Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

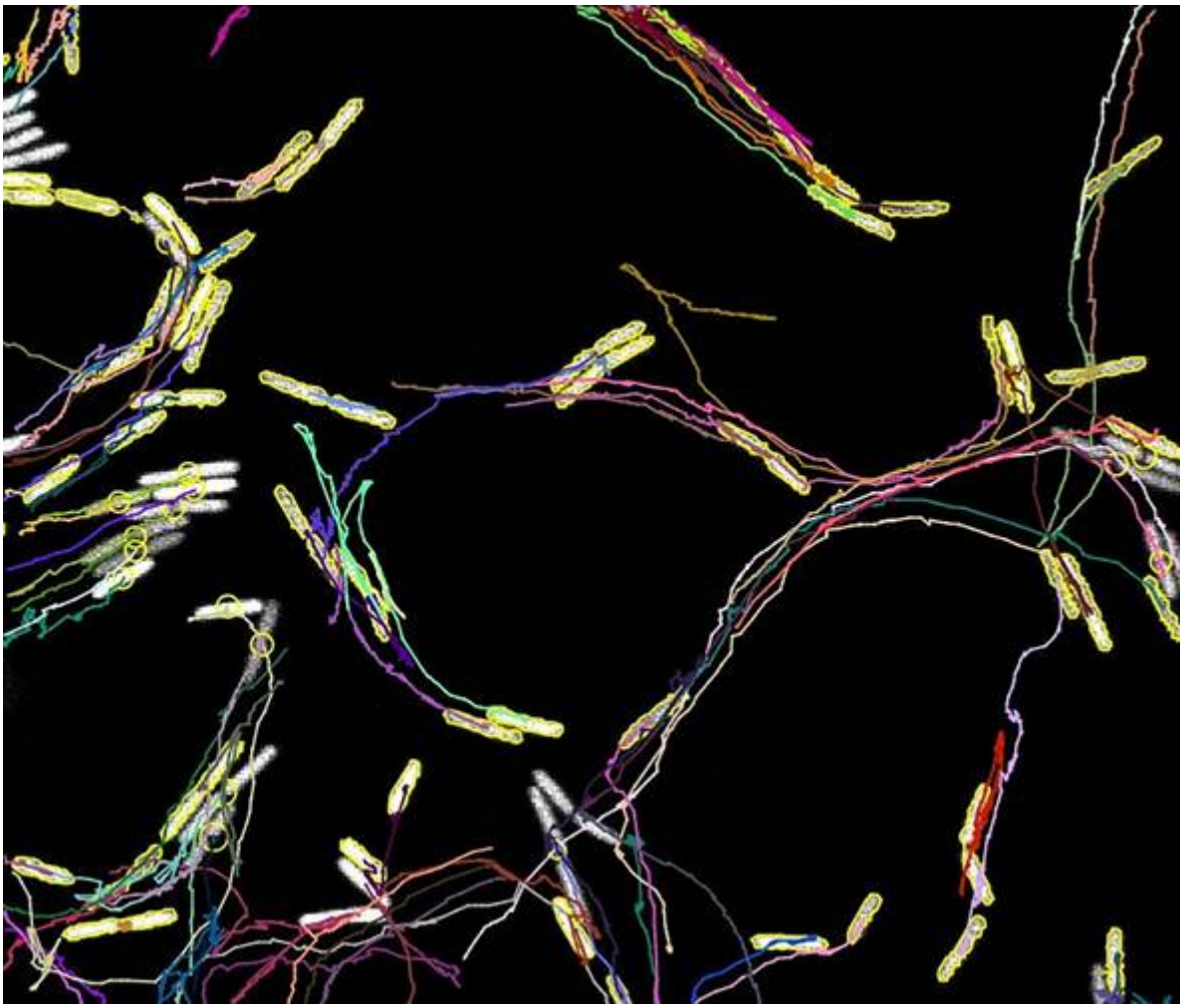

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

DEVELOPMENT OF A LAMP-BASED DIAGNOSTIC METHOD FOR THE UNIVERSAL DETECTION OF *Escherichia coli* ENTEROHEMORRHAGIC (EHEC).

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Enterohemorrhagic *Escherichia coli* (EHEC) is a zoonotic pathogen responsible for a severe disease characterized by hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS). Early and accurate detection of this pathogen is crucial for preventing clinical complications and ensuring food safety. Current diagnostic status shows that available methods present limitations, mostly concerning the impaired detection of the less frequent EHEC serotypes. Consequently, in this work, we focus on the development of a novel diagnostic tool based on Loop-Mediated Isothermal Amplification (LAMP), using the *ecf1* gene as a universal molecular marker allowing the detection of all relevant EHEC serotypes. The LAMP technique enables the rapid and specific amplification of bacterial DNA under isothermal conditions, making it ideal for Point-Of-Care Tests (POCT), particularly suitable in resource-limited settings. In addition, we aimed to develop a colorimetric detection system to easily visualize LAMP results, further directing its application as a POC test. To set the LAMP test conditions, assays were initially performed in a real-time PCR device, using DNA intercalating dyes to monitor the reaction in real time. To specifically amplify an ecf1 fragment, we designed and tested a set of six primers using the PrimerExplorer software. We further tested two different enzymes with strand displacement activity and adjusted several factors, such as buffer composition, and primers, Mg^{2}?, and dNTPs concentration. The addition of additives such as DMSO, the reaction temperature, and the time for amplification product appearance in positive controls were also evaluated. As a result, we established a specific LAMP reaction for *ecf1* which demonstrated to be capable of detecting up to 60 femtograms of the target gene within approximately 30 minutes, being highly sensitive and comparable to conventional techniques such as PCR and qPCR. Furthermore, we evaluated different dyes that respond to chemical changes during the reaction, such as pH decrease, free Mg^{2}? concentration reduction, and magnesium pyrophosphate precipitation. The tests were performed either in a thermocycler or a lab thermoblock to guarantee constant incubation temperatures. Dye concentration and color development time were optimized to obtain maximum signal clarity and sensitivity. Preliminary results indicate that the colorimetric method allows the visual detection of successful amplification in LAMP reactions. This suggests that the LAMP test we are currently developing can be an effective tool for detecting EHEC in clinical, veterinary, and

environmental samples, with potential applications in preventing HUS outbreaks. In conclusion, this method not only might offer a rapid and effective alternative for EHEC detection but also would be suitable for the implementation of preventive measures in the food chain and livestock management.

Palabras clave: Enterohemorrhagic Escherichia coli - Hemolytic Uremic Syndrome - LAMP - POC diagnostics - Colorimetric LAMP.