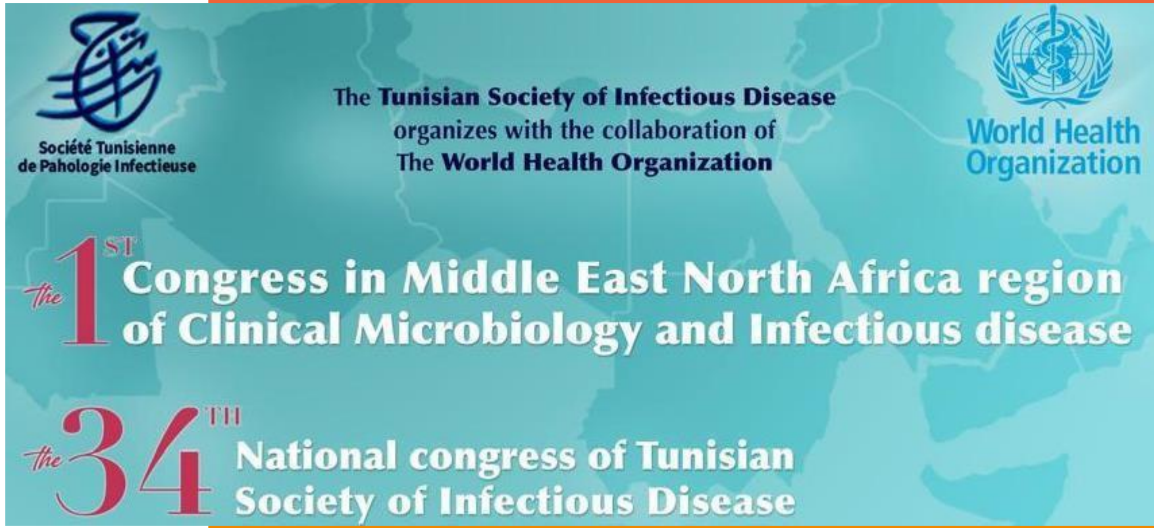
Illumina sequencing technology application to Infectious Disease Surveillance


Société Tunisienne
de Pahologie Infectieuse

The **Tunisian Society of Infectious Disease**
organizes with the collaboration of
The **World Health Organization**

World Health
Organization

the 1ST Congress in Middle East North Africa region of Clinical Microbiology and Infectious disease

the 34TH National congress of Tunisian Society of Infectious Disease

Lydia Hadjeras, PhD

Genomic specialist at Illumina

MEDIBIO

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Agenda

1. **Genomic utility in enhanced infectious diseases surveillance & AMR profiling**

2. **Illumina NGS solutions for infectious diseases surveillance & Microbiology**

Genomic utility in enhanced infectious disease surveillance & AMR profiling

1

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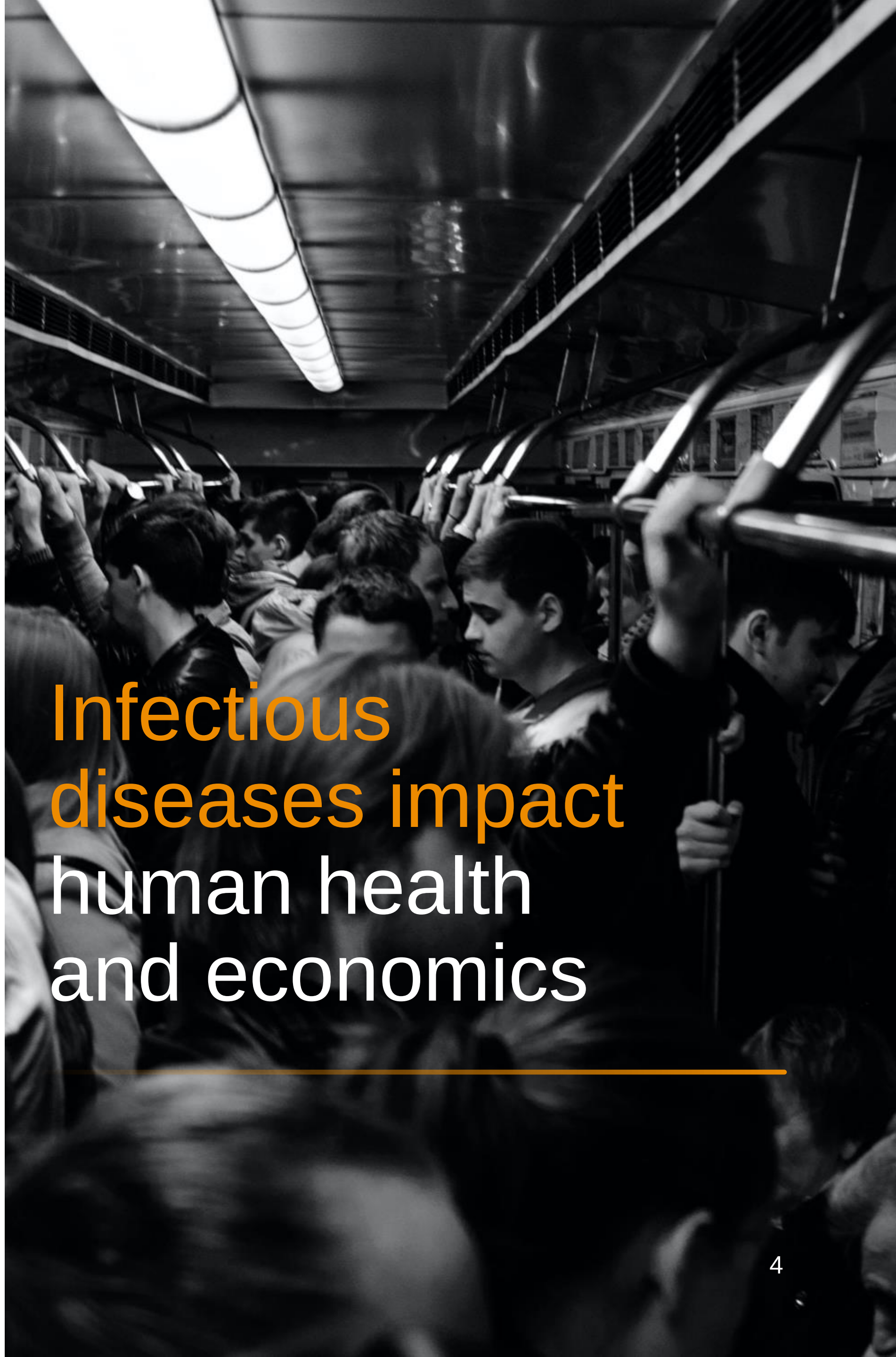
Infectious Diseases by the Numbers

770M	Cumulative cases of **COVID-19**[3]
400K	Respiratory deaths each year caused by **influenza virus**[4]
10.6M	People that fell ill from **tuberculosis** in 2021[5]
39M	People living with **HIV** in 2022[6]
3.8%	Global GDP lost due to **antimicrobial resistance** by 2050[7]
247M	**Malaria** cases in 84 malaria-endemic countries in 2021[5]
1.5M	New **hepatitis B** infections globally in 2019[5]
$28-45B	Direct annual cost of treating **healthcare associated infections** in the US[8]



Infectious diseases impact human health and economics

3. https://covid19.who.int/ (Accessed Sept. 7, 2023); 4. https://ourworldindata.org/influenza; 5. World health statistics 2023: monitoring health for the SDGs, Sustainable Development Goals. Geneva: World Health Organization; 2023.; 6. https://www.unaids.org/en/resources/fact-sheet; 7. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: World Health Organization; 2022.; 8. Gidey K, et al. Clinical and economic burden of healthcare-associated infections: A prospective cohort study. PLoS One. 2023 Feb 23;18(2):e0282141.

The increasing threat of infectious diseases

1. **Globalization and increased international travel** facilitate the rapid spread of infectious agents across borders.
2. **Expanding urbanization and increasing population density** create ideal conditions for the transmission of infectious agents.
3. **Emergence of drug-resistant strains of bacteria and viruses** poses a significant challenge in the treatment and control of infections.
4. **Closer contact with animal reservoirs of infectious diseases** due to factors such as climate change, deforestation, and high-intensity farming, can increase the risk of animal-to-human transmission.[9]

Beyond COVID:

Lessons learned from a global pandemic

The pandemic showcased effectiveness in public health surveillance:

1. Rapidly characterize a novel infectious agent
2. Monitor the spread of a global disease
3. Facilitate research that ultimately led to an effective vaccine in record time

> “Genomic surveillance is transforming public health action by providing a deeper understanding of pathogens, their evolution and circulation”

World Heath Organization[19]

Antimicrobial Resistance (AMR)

An Important Target for Surveillance

- WHO declared AMR "One of the top 10 global public health threats facing humanity"[1]
- AMR complicates the treatment of microbial infections and can lead to multi-drug resistant organisms
- Currently majority of AMR surveillance relies on commonly available microbiological methods and phenotypic drug susceptibility testing, with some molecular testing
- NGS can bring a significant improvement in AMR characterization
  - Quicker more accurate outbreak identification
  - Help guide development of new treatments
  - Identification of comprehensive AMR in multi-drug resistant organisms such at tuberculosis[2]

[1] Antimicrobial Resistance: Addressing a Global Threat to Humanity. 2023. PLoS Med. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10317217

[2] Targeted NGS directly from sputum for comprehensive genetic information on drug resistant Mycobacterium tuberculosis. 2021. Tuberculosis. https://www.sciencedirect.com/science/article/abs/pii/S1472979221000019?via%3Dihub

The One Health Approach

NGS-based infectious disease surveillance has already supported several public health initiatives, including **One Health** – an interconnective approach that promotes human health by ensuring the health of animals and plants within the shared environment.[21]

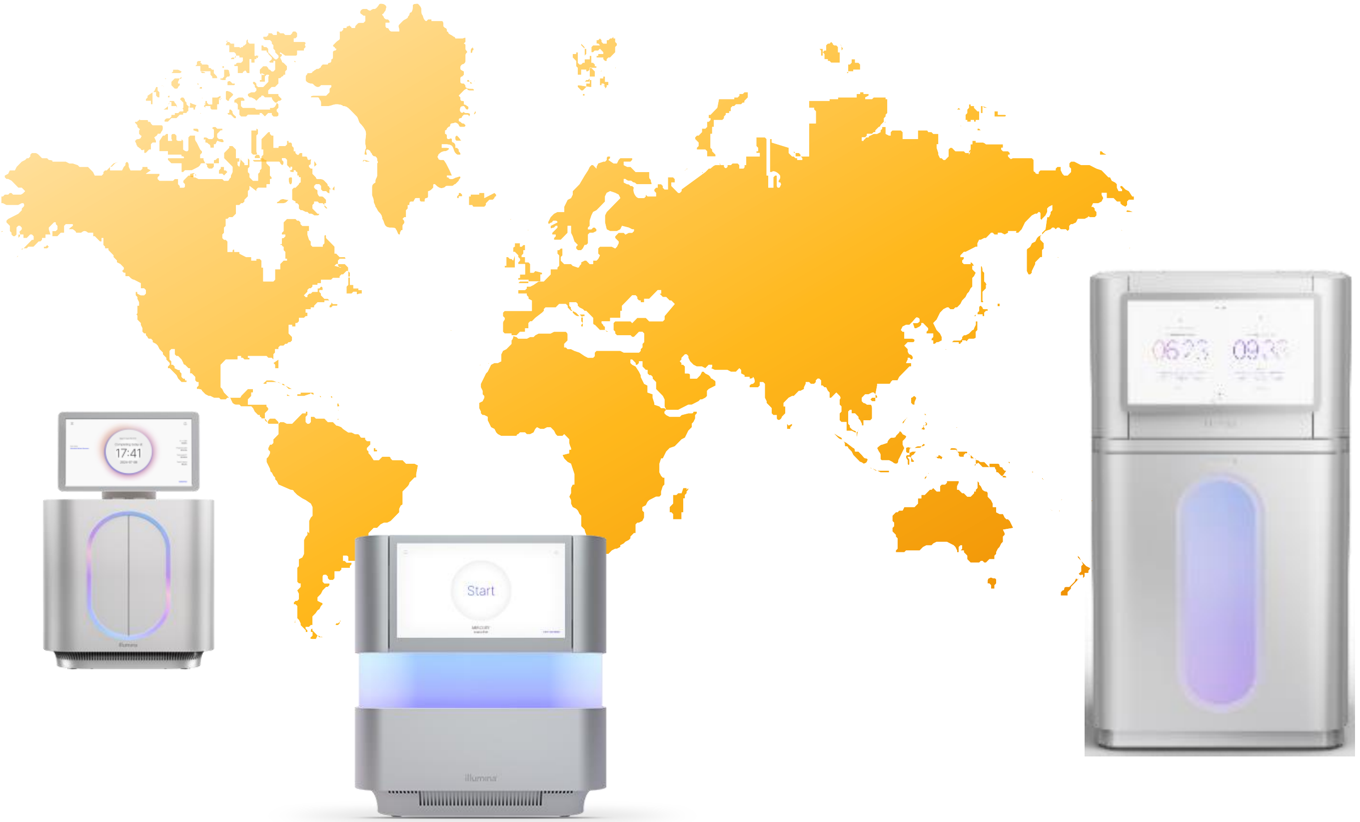
NGS allows to monitor threats to both humans and animals, including:

- Mobile genetic elements encoding antimicrobial resistance (AMR).
- Genetic mutations associated with emerging strains of infectious agents.
- Genomic surveillance of environmental samples, such as wastewater.

Illumina NGS solutions for infectious disease surveillance & Microbiology

2

Illumina technologies – a unique advantage against infectious diseases

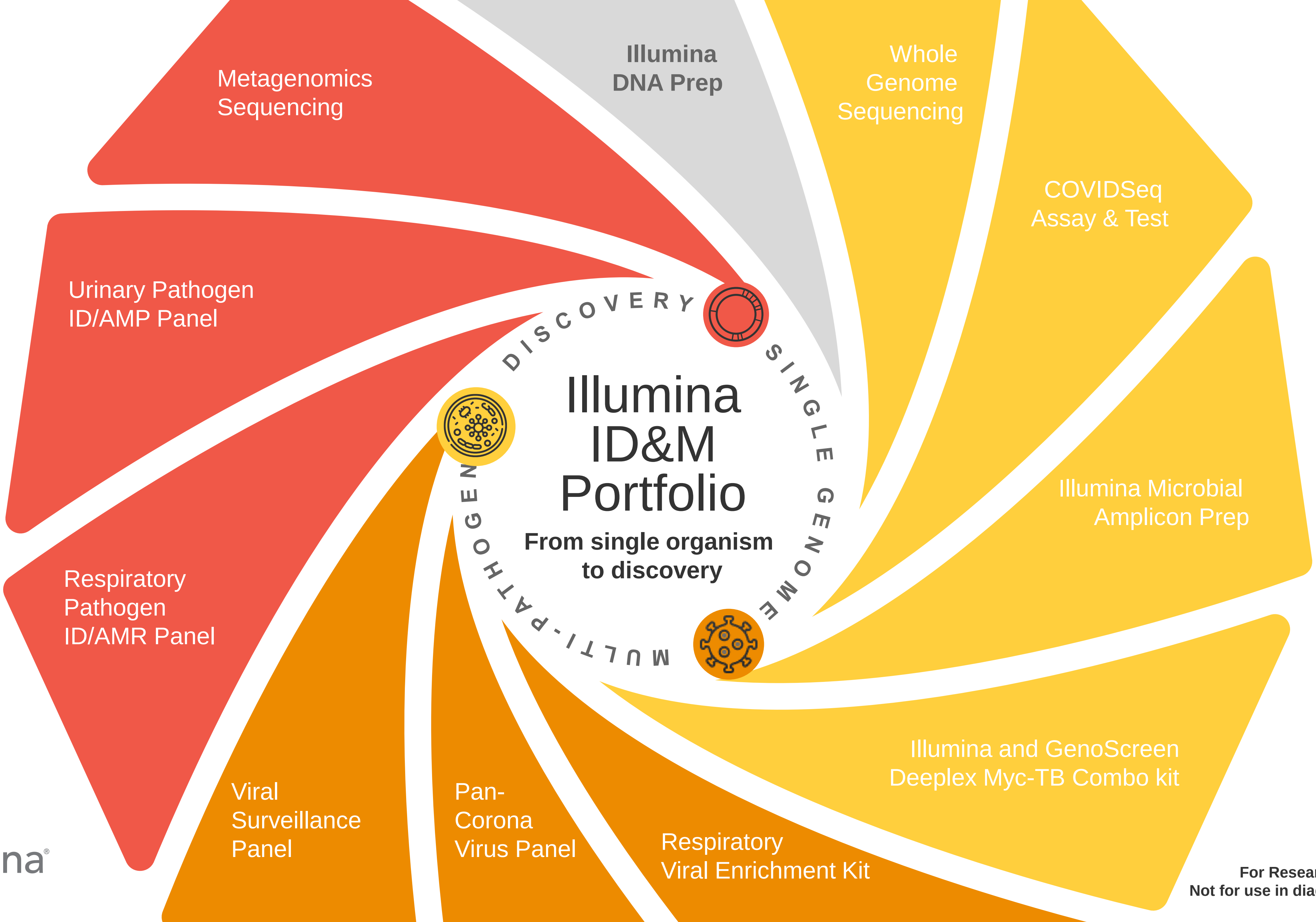


Flexible and scalable solutions to enable accessibility worldwide

- ✓ Novel pathogen ID and drug resistance characterization
- ✓ Insights for clinical assay development
- ✓ Pathogen monitoring and genomic surveillance
- ✓ Host genetics and immune response
- ✓ Drug and vaccine development

Illumina ID&M Portfolio

From single organism to discovery



DISCOVERY

Metagenomics Sequencing

Urinary Pathogen ID/AMP Panel

Respiratory Pathogen ID/AMR Panel

MULTI-PATHOGEN

Viral Surveillance Panel

Pan-Corona Virus Panel

Respiratory Viral Enrichment Kit

SINGLE GENOME

Illumina DNA Prep

Whole Genome Sequencing

COVIDSeq Assay & Test

Illumina Microbial Amplicon Prep

Illumina and GenoScreen Deeplex Myc-TB Combo kit

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NGS Methods Enable Genomic Investigation

Whole-Genome Sequencing of Isolates

- Analyze full microbial and plasmid genomes from cultured isolate
- **Discover new variants**
- Study anti-microbial resistance, virulence, or other **functional gene analysis**

Metagenomic or Metatranscriptomic

- **Culture independent method**
- Analyze all microbes, genes, or gene expression present in complex samples
- **Analyze gene expression data**
- Can require high read-depth

Hybrid Capture Enrichment

- **Culture independent method**
- Can provide whole genome results
- Enables enrichment and **analysis of large numbers of organisms** with greater sensitivity than shotgun metagenomics

Amplicon

- **Culture-independent method**
- Can provide whole genome results
- Focused streamlined analysis
- **Fast and inexpensive**
- Risk of mutations affecting primer binding

Illumina Viral Surveillance Panel (VSP)

Enables Whole genome sequencing (WGS) with targeted enrichment of 200+ viruses of high risk to public health[1]

Arthropod transmitted/ febrile tropical

Bourbon
Chikungunya
Colorado Tick Fever
Dengue 1 - 4
Heartland
Mayaro
Onyong-nyong
Oropuche
Punta Toro
Ross River
Sandfly fever
Semliki Forest
Sindbis
Tacheng tick 2
Usutu
West Nile
Yellow Fever
Zika

Bloodborne

Adeno-associated 2
Hepatitis A
Hepatitis B
Hepatitis C
Hepatitis D
Hepatitis E
HIV 1 - 2
Torque Teno

Encephalitis

Cache Valley
CA encephalitis
Cedar valley
Eastern equine enceph
Ghana
Hendra
Henipah
Jamestown Canyon
Japanese enceph
La Crosse
Langya
Lyssavirus (6)
Mojiang
Murray Valley enceph.
Nipah
Powassan
Rabies
Snowshoe hare
St. Louise enceph.
Tahyna
Tickborne enceph.
Toscana
Venezuelan equine enceph.
Western equine enceph

Respiratory/ Cardiopulmonary

Adenovirus A -G
Bocavirus
Coronavirus (4)
Hantaviruses (15)
Influenza A (22)
Influenza B (3)
Influenza C
MERS
Metapneumovirus
Parainfluenza 1 – 4
Parechovirus
Parvovirus B19
RSV A
RSV B
Rhinovirus A – C
SARS
SARS-CoV-2

Hemorrhagic fever

Aigai
Bombali
Bundibugyo
Chapare
CCHF
Ebola
Guanarito
Hantaviruses (7)
Junin
Kyasanur Forsest
Lassa
Lloviu
Lujo
Machupo
Marburg
Omsk hemorrhagic fever
Ravn
Reston
Rift Valley
Sabia
SFTSV
Sosuga
Sudan
Tai Forest

Enteric

Aichi
Coxsackieviruses
Echovirus
Enterovirus A – D
Mamastrovirus (4)
Norovirus
Poliovirus
Rotavirus (4)
Salivirus
Sapovirus

Oncolytic

Epstein-Barr
Cytomegalovirus
HPV (28)
Polyomavirus (13)

Rash/Lesion

Herpes Simplex 1-2
Measles
Menangle
Mpox
Mumps
Rubella
Varicella-zoster
Variola

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[1] - https://www.idsociety.org/public-health/public-health/ (Access 05/07/2024)

Respiratory Pathogen ID/AMR (RPIP) Enrichment Kit for Comprehensive Detection of Respiratory Pathogens and Antimicrobial Resistance Genes

>280 Pathogens

180+
Bacteria

40+
Viruses*

50+
Fungi

>16 Antibiotic Classes

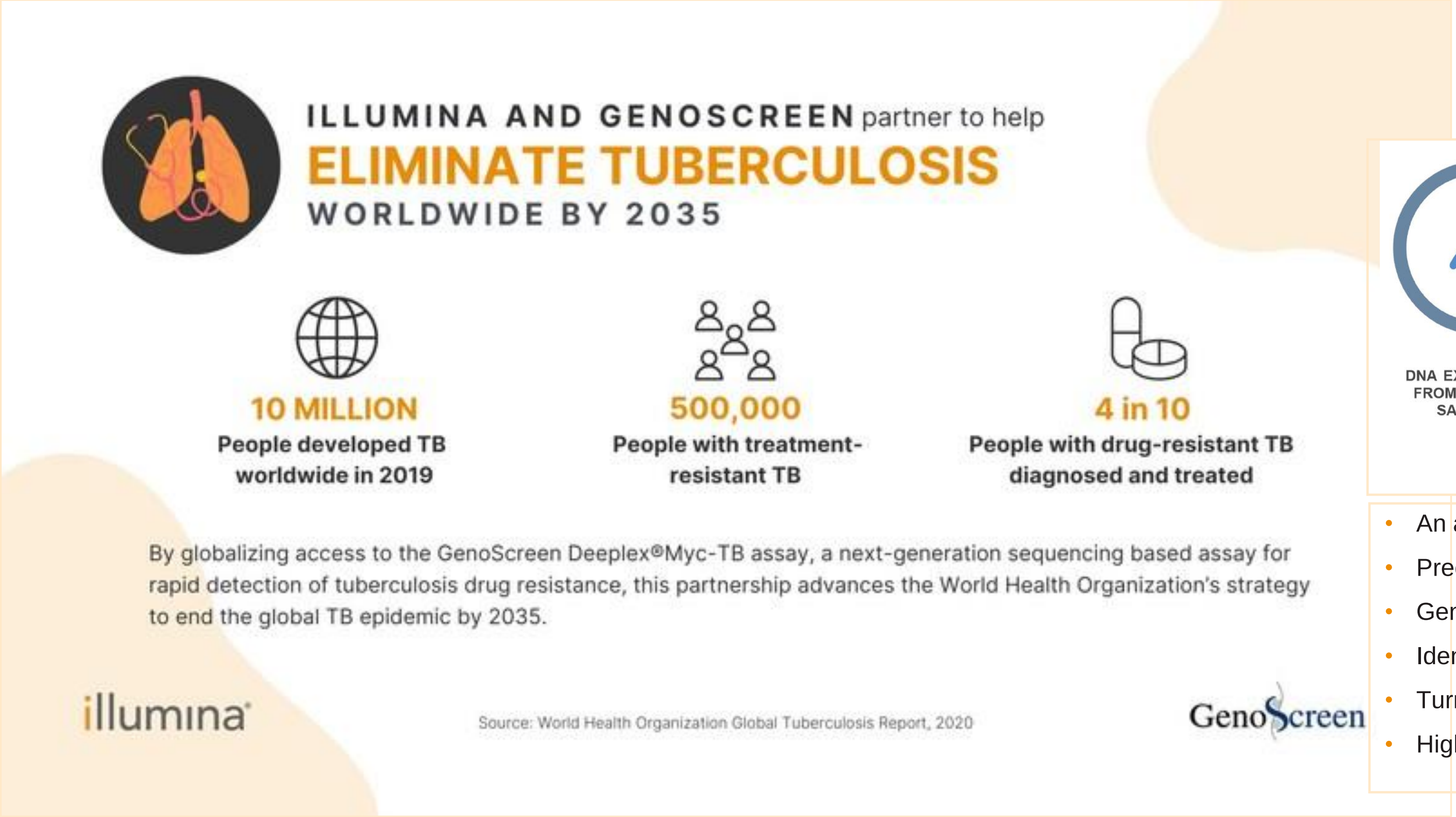
1200+
Antimicrobial resistant alleles

Catalog Number: 20046969

*full genome coverage available for SARS-CoV-2 and flu viruses

Deeplex® Myc-TB

Rapid, Culture-free, Targeted Sequencing for *M. tuberculosis* Drug Resistance Profiling



ILLUMINA AND GENOSCREEN partner to help

ELIMINATE TUBERCULOSIS

WORLDWIDE BY 2035

10 MILLION
People developed TB worldwide in 2019

500,000
People with treatment-resistant TB

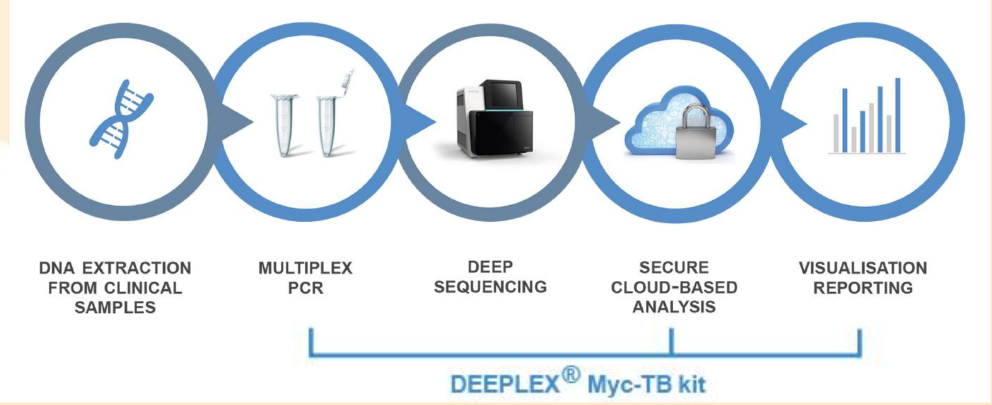
4 in 10
People with drug-resistant TB diagnosed and treated

By globalizing access to the GenoScreen Deeplex®Myc-TB assay, a next-generation sequencing based assay for rapid detection of tuberculosis drug resistance, this partnership advances the World Health Organization's strategy to end the global TB epidemic by 2035.

illumina

Source: World Health Organization Global Tuberculosis Report, 2020

GenoScreen



DNA EXTRACTION FROM CLINICAL SAMPLES

MULTIPLEX PCR

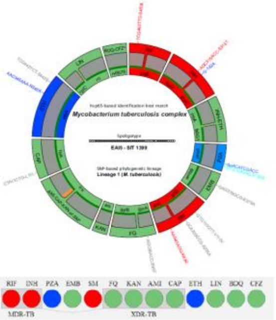
DEEP SEQUENCING

SECURE CLOUD-BASED ANALYSIS

VISUALISATION REPORTING

DEEPLEX® Myc-TB kit

- An all-in-one assay based on targeted sequencing
- Prediction of Resistance to 15 anti-TB drugs
- Genotyping and spoligotyping of MTBC strains
- Identification of more than 140 mycobacterial species
- Turnaround time of less than 48 hours
- Highly sensitive



For Research Use Only. Not for use in diagnostic procedures.

WHO Rapid Communication: Use of Targeted Next Generation Sequencing (NGS) to detect DR-TB

WHO has provided its first ever guidance on the use of NGS for clinical use

Targeted next-generation sequencing (tNGS)

- *Accurate*
- *Cost-effective depending on context*
- *Acceptable and implementable under routine conditions*

World Health Organization

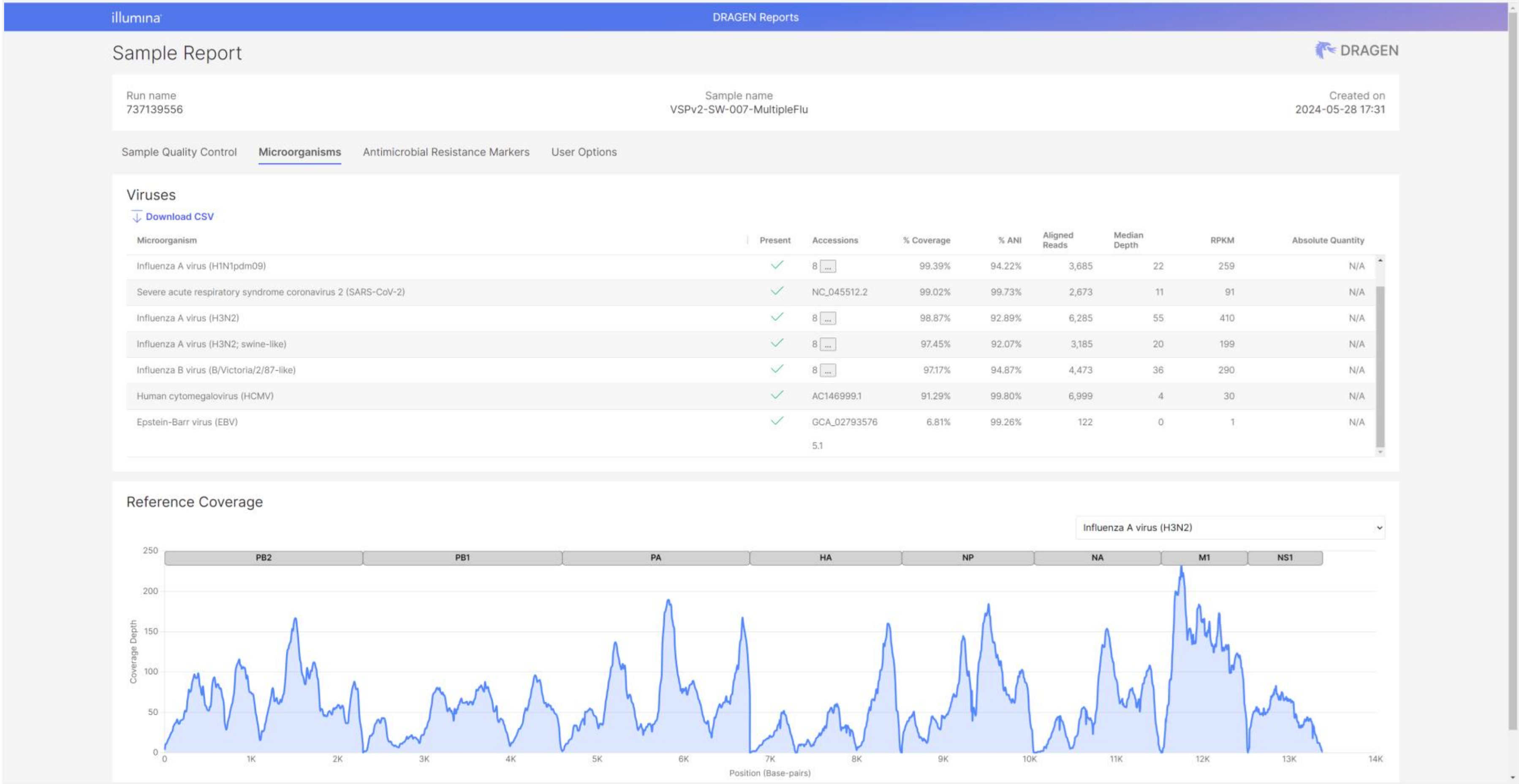
Use of targeted next-generation sequencing to detect drug-resistant tuberculosis

Rapid communication, July 2023

Goal: inform national TB programs and other stakeholders of key changes in the available options for detection of TB drug resistance and to allow for rapid transition and planning at the country level.

WHO Rapid Communication: Use of targeted next-generation sequencing to detect drug-resistant tuberculosis. July 2023 https://www.who.int/publications/i/item/9789240076372

On board DRAGEN .html report



Sample Report

Run name
737139556

Sample name
VSPv2-SW-007-MultipleFlu

Created on
2024-05-28 17:31

Viruses

Microorganism	Present	Accessions	% Coverage	% ANI	Aligned Reads	Median Depth	RPKM	Absolute Quantity
Influenza A virus (H1N1pdm09)	✓	8 ...	99.39%	94.22%	3,685	22	259	N/A
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	✓	NC_045512.2	99.02%	99.73%	2,673	11	91	N/A
Influenza A virus (H3N2)	✓	8 ...	98.87%	92.89%	6,285	55	410	N/A
Influenza A virus (H3N2; swine-like)	✓	8 ...	97.45%	92.07%	3,185	20	199	N/A
Influenza B virus (B/Victoria/2/87-like)	✓	8 ...	97.17%	94.87%	4,473	36	290	N/A
Human cytomegalovirus (HCMV)	✓	AC146999.1	91.29%	99.80%	6,999	4	30	N/A
Epstein-Barr virus (EBV)	✓	GCA_02793576 5.1	6.81%	99.26%	122	0	1	N/A

Reference Coverage

Influenza A virus (H3N2)

Example report from **DRAGEN Microbial Enrichment Plus:**

- .html dynamic report with microbial identification, coverage metrics and quantity (if using internal controls)
- Coverage plots for each genome with segments/genes (for segmented viruses)
- Each report includes QC metrics
- Delivers sufficient microbial data for quick, actionable decisions by public health labs and organizations

For Research Use Only. Not for use in diagnostic procedures.

Examples of how NGS methods have been applied across various infectious disease surveillance projects

Early detection

Outbreak response

Clinical research

Characterizing new and emerging pathogens

Identification of the SARS-CoV-2 virus

Conducted by:
Shanghai Public Health Clinical Center,
The Central Hospital of Wuhan

Funded by:
Center for Disease Control and Prevention (China),
National Institute for Communicable Disease Control and Prevention (China)

NGS methodology used:
Metatranscriptomic RNA sequencing

Sequencing platform:
MiniSeq™ System

After molecular tests ruled out other etiological agents, NGS analysis of a bronchoalveolar lavage fluid sample isolated from a patient in Wuhan, China suffering from severe respiratory disease identified a novel coronavirus in December 2019.

Originally named “WH-Human 1” coronavirus after initial phylogenetic analysis revealed the virus was most closely related to a group of SARS-like coronaviruses previously identified in Chinese bats.

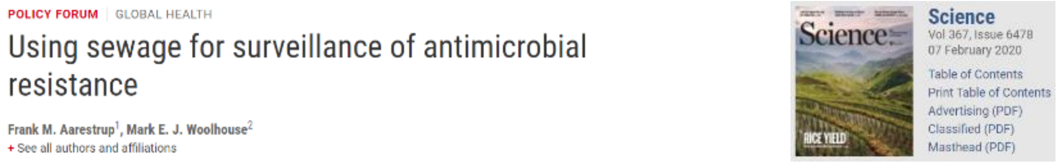
Rapid identification of this causative agent, later named SARS-CoV-2, marked the start of the COVID-19 pandemic.[30]

Sequencing Enables Genomic AMR Surveillance in Wastewater and Wildlife

Sequencing provides rich information on AMR environmental transmission through wastewater AMR surveillance

- **Metagenomics** is an improved surveillance approach that could potentially limit the global burden of AMR
- **Metagenomic analysis of sewage** is an ethically acceptable and economically feasible approach for continuous global surveillance and prediction of AMR
- Globally applying **NGS** to human sewage will be helpful for large scale AMR surveillance
- Sequencing and bioinformatics methods are easily standardized in a central facility

Using sewage for surveillance of antimicrobial resistance
BY FRANK M. AARESTRUP, MARK E. J. WOOLHOUSE | SCIENCE07 FEB 2020 : 630-632



POLICY FORUM | GLOBAL HEALTH

Using sewage for surveillance of antimicrobial resistance

Frank M. Aarestrup[1], Mark E. J. Woolhouse[2]
+ See all authors and affiliations

Science
Vol 367, Issue 6478
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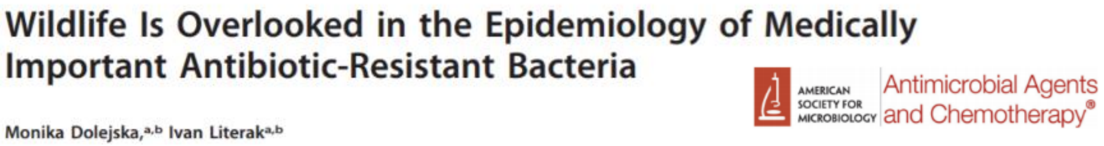
Metagenomics will give a complete picture of the AMR pathogen transmission in wildlife

- Successful AMR pathogens transmit between wildlife, humans, and companion animals
- The role of globally moving wildlife in the dissemination of AMR bacteria over long distances remains unclear
- **Metagenomics** can identify the abundance of AMR bacterial genes and mobile genetic elements in the gut of wild animals
- Continuous large-scale surveillance with metagenomics approach can provide a more complete picture of global AMR transmission in future

Wildlife Is Overlooked in the Epidemiology of Medically Important Antibiotic-Resistant Bacteria
Monika Dolejska, Ivan Literak | Antimicrobial Agents and Chemotherapy Jul 2019, 63 (8) e01167-19; DOI: 10.1128/AAC.01167-19



Wildlife Is Overlooked in the Epidemiology of Medically Important Antibiotic-Resistant Bacteria

Monika Dolejska,[a,b] Ivan Literak[a,b]

Transmission Tracking

Genomic investigation of two concurrent outbreaks of *Salmonella Enteritidis* in South Africa

Conducted by:
Centre for Enteric Diseases, National Institute for Communicable Diseases (Johannesburg, South Africa)

Supported by:
Centres for Disease Control and Prevention (Africa), Centre for Enteric Diseases (South Africa), the National Institute for Communicable Diseases (South Africa)

NGS methodology used: WGS of isolates

Sequencing platform: MiSeq™ System

Outbreak A
children at a day-care center

Outbreak B
adults who all ate at the same restaurant

WGS analysis of bacterial isolates from patients affected by the outbreaks revealed that contaminated eggs from the restaurant were the likely source of the *Salmonella Enteritidis* that caused Outbreak B.

Comparative analysis of the genomes of the *Salmonella Enteritidis* strains that caused Outbreak A and Outbreak B revealed high genetic similarity, suggesting a high probability of epidemiological relatedness.

This finding indicated the **likelihood of a larger contamination problem** with the same clone of *Salmonella Enteritidis* within the wider chicken- and egg-producing industry of South Africa.[38]

Hendra virus surveillance in Australian horses for the detection of novel variants

Genomic surveillance for the highly pathogenic zoonotic Hendra virus (HeV), which naturally infects bats, has identified several spillover events during which HeV was transmitted from bats to horses.

Although horse-to-human transmission of HeV is very rare (only seven human cases have been reported), the fatality rate in humans is extremely high at 57%.

Horse HeV vaccine developed (Equivac HeV) to prevent horse-to-human transmission[2] as well as monoclonal antibodies for emergency post-exposure prophylaxis

September 2020, an Australian horse exhibiting HeV symptoms was humanely euthanized and tested for HeV. However, routine quantitative RT-PCR and ELISA testing did not identify HeV genomic material or proteins, respectively.

Through metagenomic sequencing, researchers identified a novel variant of HeV that evaded detection by routine sentinel surveillance.

NGS supports the need for updated routine molecular testing used in surveillance and exemplifies the power of genomic surveillance.

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1. 1- Marsh GA et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. Emerging Infect Dis. 2010;16(11):1767-1769. doi:10.3201/eid1611.100501
2. 2- Annand EJ et al. Novel hendra virus variant detected by sentinel surveillance of horses in australia. Emerging Infect Dis. 2022;28(3):693-704. doi:10.3201/eid2803.211245

Merci

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