biosurfit

specific detection of nucleic acids for point-of-care diagnosis of tropical diseases

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Summary

Development of a test for rapid diagnosis of febrile syndromes caused by infectious diseases. This test utilizes isothermal amplification techniques specifically Recombinase polymerase amplification (RPA) in combination with nucleic acid detection through fluorescence microscopy to visualize the amplification products. The developed procedures for sample processing, amplification, and detection will be implemented on a disc from the spinit platform, which includes microarchitectures designed for handling small volumes of blood.

Methods & Results

Instrumentation: A bench top prototype for fluorescence reading was preliminarily developed. Filters compatible with the fluorescent molecules used to detect the amplified product were used. No autofluorescence was detected in the material commonly used in spinit platform discs. The possibility of integrating different filters to detect several fluorophores and thus having a multiplex assay was also considered.

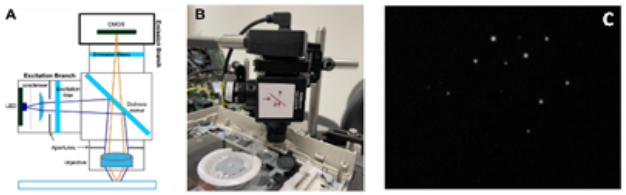

Fig 1 – A) Schematic of the fluorescence microscope prototype. B) Fluorescence microscope prototype. C) Fluorescent beads observed in the fluorescence microscope prototype.

Software: In order to be able to integrate into the spinit platform, an algorithm was developed specifically designed to control the spinit and the rotation of the disc necessary for the performance of the automated microfluidic system. An algorithm for counting fluorescent events was also developed, with the following requirements: 1) Counting based on brightness level (blue squares seen in Fig 2), 2) Exclusion rules (red squares seen in Fig 2) based on size minimum/maximum and minimum brightness.

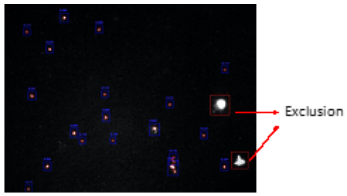

Fig 2 – Example of image processing by the developed algorithm. Blue squares are considered positive signals and red squares identify objects excluded from fluorescence analysis.

Disc: In the work of integrating a disc into the spinit platform, microfluidic structures were developed and some enable placing membranes, which will allow the detection of fluorescence both in the membrane and in solution. The possibility of detecting several targets in parallel was also designed and for this purpose microfluidic layouts were developed with the aim of carrying out multiplex detection.

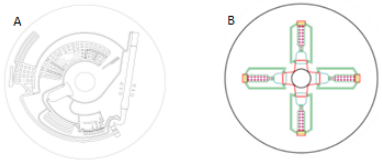

Fig 3 – Disc for detecting: A) One target B) Four targets.

Performance assessment: Short DNA sequences (10-30 nucleotides) with the necessary modifications for capture and detection across membranes were used to allow a preliminary assessment of the platform's performance. The two synthetic sequences are complementary to each other and represent the product of an amplification. Modifications to the 3′ end with characteristics appropriate to the tested membrane were included so that one side was captured on the membrane surface and the other side was marked by the fluorophores (Fig 4). Two different membranes were studied and characterized. After calibration, preliminary tests were conducted where the DNA was amplified using the RPA isothermal technique and the amplified product was detected using the membrane. Only the membrane that demonstrated the best detection limit was used.

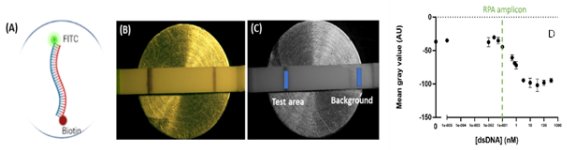

Fig 4 – A) Schematic of detection molecule used as a control for testing the membranes. B) Membrane detection of the control solution. C) Microscopy image processing and identification of areas of analysis. D) Result of amplification by RPA from an initial target DNA concentration of 5 pM. After amplification, the membrane detected the amplified product and using the calibration curve it was possible to conclude that, after DNA amplification for 15 min at 37 ℃, 100 pM of DNA was .detecte

Conclusions

The results achieved so far with this project allow us to predict the expansion of the potential and versatility of the spinit® platform, positioning biosurfit at the forefront of the development of "point-of-care" diagnostic solutions in the field of infectious diseases that can be detected through isothermal nucleic acid amplification techniques, opening up the molecular diagnostics market as a possibility. While the results achieved demonstrate the feasibility of detecting genetic material in a sample, there remains room for further optimization and refinement in the integration of the entire testing process.