Sociedad Argentina de Microbiología General

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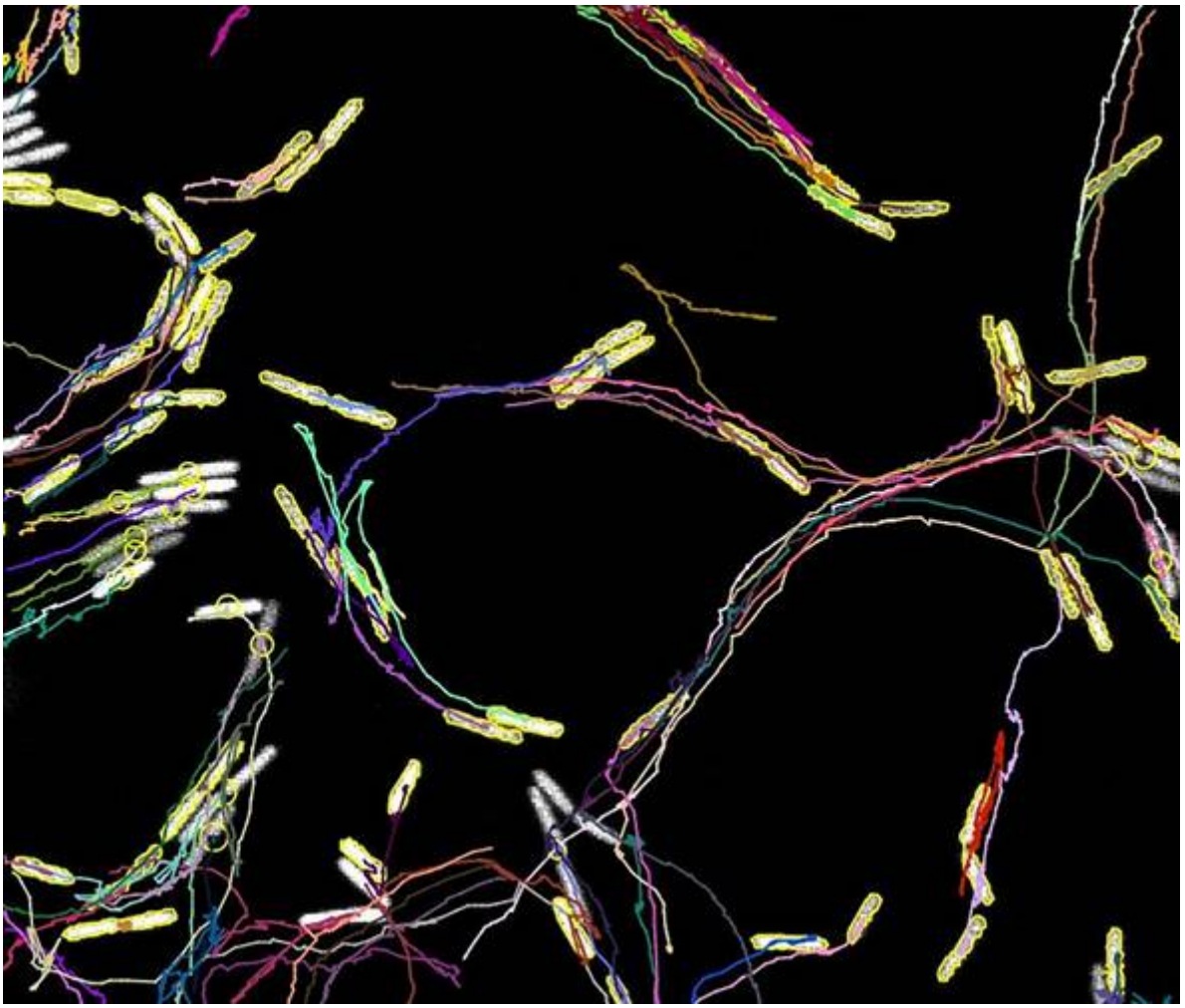

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

LOCAL DEVELOPMENT OF A DENGUE SEROTYPE-SPECIFIC DETECTION METHOD BASED ON LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR EPIDEMIOLOGICAL OUTBREAKS USING PUBLIC DOMAIN TECHNOLOGIES

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Dengue virus (DENV) is a highly relevant pathogen for public health in the northern and central areas of Argentina, as well as in tropical countries. Although rapid diagnostic tests are available, these are not usually sensitive enough and do not identify among DENV serotypes. Being able to identify DENV serotype has implications for case management and epidemiological purposes. Furthermore, access to validated tests remains difficult or cost-prohibitive in countries where this virus is endemic. Particularly, in the context of the 2023-2024 outbreak in Argentina, the lack of diagnostics tests was evident, as many of the available kits were hard to obtain due to accessibility or cost issues. To meet the demands during regional epidemiological outbreaks, we have decided to optimise a diagnostic method, based on reverse transcription followed by loop-mediated isothermal amplification (RT-LAMP) using exclusively public domain technology. This will allow not only to improve diagnostic accuracy and clinical management of dengue but also will enhance our country's technological independence through the local production of essential reagents and reducing the need for imported supplies. Acquiring these tools is important as it will facilitate the implementation of accessible solutions and provide the necessary know-how to adapt technologies to other local needs, especially in public health emergencies. In this study, a proof of concept for the LAMP reaction was conducted for the detection of each DENV serotype. The activity of an open-source and codon-optimised Bst-LF DNA polymerase enzyme (the polymerase usually used in LAMP reactions), which was expressed and purified in our lab, was compared with a commercially available enzyme. These preliminary tests were carried out using plasmids encoding for the genome of each DENV serotype (DENV1-4). In these assays, the activity of the in-house produced enzyme was analysed using serotype-specific LAMP primers. The results showed detection of DENV1, DENV2, and DENV3 serotypes when using the in-

house purified enzyme, and its efficiency was comparable to the commercial enzyme. The amplification efficiency for DENV4 was not optimal under the tested conditions, even with the commercial enzyme, which would indicate the need of primer optimization or redesign. Finally, dilutions of DENV1 plasmid DNA were performed to estimate the technique's limit of detection (LOD), reaching approximately 9000 copies per ml of serum. Given that low viremia is around 1000-10000 copies/ml, it is possible to conclude that the proposed technique has significant potential as a diagnostic tool.

Palabras clave: DENV – diagnostics – LAMP - open source bioreagents - serotype-specific