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LABORATORY OF CLINICAL MICROBIOLOGY, VIROLOGY AND BIOEMERGENCIES

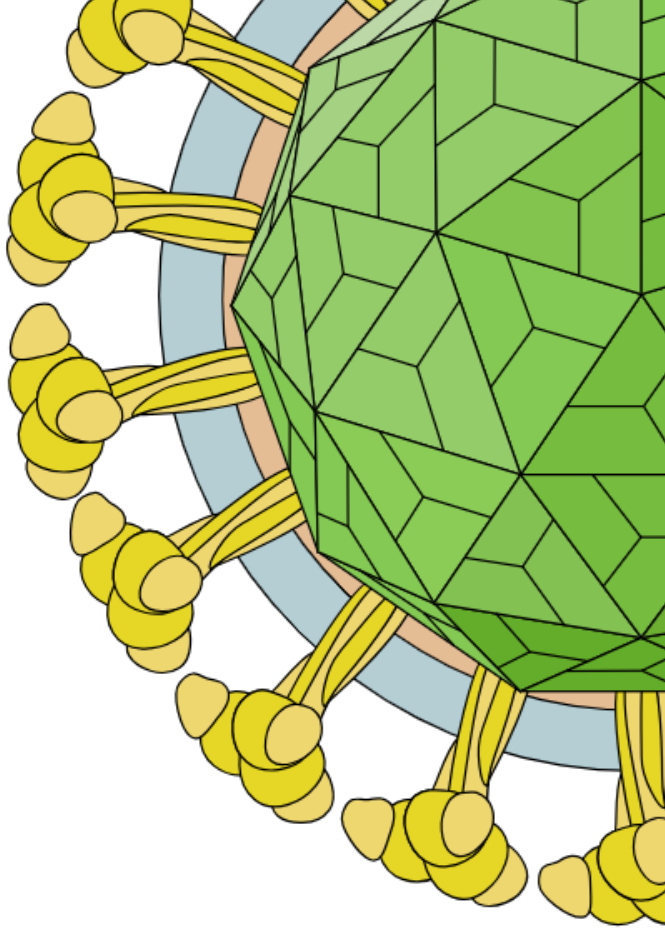
SCUOLA DI SPECIALIZZAZIONE IN MALATTIE INFETTIVE E TROPICALI

PANDORA ID - 24

TIME TO WEBINAR IN INFECTIOUS DISEASES

PROGETTO DI FORMAZIONE

Anno Accademico 23/24



ECCMID: diagnostica in malattie infettive

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viralzone.it/Alphavirus

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Evoluzione della Microbiologia Clinica

La microbiologia clinica ha subito profondi cambiamenti negli ultimi decenni.

Dagli anni '60, sono state introdotte e utilizzate numerose innovazioni tecnologiche nei laboratori di microbiologia clinica; nonostante ciò, per molte diagnosi, le nuove tecnologia non hanno permesso ai microbiologi di abbandonare il dogma della coltura pura.

Isenberg, J Clin Microbiol. 2003

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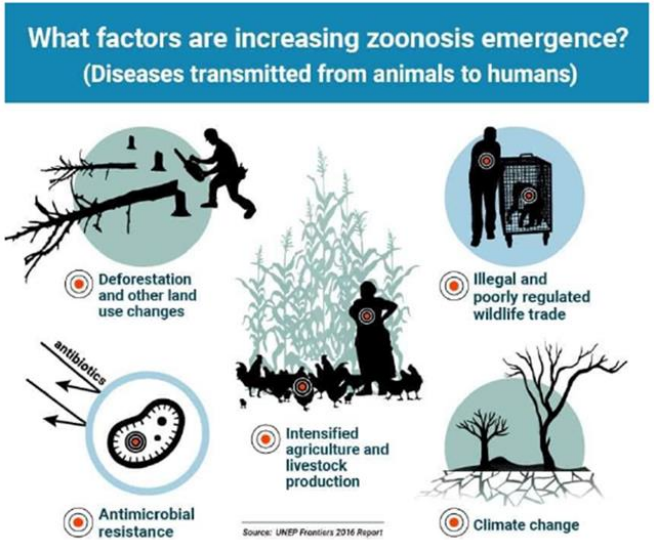
Evoluzione della Microbiologia Clinica

Evoluzione come risposta e nuove necessità:

1. Risposta alle esigenze cliniche



2. Comparsa di microrganismi e virus finora sconosciuti, spesso come risultato di attività umane con impatti ambientali

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Batteriologia

Percorso Manuale

Percorso Automatizzato

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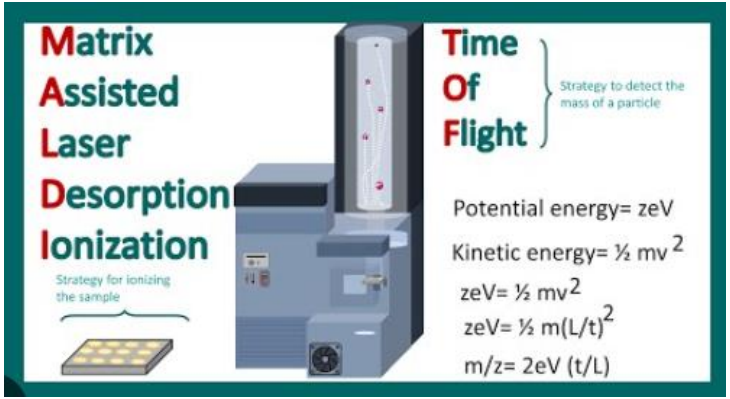
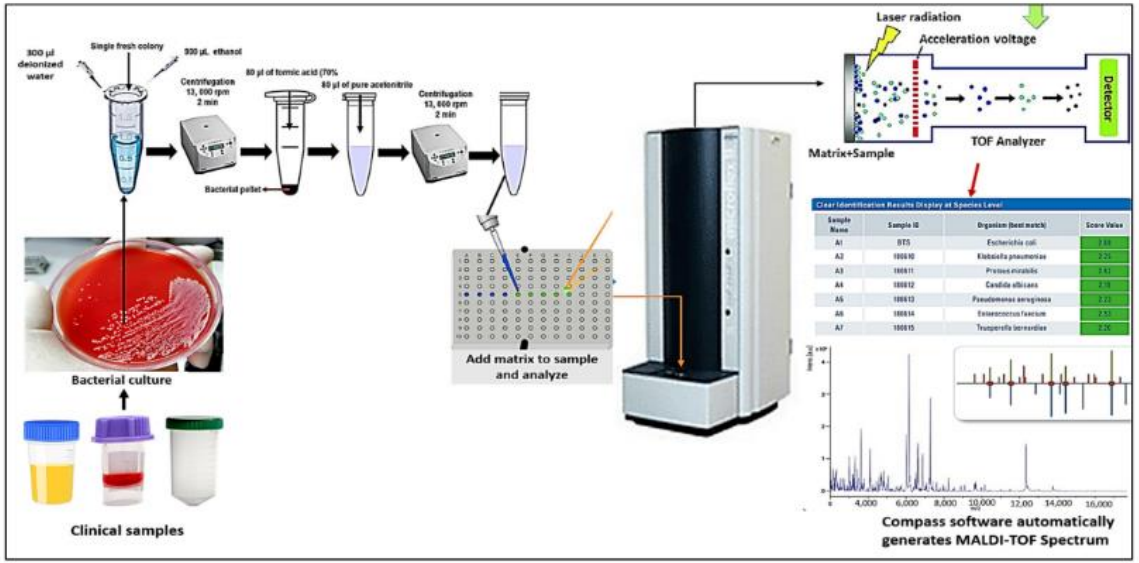
Batteriologia





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Batteriologia

RAST mediante disco-diffusione da emocoltura positiva (RAST EUCAST)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Rapid AST in bloodcultures

- Organization
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
  - Methods
  - Breakpoints for short incubation
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- Consultations - New!
- MIC and zone distributions and ECOFFs
- AST of bacteria
- AST of mycobacteria

Rapid AST directly from blood culture bottles

EUCAST has published recommendations for short incubation (4, 6 and 8 hours) AST directly from positive blood culture bottles:

- direct inoculation of disk diffusion plates (MH, MH-F) using 100 - 150 µL directly from a positive blood culture bottle (BD, bioMerieux and Thermo Fisher).
- no centrifugation or dilution of the inoculum - streak plates as for standard EUCAST disk diffusion.
- shortened incubation - 4, 6 and 8 hours with breakpoints adapted to each incubation time.
- zone diameters are read from the front of the plate after removal of the lid.
- breakpoints for each species and each reading time.
- identity of species must be known prior to interpretation of AST results.

Rapid AST directly from positive blood culture bottles

- ✓ EUCAST has validated a method for **direct** plating of disk diffusion MH and MHF agar plates for reading after **4, 6 and 8 hours of incubation**
- ✓ Incubation can not be prolonged - the method is only validated for the **short incubation time.**
- ✓ Currently the method is **validated** for:
  - *E. coli*
  - *K.pneumoniae*
  - *Ps. aeruginosa*
  - *S. aureus*
  - *E. faecalis and faecium*
  - *S.pneumoniae*

and for a limited panel of antimicrobials.

Volume 75, Issue 4
April 2020

JOURNAL ARTICLE

The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles

Emma Jonasson ✉, Erika Matuschek, Gunnar Kahlmeter

Journal of Antimicrobial Chemotherapy, Volume 75, Issue 4, April 2020, Pages 968–978, https://doi.org/10.1093/jac/dkz548
Published: 04 February 2020 **Article history ▾**

Received 25 September 2019; returned 24 October 2019; revised 3 December 2019; accepted 9 December 2019

Objectives: With increasing antimicrobial resistance, rapid antimicrobial susceptibility testing (RAST) becomes important, especially in patients with bloodstream infections. EUCAST decided to develop a standardized rapid method, based on EUCAST disc diffusion, to offer susceptibility reports within 4–8 h of a positive blood culture (BC).

Methods: BC bottles were spiked with clinical isolates ($n = 332$) of the seven most relevant sepsis pathogens with a variety of resistance mechanisms. RAST was performed directly from the bottle and zones read after 4, 6 and 8 h. Several variables were investigated, including the effect of using different BC bottles and of a 0–18 h delay between a positive signal and the performance of RAST.

Results: For five species, most inhibition zones could be read after 4 h. The proportion of results that could be interpreted increased from 75% a
was good, with error rates of false
1.2%, 0.2% and 0.1% at 4, 6 and 8

Conclusions: With the EUCAST RAS
tles for seven important bloodstrear
ability caused by using a non-stand
delays, we have introduced an area

Volume 78, Issue 12
December 2023

< Previous Next >

JOURNAL ARTICLE

Evaluation of prolonged incubation time of 16–20 h with the EUCAST rapid antimicrobial susceptibility disc diffusion testing method

Get access >

Emma Jonasson, Erika Matuschek ✉, Gunnar Kahlmeter

Journal of Antimicrobial Chemotherapy, Volume 78, Issue 12, December 2023, Pages 2926–2932, https://doi.org/10.1093/jac/dkad332
Published: 21 October 2023 **Article history ▾**

Objectives

Antimicrobial resistance rates are continuously increasing, driving the need for rapid antimicrobial susceptibility testing (RAST) results, especially in the treatment of bloodstream infections. The EUCAST RAST method performed directly from positive blood cultures with incubation times from 4 to 8 h was developed in 2018 and is now used in many laboratories. To increase the practicality of the method, an extended incubation time of 16 and 20 h was evaluated in this study.

Method

Blood culture bottles were spiked with clinical isolates ($n = 325$) of the seven most important sepsis pathogens. The EUCAST RAST method was performed, extending the incubation time to 16 and 20 h. Broth microdilution (BMD) was used as a reference, except for screening tests where standard disc diffusion or presence of resistance genes was used.

Results

Inhibition zones were possible to read for all species–agent combinations. For 16 and 20 h, the MIC zone diameter correlations were sufficiently similar to allow establishment of common breakpoints for the time interval of 16–20 h. The proportion of isolates in the area of technical uncertainty was, on average, 6% for all species and the number of errors were low, with <1% false-resistant and <0.5% false-susceptible results.

Conclusions

This study shows that, for EUCAST RAST, prolonging the recommended incubation to 16–20 h is possible and can be used as a complement when the intended shorter incubation is not possible to achieve. The introduction of the prolonged incubation will increase the usefulness of the EUCAST RAST method in clinical laboratories with limited opening hours.

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Preparation of blood culture bottles

The EUCAST RAST method has been validated using blood culture bottles for BACTEC (Becton Dickinson), BacT/ALERT (bioMérieux) and VersaTREK (Thermo Fisher). The RAST method can be performed 0 – 18 hours after blood culture bottles have signalled positive. Do not remove positive bottles from the blood culture instrument until you are ready to proceed with the RAST. However, to allow for transport of positive bottles from one site to another, we have evaluated the impact of keeping bottles at room temperature after having removed them from the instrument. RAST results were not affected by a "delay" of up to 3 hours.

- Metodo da eseguire entro 18 ore dalla positivizzazione del flacone
- Entro 3 ore dallo scarico del flacone dall'incubatore

Inoculation of agar plates directly from blood culture bottles

Take 125±25 µl of undiluted blood culture broth from the positive blood culture bottle to each 90-mm circular MH/MH-F agar plate. Spread the broth gently over the agar surface by swabbing in three directions or using an automatic plate rotator and apply disks as for standard AST.

Incubation and reading of plates

Incubate plates as described in Table 1. Read inhibition zones at ± 5 minutes of the stated reading time (4, 6 and/or 8 hours). If needed, re-incubate the plates within 10 minutes to enable reading at a later time (6 and/or 8 hours). Do not incubate or read plates beyond 8 hours.

- Prelevare **125 +/- 25 microlitri di brodo di emocoltura positiva non diluita**
- Seminare su **piastra di MH o MH-F da 90 mm** coprendo tutta la superficie (semina manuale o automatica)
- Applicare i dischetti di antibiotico
- **Incubare e leggere la crescita a 4-6-8 ore (più o meno 5 minuti dall'orario previsto)**
- **Reincubare entro 10 minuti per la lettura successiva**

Impact of the EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) on patients with bacteremia on clinical outcomes and Antimicrobial Stewardship: a real-life study in an Italian university hospital.

A. Santoro [1], V. Todisco [1], A. Di Marcello [2], V. Menozzi [1], D. Chemello [1], A. Spadoni [1], K. Falasca [2], J. Vecchiet [2], A. Bedini [1], M. Sarti [3], E. Franceschini [1], C. Mussini [1], M. Meschiari [1].

[1]AOU Policlinico di Modena, Infectious Disease Department - Modena (Italy), [2]Università di Chieti, Infectious Disease Department - Chieti (Italy), [3]AOU Policlinico di Modena, Microbiology Department - Modena (Italy)

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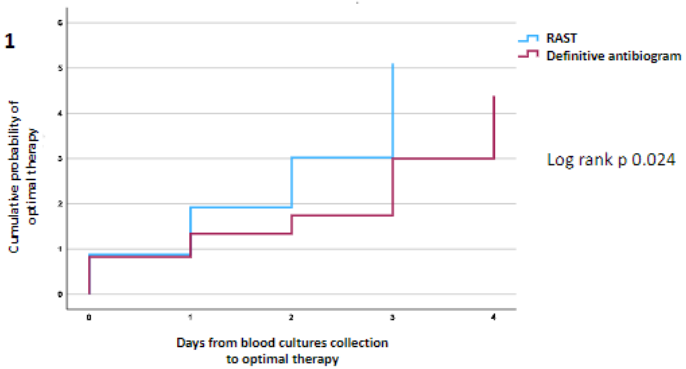
TABLE 1. Population characteristics

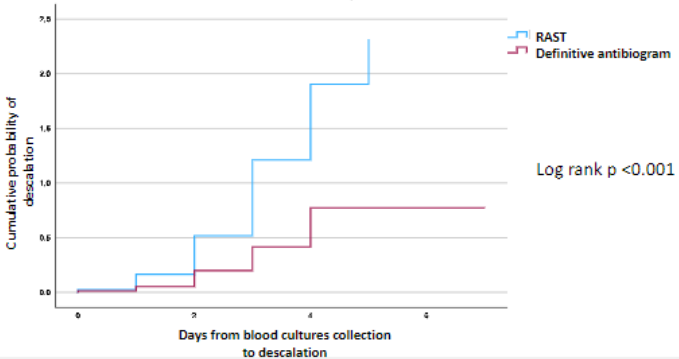
Variables	Total	Species identification	RAST	Definitive antibiogram	p-value
Number of patients	333	87 (25.5)	166 (48.7)	80 (23.5)	
Age in years ± SD	71.26 (± 15.52)	68.38 (±15.97)	73.60 (±14.58)	69.54 (±16.31)	0.020
Female gender (%)	149 (44.7)	35 (40.2)	76 (45.8)	38 (47.5)	0.596
Charlson comorbidity index ± SD	3.12 (± 2.37)	2.70 (± 2.31)	3.11 (± 2.30)	3.12 (± 2.37)	0.054
Neutropenia	10 (3.0)	2 (2.3)	7 (4.2)	1 (1.3)	0.400
HSCT	4 (1.2)	2 (2.3)	1 (0.6)	1 (1.3)	0.500
Solid Organ Transplant	22 (6.6)	7 (8.0)	13 (7.8)	2 (2.5)	0.237
Mechanical ventilation	20 (6.0)	3 (3.4)	7 (4.2)	10 (12.5)	0.019
Severity of illness:					
Pitt bacteriemia score ± SD	1.13 (± 1.38)	0.87 (±1.30)	1.12 (±1.34)	1.44 (±1.52)	0.031
Sepsis|,9	160 (48.0)	37 (42.5)	86 (51.8)	37 (46.3)	0.641
Septic shock	41 (12.3)	9 (10.3))	21 (12.7)	11 (13.8)	0.798
Gram					
• Gram+	123 (36.9)	50 (57.5)	51 (30.7)	22 (27.5)	<0.001
• Gram-	201 (60.4)	31 (35.6)	115 (69.3)	55 (68.8)	<0.001
Resistance Mechanism					
• Methicillin Resist.	19 (5.7)	4 (4.6)	9 (5.4)	6 (7.5)	0.704
• ESBL/AMPc	41 (12.3)	3 (3.4)	27 (16.3)	11 (13.8)	0.012
• Carbapenemases	8 (2.4)	2 (2.3)	3 (1.8)	3 (3.8)	0.646
• VRE	2 (0.6)	1 (1.1)	1 (0.6)	0	0.631
AMS Intervention:					
• Start Therapy	45 (13.5)	24 (27.6)	20 (12.0)	1 (1.3)	0.001
• Confirmed therapy	96 (28.8)	25 (28.7)	40 (24.1)	31 (38.8)	0.046
• Descalation	113 (33.9)	15 (17.2)	70 (42.2)	28 (35.0)	0.001
• Escalation	46 (13.8)	11 (12.6)	23 (13.9)	12 (15.0)	0.907
• Switch	30 (9.0)	11 (12.6)	13 (7.8)	6 (7.5)	0.386
Time to effective therapy (in days ± SD)	0.74 (± 1.078)	0.80 (±1.14)	0.61 (±0.90)	0.94 (±1.31)	0.072
Time to optimal therapy (in days ± SD)	2.00 (± 1.85)	2.20 (±2.06)	1.73 (±1.61)	2.35 (±2.01)	0.027
Length of hospitalization (in days ± SD)	26.11 (± 25.95)	31.18 (±33.32)	24.13 (±23.04)	24.62(±21.52)	0.118
Duration of antibiotic therapy (in days ± SD)	12.38 (± 9.42)	13.97 (±10.33)	11.76 (±8.30)	11.95 (±10.46)	0.190
Mortality at 14 days	31 (9.3)	4 (4.6)	18 (10.8)	9 (11.3)	0.211
Mortality at 30 days	53 (15.9)	18 (20.7)	23 (13.9)	12 (15.0)	0.357
Mortality at 90 days	76 (22.8)	28 (32.2)	32 (19.3)	8 (27.6)	0.053
Re-hospitalization at 90 days	76 (22.8)	27 (31.0)	36 (21.7)	16 (20.0)	0.067

Figure 1



Log rank p 0.024

Figure 2



Log rank p <0.001

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Conclusion: EUCAST RAST offers a promising approach to test antimicrobial susceptibility, enabling earlier initiation of optimal therapy, especially in cases of bacteremia caused by multidrug-sensitive and methicillin-resistant bacteria, thereby promoting early de-escalation strategies. Our findings suggest that integrating strategies that combine AMS interventions with diagnostic stewardship can enhance prescription appropriateness. Considering these results, the cost-effectiveness, simplicity of execution, and strong concordance of RAST, identify this test as a viable option in clinical microbiology.

RAST mediante disco-diffusione da emocoltura positiva (RAST EUCAST)

VANTAGGI

- Costi limitati
- Flessibilità
- Applicabilità a tutti i pazienti
- Possibile allestimento subito dopo scarico flacone positivo
- BP per i principali patogeni e antibiotici

LIMITI

- Lettura a 4-6-8 ore (gestione refertazione?)
- Lettura operatore dipendente
- Risultato fenotipico quantitativo che non fornisce la MIC
- % ATU (soprattutto per piperacillina-tazobactam)

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Conclusioni

- Metodi **potenzialmente** utili per ridurre il tempo di impostazione di una terapia antimicrobica appropriata

- Diversi metodi attualmente disponibili (EUCAST RAST, RAST automatizzati), necessario valutare sulla base di:

- risorse economiche, strutturali, di personale
- organizzazione ed orari di apertura del laboratorio (flusso di lavoro 24/24 su 7gg)
- percorsi dedicati, tipologia pazienti ed epidemiologia locale

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03. Bacterial susceptibility & resistance

Evolutionary dynamics of antimicrobial resistance: from phylogenetic drivers to plasmid-mediated resistance

CME

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03. Bacterial susceptibility & resistance

Genomic surveillance to track emerging antibiotic resistance

CME

04. Diagnostic microbiology

New solutions for susceptibility testing, pathogen detection and surveillance

CME

04. Diagnostic microbiology

Advances in drug resistance testing

CME

Add to my agenda

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Advances in drug resistance testing

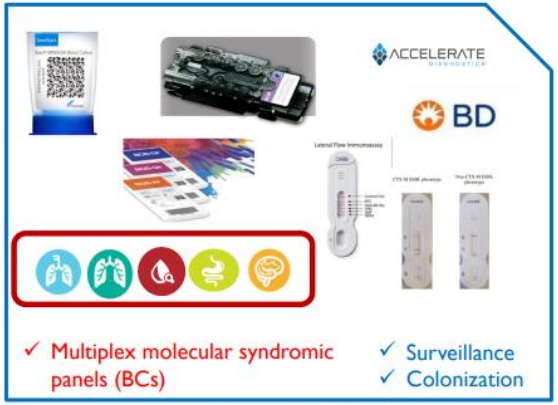
CME

Add to my agenda

RAPID MOLECULAR (BIOCHEMICAL) DIAGNOSTICS FOR AMR DETECTION → GAME-CHANGER

Although termed as "molecular", are based on a variety of technologies:

- *RT-PCR*
- *PNA-FISH*
- *Nested-PCR*
- *Microarrays*
- *DNA-hybridization*
- *Immunochromatography (enzyme)*



FDA-cleared diagnostics identify ID and detect AMR genes (simultaneously or not)

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PROS.....

Technical efficacy

- Faster Tourn-Around-Times **(TAT)**
- Simultaneously **ID/AMR** gene
- **Multiplex** targeting of AMR

Identification (ID) accuracy

- In case of **difficult or not possible growth** Independent on live cells
- Previous/under **antibiotic therapy**

Antimicrobial resistance (AMR)

- **Specific** AMR targets
- Insights into **mechanism of R**
- **Prediction** of phenotypic AST

Patient Outcome & lower hospital cost

- ***Reduced Time*** *to effective therapy*
- *Reduce* ***antibiotic consumption***
- *Reduce Hospital* ***costs***
- *Improve existing* ***stewardship*** *programs*

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CONS.....

Technical efficacy

- CANNOT define **MICs**
- Predict **R NOT S**
- No standardization in clinical practice
- Higher cost

Identification (ID) accuracy

- Less accurate in **polymicrobial** detection

Antimicrobial resistance (AMR)

- NO **Off-panel** - new – AMR variants
- MAY NOT DETECT **non-enzymatic** mechanisms (porin loss; efflux-pump up-regulation).
- May overcall R (AMR genes detected but **NOT expressed**)

Impact on patient outcome

- **Limited** to some antibiotics
- **Cannot rule** AM therapy options
- **Inappropriate** de-escalation or unnecessary broad-spectrum therapy.

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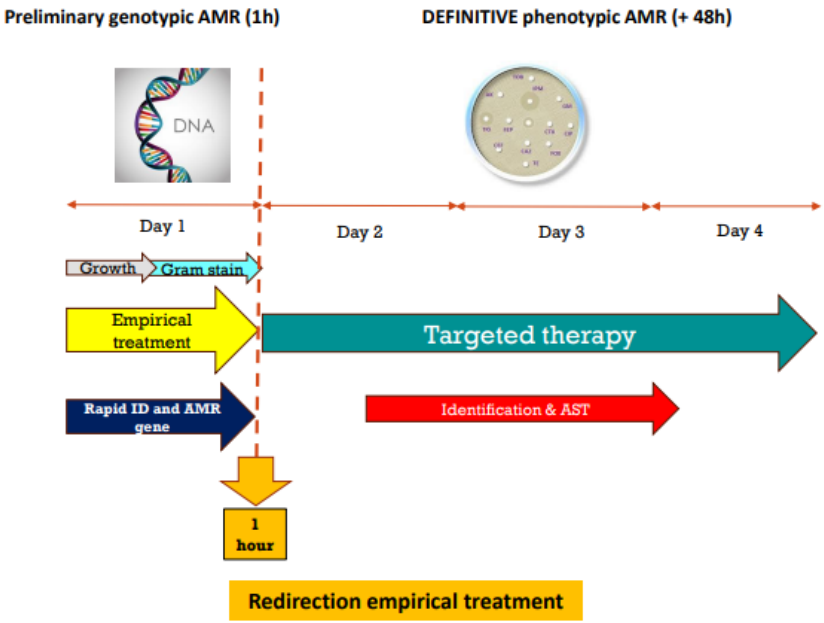
THE GENOTYPE-TO-PHENOTYPE DILEMMA



WHAT THE POSSIBLE SCENARIOS?

GENETIC ALTERATIONS RESULTING IN GENOTYPIC/PHENOTYPIC DISCREPANCIES

Staphylococcus aureus

Genotype	Phenotype	Description	Discrepancy reason
mecA detected	Methicillin susceptible	*mec*A inactivation → PBP2a aminoacidic substitution	Loss of PBP2a transpeptidase activity
mecA detected	Methicillin susceptible	Heterogeneously resistant large inoculum led to resistant phenotype	Oxacillin induction increased MIC
mecA not detected	*S. aureus with* borderline-oxacillin resistance (BORSA)	• Beta-lactamases over-expression • PBP alterations • Adaptative mutations in cell-wall genes	Oxa R Fox S

*Take home message

WHAT CAN LABS DO TO SOLVE DISCREPANCIES?

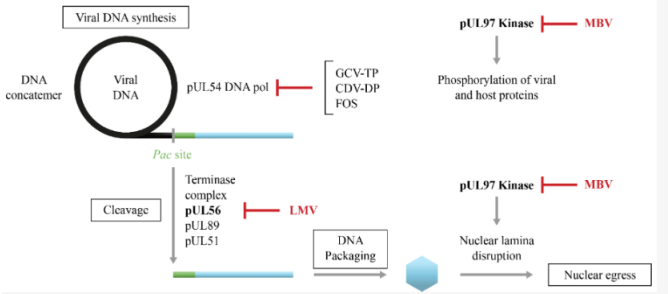
- ✓ HARMONIZE GENOTYPE-TO-PHENOTYPE RESULTS
- ✓ CRITICALLY JUSTIFY DISCREPANCIES
- ✓ REPORT AS "R" IF A DISCREPANCY IS NOT RESOLVED
- ✓ CLEAR RESULT COMMUNICATION

J Clin Microbiol. 2024 May 8;62(5):e0163023. doi: 10.1128/jcm.01630-23. Epub 2024 Mar 27.

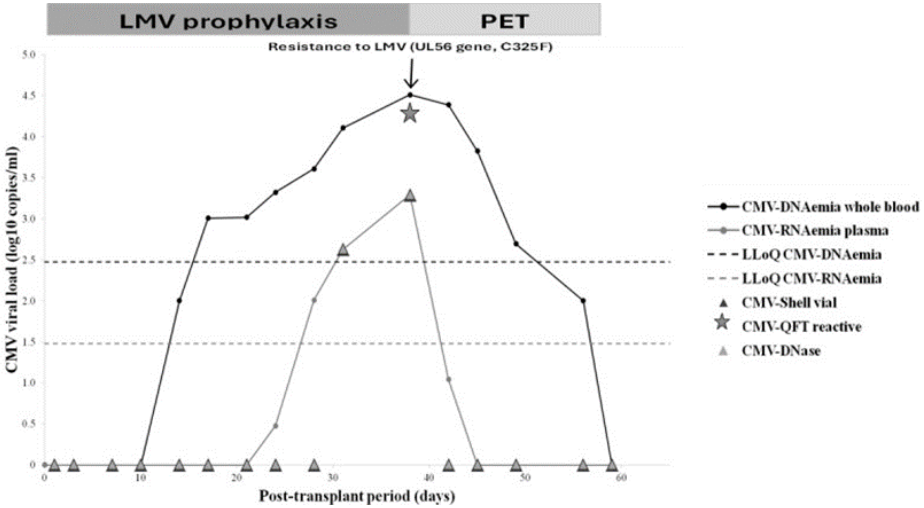
CMV-RNAemia as new marker of active viral replication in transplant recipients

Giulia Piccirilli [1], Federica Lanna [2], Liliana Gabrielli [1], Vincenzo Motta [2], Martina Franceschiello [1], Alessia Cantiani [2], Matteo Pavoni [2], Marta Leone [2], Eva Caterina Borgatti [2], Dino Gibertoni [3], Renato Pascale [4], Maddalena Giannella [4], Francesca Bonifazi [5], Tiziana Lazzarotto [1 2]

concatemers at Pac site and their packaging into capsids. Letermovir (LMV) is an inhibitor of the viral terminase complex and more specifically of the pUL56 subunit. On the other hand, pUL97 kinase is involved in the phosphorylation of several viral and host proteins. pUL97 kinase also participates in the disruption of the nuclear lamina and in the nuclear egress of virions. Maribavir (MBV) is a selective inhibitor of the pUL97 kinase activity.



replication (6,–9). Indeed, by blocking the terminase complex, LMV induces the release of free CMV-DNA fragments (abortive infection), which could lead to potential misinterpretation of molecular testing results (1, 6,–9). Additional methods, such as CMV-viremia (shell vial method) and CMV-



during the viral load evaluations. In the analysis, the CMV-DNAemia episodes were stratified into three groups: LMV-prophylaxis, LMV off-label treatment, and pre-emptive therapy. Focusing on cases receiving LMV prophylaxis, CMV-RNAemia resulted positive in 6/25 (24%) episodes (Table 1). Among these, 4/6 (66.7%) were related to clinically significant CMV infections (based on both clinical and laboratory findings), treated by pre-emptive therapy. In 2/4 (50%) cases, genotypic LMV resistance (LMV-R) was documented. Considering the group receiving LMV off-label, six out of seven episodes (85.7%) showed positive CMV-RNAemia (Table 1), and in 3/6 cases (50%), LMV-R was found, leading to switching the treatment to other anti-CMV agents: maribavir, foscarnet, or immunoglobulins. In the remaining three cases, the LMV treatment was

In conclusion, CMV-RNAemia, detected by a standardized user-friendly assay and an automated and integrated system, together with CMV-DNAemia, could provide accurate information on viral load kinetics during post-transplant monitoring of CMV infection, especially in patients receiving LMV. Further studies with larger numbers of samples, including patients undergoing therapy with new anti-CMV drugs such as maribavir, are needed to confirm these preliminary data.

01. Viral infection & disease (incl. COVID-19)

Emerging vector-borne and zoonotic viruses diseases

CME

Genetic characterisation of autochthonous dengue virus isolates circulating in Lazio region, Italy, August to November 2023 Abstract
Martina RUECA, Italy

Autochthonous dengue cases in Northeastern Spain, 2023: molecular analysis Abstract
Jessica NAVERO CASTILLEJOS, Spain

Epidemiology of *Corynebacterium ulcerans* infection linked to companion animals in England Abstract
Eva EMANUEL, United Kingdom

Tick-borne encephalitis virus strains unveil specific *in vitro* and *ex vivo* phenotypes Abstract
Simone LEONI, Switzerland

Maternal safety, reactogenicity, and immunogenicity of a 2-dose Ebola vaccine regimen in healthy pregnant women Abstract
Julien NYOMBAYIRE, Rwanda

Positive immunogenicity and safety profile for a virus-like particle (VLP) based chikungunya virus (CHIKV) vaccine: results from a pivotal Phase 3 trial Abstract
Hristu GACHE, United States

Tick-borne disease surveillance in Veneto, Italy: insights from a 2023 outbreak with enhanced monitoring after tick bite exposure Abstract
Giacomo STROFFOLINI, Italy

RAPID COMMUNICATION

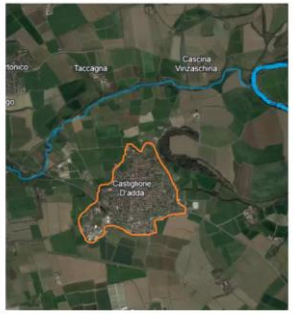
Preliminary results on an autochthonous dengue outbreak in Lombardy Region, Italy, August 2023

Focolaio autoctono di Dengue di Castiglione d'Adda

Caratteristiche del territorio

Provincia di Lodi

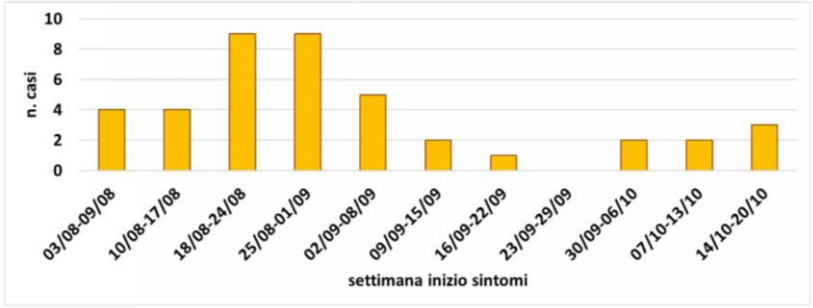
4.400 abitanti
(01/01/2023 - ISTAT)



Area a limitata urbanizzazione e bassa densità abitativa
85% del territorio: scopi agricoli

Da Agosto ad Ottobre 2023
41 confermati + 6 probabili

Sierotipo DENV-1



Cassaniti, Eurosurveillance 2023

RAPID COMMUNICATION

Outbreaks of autochthonous Dengue in Lazio region, Italy, August to September 2023: preliminary investigation

Epidemiological and laboratory parameters	DENV-1 cluster[a]				DENV-3 cluster		DENV-2
	Case 1	Case 4	Case 5	Case 6	Case 2	Case 3	Case 7
Date of notification	18 Aug	5 Sep	8 Sep	12 Sep	31 Aug	31 Aug	20 Sep
Epidemiological link with imported case	No	No	No	No	No	No	Yes
Laboratory results							
DENV NS_1 antigen	NA	Negative	Positive	Positive	Positive	Positive	Positive
DENV IgG IC	Positive	Borderline	Positive	Negative	Negative	Negative	NA
DENV IgM IC	Positive	Borderline	Positive	Positive	Negative	Negative	NA
DENV IgG IF	Positive	Positive	Positive	NA	Weak reactivity	Weak reactivity	Positive
DENV IgM IF	Positive	Positive	Positive	NA	Weak reactivity	Weak reactivity	Positive
DENV RT-PCR (Cq at diagnosis)	31	32	23	27	22	24	36
DENV serotype	1	1	1	1	3	3	2

De Carli, Eurosurveillance 2023

Istituto Superiore di Sanità
EpiCentro - L'epidemiologia per la sanità pubblica

5/12/2023 - Casi di arbovirosi in Italia: i dati aggiornati

Dal 1 gennaio al 4 dicembre 2023 al sistema di sorveglianza nazionale risultano: 347 casi confermati di Dengue (82 casi autoctoni - vedere focus); 8 casi confermati di Zika Virus; 7 casi confermati di Chikungunya; 48 casi confermati di infezione neuro-invasiva - TBE; 127 casi confermati di Toscana Virus. Per maggiori informazioni sui dati consulta la *dashboard* e la pagina generale dedicata

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Istituto Superiore di Sanità
EpiCentro - L'epidemiologia per la sanità pubblica

Arbovirosi

Ultimi aggiornamenti

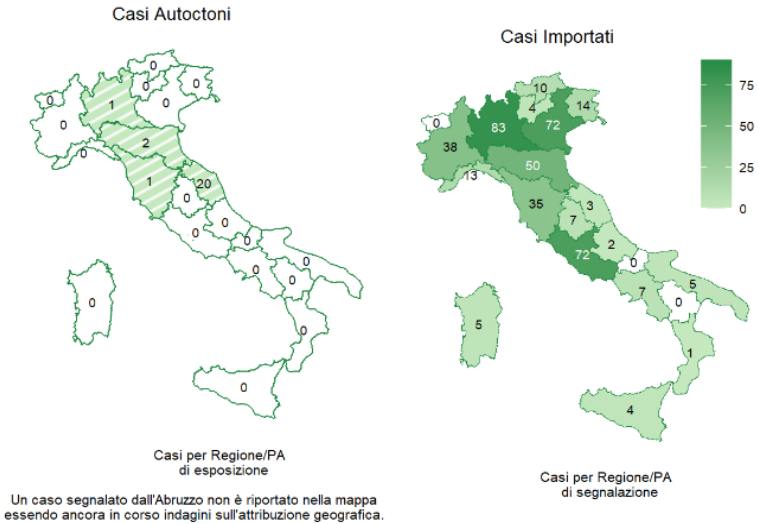
19/9/2024 - Casi di Dengue in Italia: i dati aggiornati

Sono 450 i casi confermati di Dengue dal 1 gennaio al 17 settembre 2024 e segnalati al sistema di sorveglianza nazionale. Di questi, 425 sono associati a viaggi all'estero e 25 autoctoni. L'età mediana dei casi segnalati è di 40,5 anni e il 48% è di sesso

450	48% | 52%	40.5 anni	0	25 casi | 425 casi
Casi*	Maschi | Femmine*	Età mediana*	Decessi*	Autoctoni | Importati*

*Dati in fase di consolidamento

Casi per Regione/PA*



Casi per Regione/PA di esposizione

Un caso segnalato dall'Abruzzo non è riportato nella mappa essendo ancora in corso indagini sull'attribuzione geografica.

Casi per Regione/PA di segnalazione

Sistema Socio Sanitario
Ospedale Luigi Sacco POLO UNIVERSITARIO
Regione Lombardia
ASST Fatebenefratelli Sacco

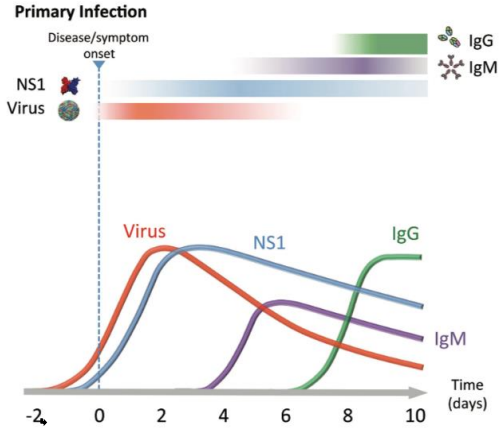
Diagnostica microbiologica

Isolamento Virale

- Substrati cellulari permissivi all'infezione

Indagini Molecolari

- Saggi molecolari (commerciali) specifici per conferma di infezione acuta
- Saggi molecolari *home-made pan*-genere in grado di rilevare sequenze conservate intra genere virale:
  - *pan-Flavivirus*
  - *pan-Alphavirus*
  - *pan-Flebovirus*
- Sequenziamento genomico (Sanger o NGS) in ausilio alle indagini molecolari pan-genere



Viremia Transitoria

Matrici Biologiche

- Sangue, Urine; Liquor (Encefaliti); Saliva e Liquido Seminale (Zika)

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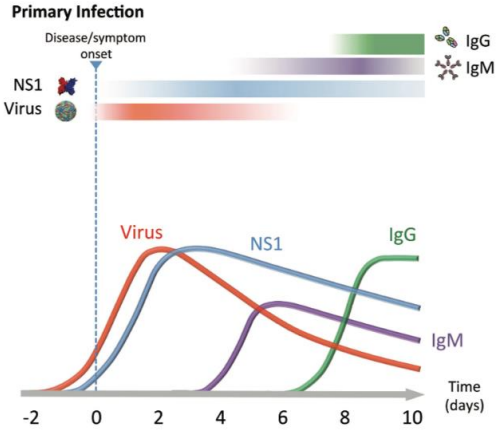
Diagnostica microbiologica

Indagini Sierologiche

- Saggi (commerciali) per la rilevazione di specifiche IgM e IgG (ELISA-CLIA-IF)

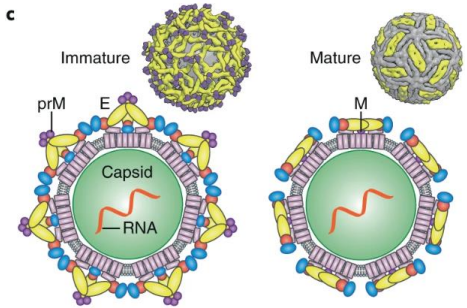
Specificità!



EDITORIAL

Diagnosis of arboviral infections – A quagmire of cross reactions and complexities

Remi, Trav. Med. 2016



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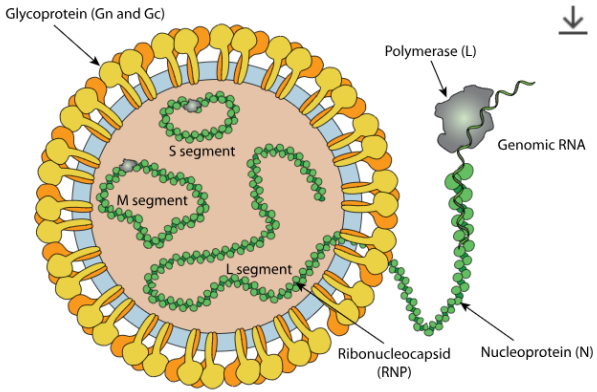
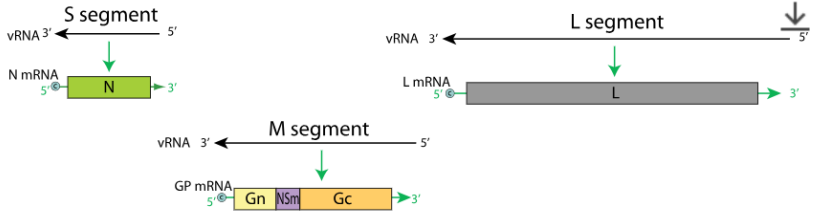
Oropouche virus

VIRION



Enveloped, spherical. Diameter from 80 to 120nm.

GENOME



TAXONOMY

Group V: Negative sense ssRNA viruses

Order:	*Bunyavirales*
Family:	***Peribunyaviridae***
Genus:	*Herbevirus*
	Orthobunyavirus
	Tospovirus

Culicoides paraensis

Culex quinquefasciatus

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Epidemiological Alert - Oropouche in the Region of the Americas - 2 February 2024

Map. Distribution of Oropouche cases in the State of Amazonas, Brazil, 2024

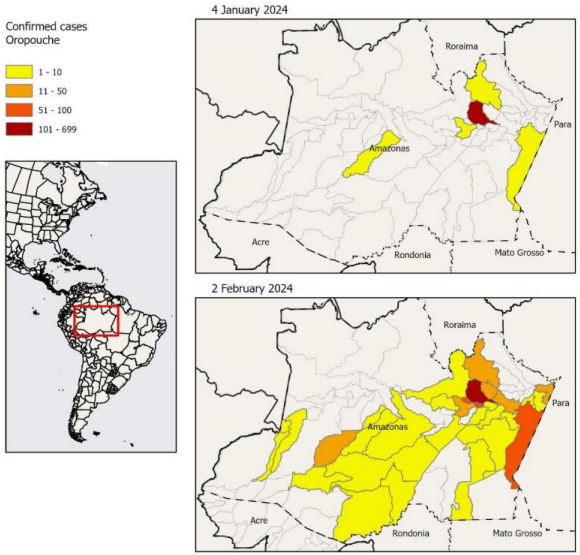

Figure 1: Number of cases of Oropouche virus disease in Cuba by province

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Published in final edited form as:

Diagn Microbiol Infect Dis. 2020 January ; 96(1): 114894. doi:10.1016/j.diagmicrobio.2019.114894.

Real-time RT-PCR for the Detection and Quantitation of Oropouche Virus

Alejandra Rojas[1], **Victoria Stittleburg**[2], **Fátima Cardozo**[3], **Nathen Bopp**[4], **César Cantero**[1], **Sanny López**[1], **Cynthia Bernal**[1], **Laura Mendoza**[3], **Patricia Aguilar**[4], **Benjamin A. Pinsky**[5,6], **Yvalena Guillén**[1], **Malvina Páez**[3], **Jesse J. Waggoner**[2,7,*]

[1]Universidad Nacional de Asunción, Instituto de Investigaciones en Ciencias de la Salud, Departamento de Producción, Paraguay

[2]Emory University, Department of Medicine, Division of Infectious Diseases, Atlanta, Georgia, USA

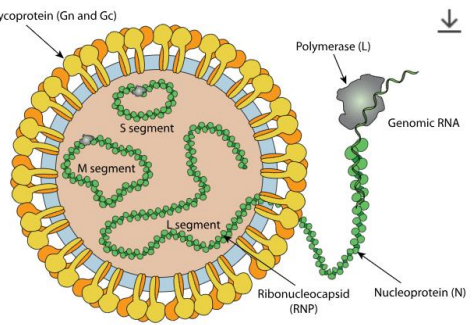
[3]Universidad Nacional de Asunción, Instituto de Investigaciones en Ciencias de la Salud, Departamento de Salud Pública, Paraguay

[4]Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA

[5]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA

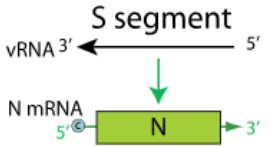
[6]Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California, USA

VIRION



Enveloped, spherical. Diameter from 80 to 120nm.

GENOME



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RAPID COMMUNICATION

Oropouche fever cases diagnosed in Italy in two epidemiologically non-related travellers from Cuba, late May to early June 2024

Concetta Castilletti[1], Antonio Mori[1], Andrea Matucci[1], Niccolò Ronzoni[1], Lukas Van Duffel[2], Giada Rossini[3], Pietro Sponga[1], Maria Luca D'Errico[1], Paola Rodari[1], Francesco Cristini[2,4], Ralph Huits[1], Federico Giovanni Gobbi[1,5]

1. Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy
2. Infectious Diseases Unit, AUSL Romagna, Forlì and Cesena Hospitals, Forlì, Italy
3. Unità Operativa Complessa Microbiologia, IRCCS Azienda Ospedaliero, University of Bologna, Bologna, Italy
4. Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy
5. Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

Correspondence: Concetta Castilletti (concetta.castilletti@sacrocuore.it)

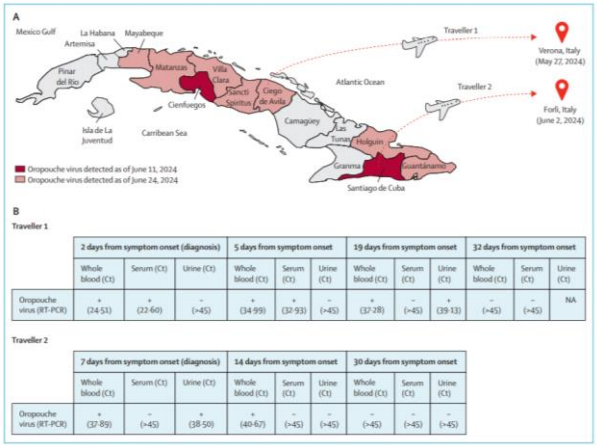

Figure: Oropouche virus in Cuba and Italy
(A) Map of Cuba in which the presence of the Oropouche virus in nine provinces and 23 municipalities was confirmed by the national reference laboratory of the Pedro Kouri Institute of Tropical Medicine. (B) Virological findings in two people with Oropouche fever during 1-month follow-up. Oropouche virus-specific molecular

J Travel Med. 2024 Aug 29:taae115. doi: 10.1093/jtm/taae115. Online ahead of print.

Oropouche fever diagnosed in Milan, Italy in returning travellers from Rio de Janeiro, march 2024, and Cuba, July 2024

Alessandro Mancon [1], Gloria Gagliardi [2], Andrea Giacomelli [2 3], Luigi Vezzosi [4], Andrea Gori [5 6], Spinello Antinori [2 3], Danilo Cereda [4], Maria Rita Gismondo [1 2], Davide Mileto [1 7]

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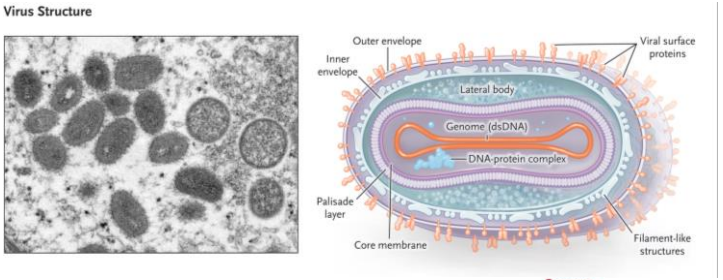
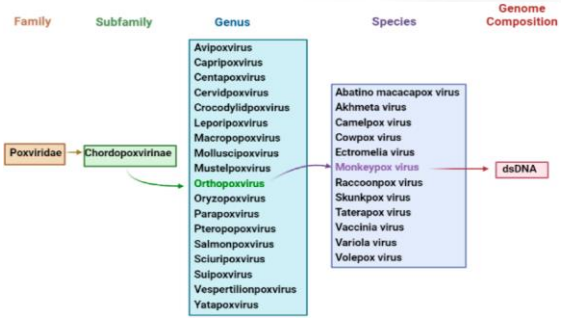
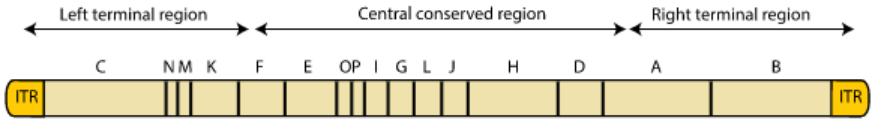
Monkeypox virus (Mpox)



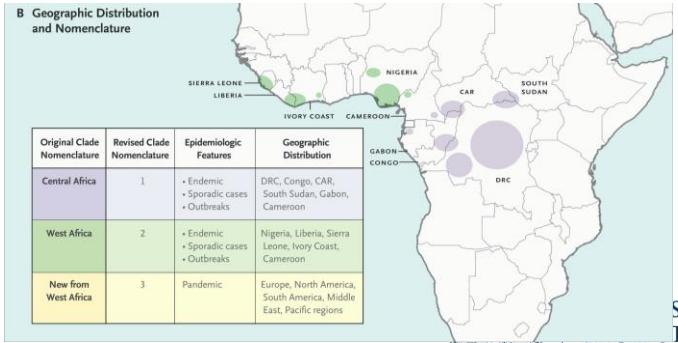
- Family ***Poxviridae***, subfamily Chordopoxvirinae, genus ***Orthopoxvirus***
- Enveloper virus, Linear dsDNA genome of ~ 200kb

- Two different genetic clades, with genomes differing by less than 1%. **Clade one** (I) is endemic in Central Africa (Congo Basin), while **Clade two** (II) in West Africa. Additionally, it was agreed that Clade II consist of two subclades, **IIa** and **IIb**
- Clade I (Congo Basin Clade) is more clinically severe, with higher mortality rates and exhibited increased transmissibility than Clade II





ITR= Inverted terminal repeats



Original Clade Nomenclature	Revised Clade Nomenclature	Epidemiologic Features	Geographic Distribution
Central Africa	1	• Endemic • Sporadic cases • Outbreaks	DRC, Congo, CAR, South Sudan, Gabon, Cameroon
West Africa	2	• Endemic • Sporadic cases • Outbreaks	Nigeria, Liberia, Sierra Leone, Ivory Coast, Cameroon
New from West Africa	3	Pandemic	Europe, North America, South America, Middle East, Pacific regions

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A. Gessain et al., N Engl J Med 2022

N. Luna et al., Travel Med Infect Dis. 2022

F. Anwar et al., Life 2023

SITÀ TUDI DI MILANO

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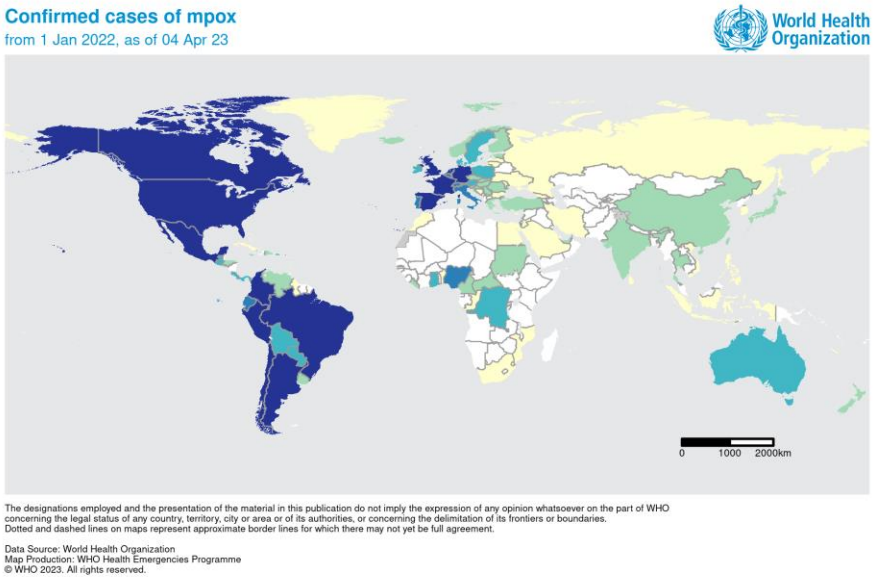
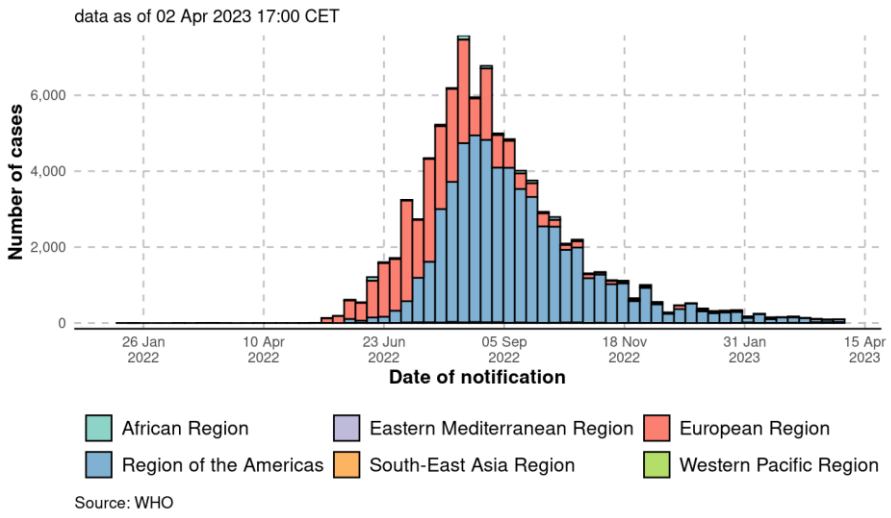
2022-23 Mpox Outbreak

Since **1 January 2022**, cases of mpox have been reported to WHO from 110 Member States across all 6 WHO regions. As of **03 April 2023** a **total of 86,838** laboratory confirmed cases and 1,050 probable cases, including **112 deaths**, have been reported to WHO

Since **13 May 2022**, a high proportion of these **cases** have been reported **from countries without previously documented mpox transmission**. This is the **first time** that cases and sustained chains of transmission have been reported in countries without direct or immediate epidemiological links to areas of West or Central Africa





https://worldhealthorg.shinyapps.io/mpx_global/

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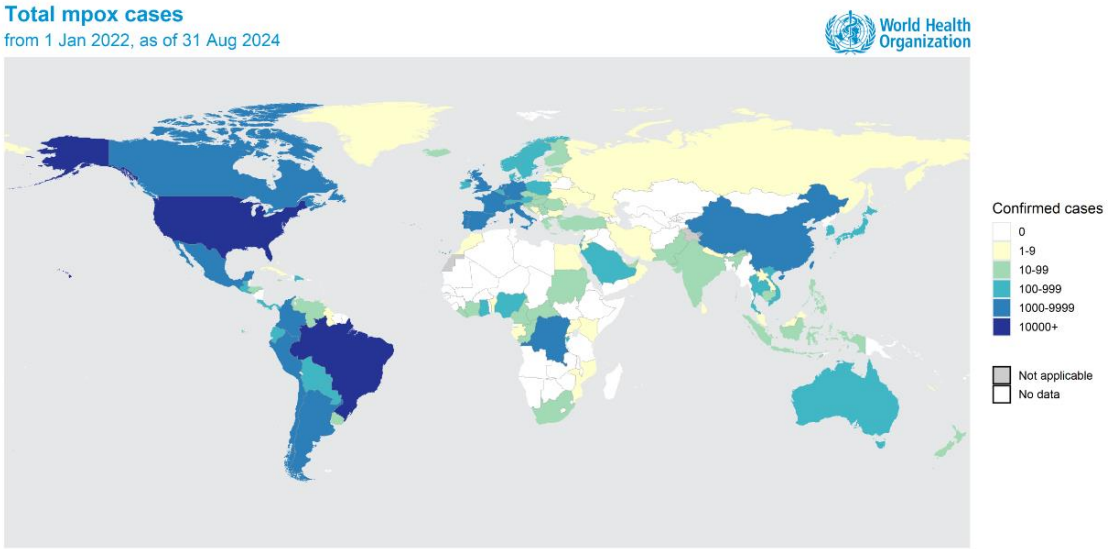
3. Global situation update

All of the following data in this report are presented in the context of the ongoing global mpox outbreak. The data presented here are based on the most recent complete month of data reported to WHO as of 31 August 2024.

Since 1 January 2022, cases of mpox have been reported to WHO from **123 Member States across all 6 WHO regions**. As of 31 August 2024 , a total of **106 310 laboratory confirmed cases** and **0 probable cases**, including **234 deaths**, have been reported to WHO.



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Sistema Socio Sanitario Regione Lombardia ASST Fatebenefratelli Sacco

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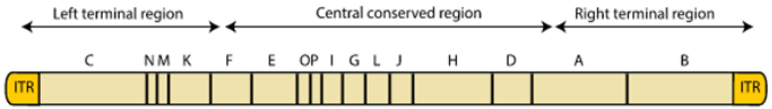
Ongoing mpox outbreak in Kamituga, South Kivu province, associated with monkeypox virus of a novel Clade I sub-lineage, Democratic Republic of the Congo, 2024

Leandre Murhula Masirika[1,2], Jean Claude Udahemuka[3,4], Leonard Schuele[5], Pacifique Ndishimye[4,6,7], Saria Otani[8], Justin Bengehya Mbiribindi[9], Jean M. Marekani[10], Léandre Mutimbwa Mambo[11], Nadine Malyamungu Bubala[9], Marjan Boter[5], David F. Nieuwenhuijse[5], Trudie Lang[12], Ernest Balyahamwabo Kalalizi[2], Jean Pierre Musabyimana[4,13], Frank M. Aarestrup[8], Marion Koopmans[5], Bas B. Oude Munnink[5,*], Freddy Belesi Siangoli[14,*]

1. Centre de Recherche en Sciences Naturelles de Lwiro, DS Bukavu, South Kivu, Bukavu, Democratic Republic of the Congo



ITR= Inverted terminal repeats

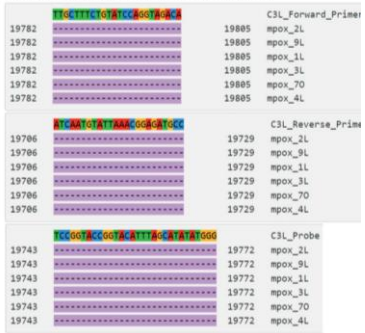
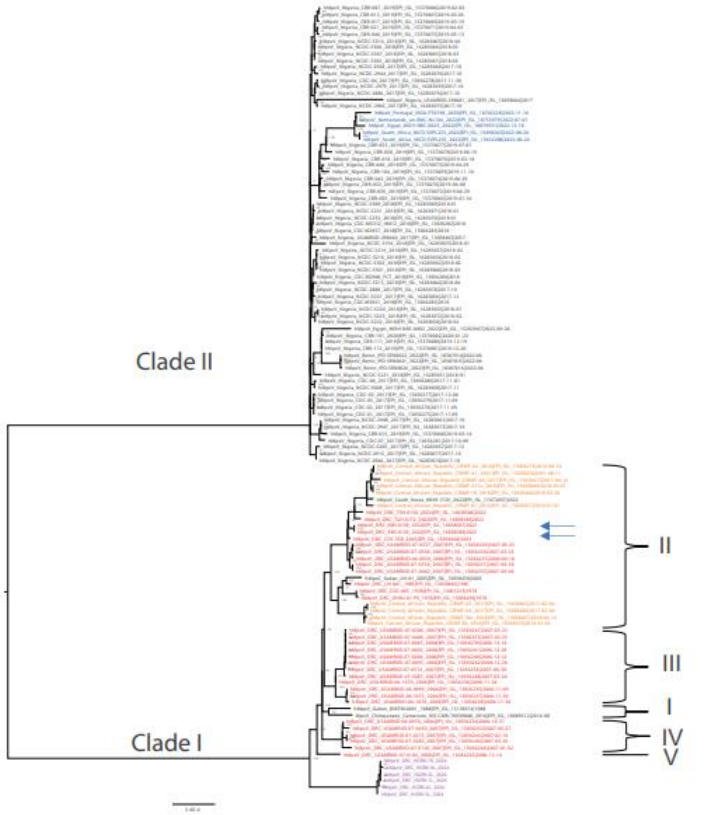


FIGURE 1

Phylogenetic analysis of all near-to-complete genome mpox sequences from Africa available on GISAID and the NCBI including the 2024 sequences from Kamituga, Democratic Republic of the Congo (n = 121 sequences)



DRC: Democratic Republic of the Congo; NCBI: National Center for Biotechnology Information.

The scale under the tree represents the number of substitutions per site. Sequences from Kamituga are presented in magenta, previous sequences from DRC in red and sequences from the 2022 outbreak in blue [14]. Arrows indicate sequences which have been shown in a prior publication to cluster with the previous sequences from a 2023 MPXV outbreak from Kwilu Province near Kinshasa [3]. Lineages are indicated as proposed by Nakazawa et al. [19] and Berthet et al. [20].

We gratefully acknowledge all data contributors, i.e., the authors and their originating laboratories responsible for obtaining the specimens, and their submitting laboratories for generating the genetic sequence and metadata and sharing via the GISAID Initiative, on which this research is based.

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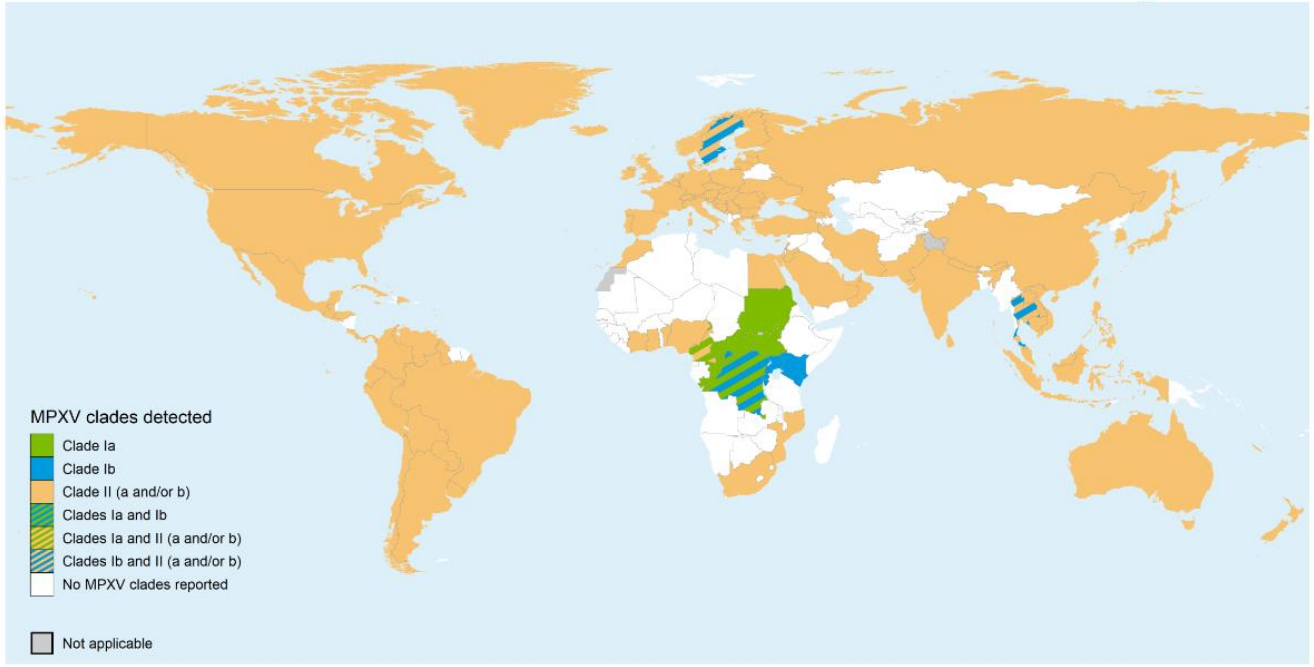
MPXV clades detected globally

includes imported cases; from 1 Jan 2022, as of 22 Sep 2024

World Health Organization



Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme

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Regione Lombardia
ASST Fatebenefratelli Sacco

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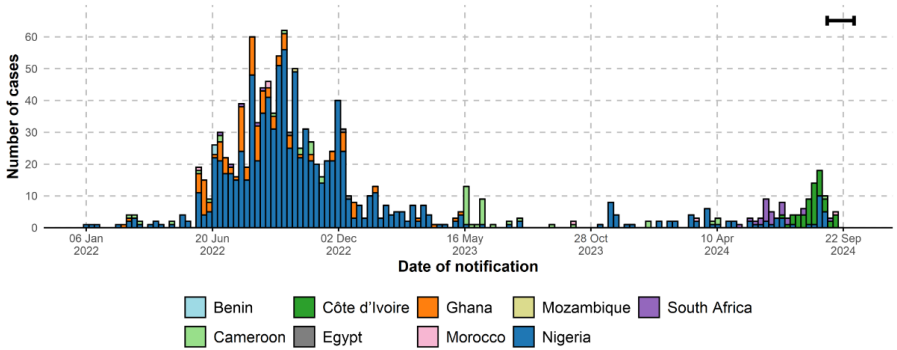
2.2.1. Confirmed cases in all countries in Africa
2.2.2. Confirmed cases in clade Ib countries
2.2.3. Confirmed cases in clade Ia countries
2.2.4. Clade II (a and/or b) countries
2.2.5. All cases in Africa

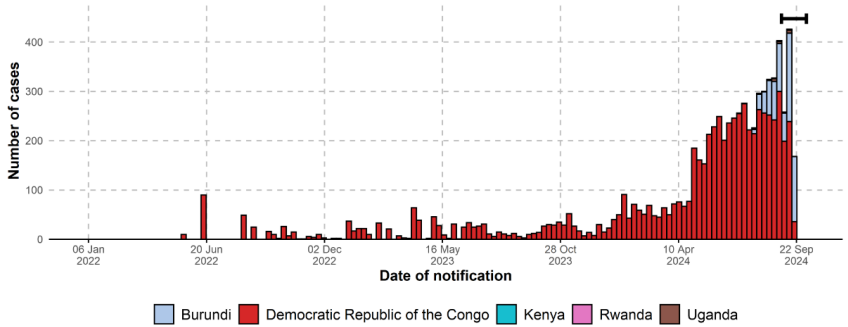
Bracket at end of curve indicates potential reporting delays in recent weeks of data.
Data as of 22 Sep 2024



Source: WHO

2.2.1. Confirmed cases in all countries in Africa
2.2.2. Confirmed cases in clade Ib countries
2.2.3. Confirmed cases in clade Ia countries
2.2.4. Clade II (a and/or b) countries
2.2.5. All cases in Africa

Bracket at end of curve indicates potential reporting delays in recent weeks of data.
Data as of 22 Sep 2024



Source: WHO

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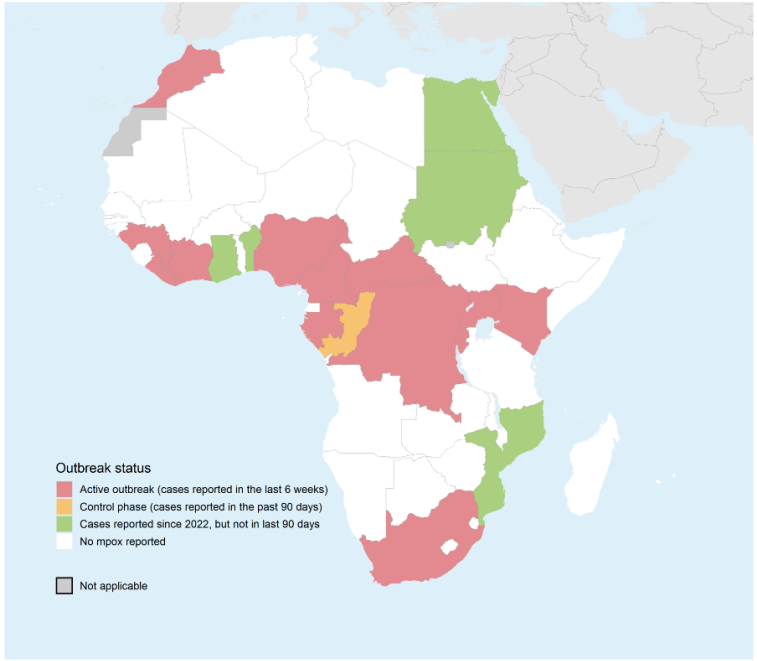
Mpox: countries affected in Africa

from 1 Jan 2022, as of 22 Sep 2024

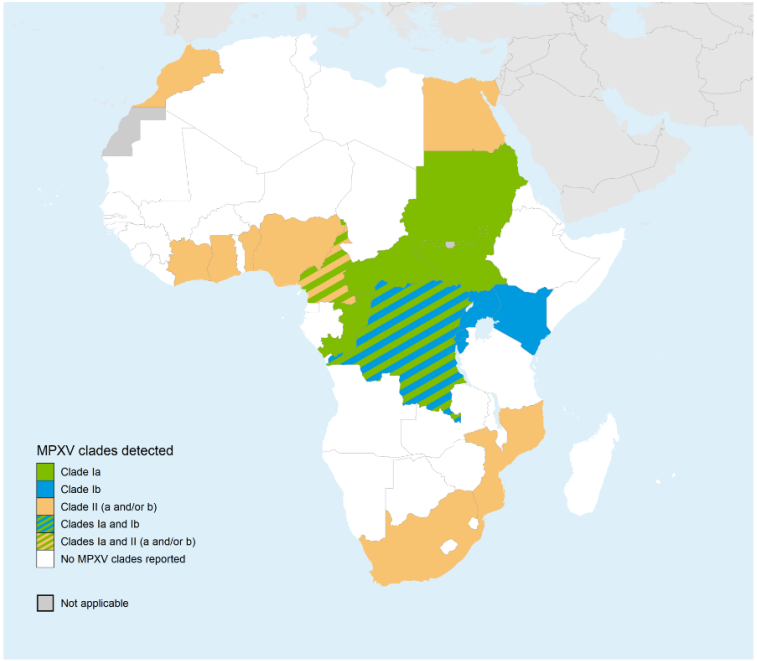
World Health Organization



MPXV clades detected in Africa

from 1 Jan 2022, as of 22 Sep 2024

World Health Organization



Sistema Socio Sanitario

Ospedale Luigi Sacco POLO UNIVERSITARIO

Regione Lombardia

ASST Fatebenefratelli Sacco

UNIVERSITÀ DEGLI STUDI DI MILANO

2022-23 Mpox Outbreak – Method Comparison Protocol Study

CLIMVIB

SD BIOSENSOR

LABORATORY OF CLINICAL MICROBIOLOGY,

33rd ECCMID Copenhagen, Denmark 15–18 April 2023

STANDARD M10 MPX/OPX

STANDARD™ M10 MPX/OPX

TEST PROCEDURE

1 Sample ID Scan

2 Cartridge Scan

3 Sample Transfer

4 Cartridge Loading

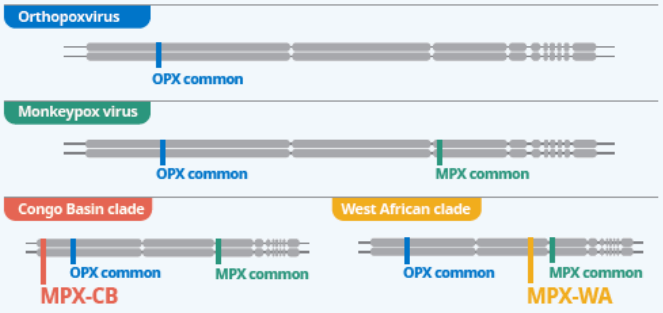
SPECIFICATONS

Cat no.	11MPX10A
Method	**All-in-one Cartridge** Viral DNA Extraction : Column Affinity Method Nucleic Acid Amplification : Real-Time PCR
Target gene	• MPX - G2R • **MPX CB Clade** – intergenic region (F3L~F4L gene) • **MPX WA Clade** – intergenic region (OPG181~OPG185 gene) • OPX – E9L • Internal Control (IC) – Exogenous gene
Sample type	Skin lesion material, Serum/Plasma, Whole blood, Nasopharyngeal swab, Oropharynegeal swab
Sample type	300㎕
Testing time	**60 minutes**
Storage temp.	2~28℃
Test / Kit	10 Tests

TARGET GENE

MPX/OPX

MPX(CB/WA)



DEGLI STUDI DI MILANO

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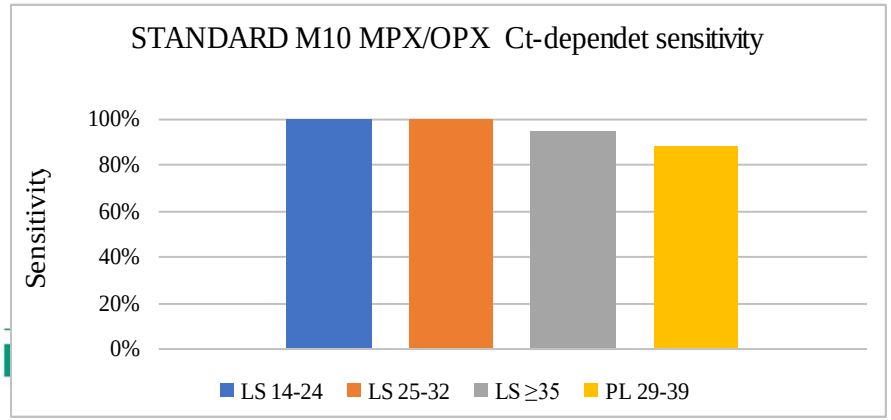
LABORATORY OF CLINICAL MICROBIOLOGY, VIROLOGY AND BIOEMERGENCIES

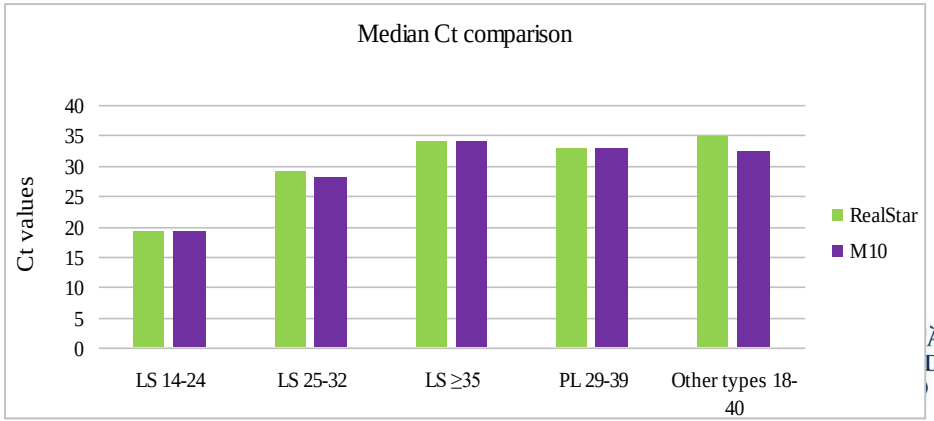
33rd ECCMID Copenhagen, Denmark 15–18 April 2023

- **100% concordance on negative samples**

- **Positive Skin lesion samples were stratified according to Ct at diagnosis in three groups:**
  - **A: Ct 14-24 (n=34)**
  - **B: Ct 25-32 (n=35)**
  - **C: Ct >35 (n=31)**

	Group A LS 14-24		Group B LS 25-32		Group C LS ≥35		plasma PL 29-39	
	RealStar® Orthopox	STANDARD™ M10 MPX/OPX	RealStar® Orthopox	STANDARD™ M10 MPX/OPX	RealStar® Orthopox	STANDARD™ M10 MPX/OPX	RealStar® Orthopox	STANDARD™ M10 MPX/OPX
median ct	19	19	29	28	34	34	33	33
q1	17	16	26	27	33	32	32	32
q3	21	21	31	30	35	34	35	35
sensitivity	100%		100%		95%		88%	





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RAPID COMMUNICATION

Real-time PCR assay to detect the novel Clade Ib monkeypox virus, September 2023 to May 2024

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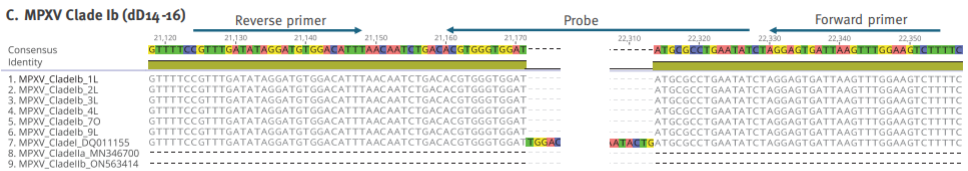
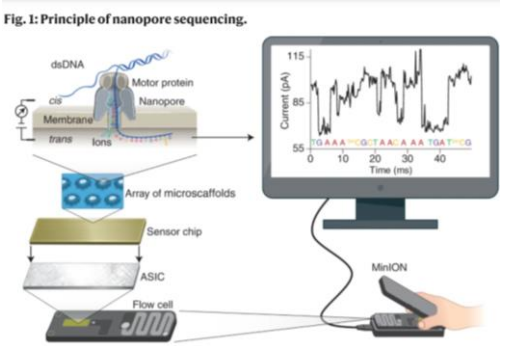


CDC: United States Centers for Disease Control and Prevention; MPXV: monkeypox virus.



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