CENTRE NATIONAL DE REFERENCE ARBOVIRUS	**Kit testé**: **RealStar® Oropouche Fever RT-PCR Kit 1.0 RUO (altona Diagnostics)**
	Date : Avril 2026
	Lieu : CNR Arbovirus, CHU Bordeaux (Pantxika Bellecave, Nael Zemali)

Détails de l'évaluation

Le test RealStar® Oropouche Fever RT-PCR Kit 1.0 RUO a été évalué à l'aide (a) d'un panel EEQ fourni par l'EURL-PH-VBV (EU Reference Laboratory for Public Health on Vector-borne Viral Pathogens) et (b) d'échantillons cliniques issus du CNR arbovirus.

a) Le panel EEQ OROV contient 6 flacons: 2 faiblement positifs, 2 fortement positifs, et 2 négatifs. Il a été préparé en utilisant du virus inactivé spiké dans du plasma négatif et lyophilisé. La souche utilisée, UVE/OROV/2020/GF/Saul-17215 (Ref-SKU: 001V-06152, https://www.european-virus-archive.com/), est phylogénétiquement proche des souches ayant circulé en Amérique Centrale et en Amérique du sud en 2024-2025.
   → Le test RealStar® Oropouche Fever a correctement identifié les 4 positifs ; aucune amplification n'a été observée pour les deux négatifs.
b) Six échantillons identifiés comme positifs avec la méthode de référence du CNR arbovirus (Naveca et al, 2017, sur Panther Fusion-HOLOGIC) ont également été testés avec le kit RealStar® Oropouche Fever.
   →Les résultats obtenus étaient comparables (<1,5 Ct d'écart).

Attention, ces résultats doivent être interprétés avec prudence en raison du nombre limité d'échantillons inclus dans cette évaluation.

Pour aller plus loin :

Altona Diagnostic a réalisé une étude de sensibilité et spécificité en collaboration avec le LACEN, Laboratorio Central Do Espirito Santo (Brésil). Les résultats sont présentés dans un poster scientifique :

Poster ID 187

Oropouche Virus: Evaluation of two RT-PCR techniques for serum sample testing in Brazil

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Background

Oropouche virus (OROV), genus Orthobunyavirus (family Peribunyaviridae) is transmitted predominantly by biting midges and possibly mosquitoes. It causes acute febrile illness and whereas most cases recover completely, severe neurological diseases can occur. The incidence and geographical spread of reported OROV infections have increased over the last years, and the Brazilian Western Amazon experienced its largest laboratory-confirmed OROV outbreak between 2022 and 2024.
Given the similar clinical presentation to other arboviruses like dengue and chikungunya, Oropouche virus disease is often unrecognized or misdiagnosed. Several laboratory tests have been developed but robust commercial tests are hardly available.

Aim

The aim of the study was to compare the performance of the commercially available RealStar® Oropouche Fever RT-PCR Kit 1.0 (altona Diagnostics) and the at LACEN ES [Laboratorio Central Do Espirito Santo] established LDT* (lab developed test) provided by the Ministry of Health of Brazil, based on primer/probes by Naveca FG et al. for comparison in serum samples.

* Naveca FG et al.; Nat Med. 2024 Dec;30(12):3509-3521.

Method

52 pre-tested samples (LDT LACEN ES) were chosen for this study: 25 serum samples tested as "OROV detected" and 27 serum samples tested as "not detected".
All serum samples were extracted according to the protocol of the EXTRACTA® KIT – DNA e RNA DE PATÓGENOS (Loccus®) in combination with the TANBead Maelstrom 9600 instrument (Taiwan Advanced Nanotech Inc.): For extraction, 300 µL of each serum sample was used, 20 µL of Proteinase K and 6 µL of internal control (IC) were added, following the recommendations in the package insert by the manufacturer of the extraction and amplification kits.
The eluates, with genetic material from the samples, were used for both real-time PCRs: The LDT and the RealStar® Oropouche Fever RT-PCR Kit 1.0 were run and analyzed on the QuantStudio 6 Flex (ThermoFisher SCIENTIFIC).

Results

The interpretation of the results of the RealStar® Oropouche Fever RT-PCR Kit 1.0 followed the instructions provided in the package insert (Table 1). Out of 25 "OROV detected" serum samples 24 were tested positive with the LDT test and the RealStar® Oropouche Fever RT-PCR Kit 1.0: It was possible to identify the presence of amplification curves (FAM), with Ct's up to 35, and the IC target (JOE). One sample was tested discordant.

25 out of 27 "not detected" samples gave concordant negative results with both assays. Two samples were tested invalid (Table 2a).

Discussion

The execution of the test demonstrated that the RealStar® Oropouche Fever RT-PCR Kit 1.0 is considered to be compatible with the equipment available in the LACEN ES technology park, presenting amplification curves suitable for the OROV target in the samples evaluated.
In two samples the internal control of the RealStar® Oropouche Fever RT-PCR Kit 1.0 failed or was heavily inhibited. The results of these 2 samples were defined as invalid. Including the invalid sample results, a sensitivity of 96% and a specificity of 93%, excluding the two invalid sample results, a sensitivity of 96% and a specificity of 100% was calculated for the RealStar® Oropouche Fever RT-PCR Kit 1.0 in comparison to the results generated with established LDT at LACEN ES (Table 2a and b).

Conclusion

Despite the divergence in one result, the RealStar® Oropouche Fever RT-PCR Kit 1.0* was found to be compatible with the routine tests performed in the laboratory and is therefore a useful tool for rapid diagnosis of Oropouche fever infections.

Table 1: Result interpretation for the RealStar® Oropouche Fever RT-PCR Kit 1.0.

Oropouche Virus	Internal Control	Negative Control	Positive Control	Interpretation
+	+/–	–	+	Oropouche virus detectable
–	+	–	+	Oropouche virus Not detectable
–	–	–	+	Experiment failure
+	+	+	–	Experiment failure

+: Amplification curve present

–: Absence of amplification curve

Table 2a: Result overview: All sample results

OROV (n=52)		LDT LACEN ES	
		pos	neg
RealStar®	pos	24	(2**)
	neg	1	25

(**) Invalid, Internal Control failed after extraction and re-extraction in RealStar® Oropouche Fever RT-PCR Kit 1.0

Table 2b: Result overview excluding invalid sample results

OROV (n=50)		LDT LACEN ES	
		pos	neg
RealStar®	pos	24	0
	neg	1	25

Calculation of diagnostic sensitivity and diagnostic specificity, based on Table 2b

		95% confidence interval
Sensitivity	96.00%	79.65% to 99.90%
Specificity	100.00%	86.28% to 100.00%

Notice: *The RealStar® Oropouche Fever RT-PCR Kit 1.0 is for research use only (RUO).

Conflits d'Intérêts

Le kit RealStar® Oropouche Fever RT-PCR Kit 1.0 a été fourni gratuitement par altona Diagnostics ; altona n'a eu aucun rôle dans la conception de l'évaluation, la réalisation des tests, l'interprétation et la diffusion des résultats.

Remerciements

Une partie de réactifs utilisés lors de cette évaluation a été fournie par EVAM (European virus archive-Marseille, https://evam.european-virus-archive.com/). Le panel EEQ OROV a été fourni par l'EURL-PH-VBV (EU Reference Laboratory for Public Health on Vector-borne Viral Pathogens).