Project funded by the Horizon 2020

Grant Agreement no. 829010

Call: H2020-FETOPEN-2018-2020
Topic: FETOPEN-01-2018-2019-2020
Type of Action: RIA (Research and Innovation action)
Name of Lead Beneficiary: CSIC, Spain
Project Start Date: 1st May 2019
Project Duration: 54-Months

DELIVERABLE 3.3:
Validated prototype of the IVD chip

Due date of Deliverable:	31/10/2023
Actual Submission Date:	**31/10/2023**
Responsible partner:	UNIZAR
Report Author(s):	Gabriel Alfranca, Lorena Ceamanos, Carlos Sánchez-Somolinos, Jesús M de la Fuente, Raquel Arroyo, Claudia Olaizola, Maria Sainz, Rosa Monge, Ignacio Ochoa
Type1[1]:	R
Dissemination Level[2]:	PU

[1] **Type**: Use one of the following codes (in consistence with the Description of the Action):
- R: Document, report (excluding the periodic and final reports)
- DEM: Demonstrator, pilot, prototype, plan designs
- DEC: Websites, patents filing, press & media actions, videos, etc.
- OTHER: Software, technical diagram, etc.

[2] **Dissemination level**: Use one of the following codes (in consistence with the Description of the Action)
- PU: Public, fully open, e.g. web
- CO: Confidential, restricted under conditions set out in the Model Grant Agreement
- CI: Classified, information as referred to in Commission Decision 2001/844/EC

DELIVERABLE D3.3: *Validated prototype of the IVD chip*

Table of Contents

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

1. DOCUMENT HISTORY

Version	Date	Authors/ who took action	Comment	Modifications made by
0.1	25-10-2023	Ignacio Ochoa (UNIZAR), Gabriel Alfranca, Lorena Ceamanos (CSIC), Rosa Monge (BOC)	First draft sent to Carlos Sánchez Somolinos (CSIC)	Carlos Sánchez Somolinos (CSIC)
0.2	30-10-2023	Ignacio Ochoa (UNIZAR)	Second draft sent to all IPs	Carlos Sánchez Somolinos (CSIC)
0.3	31-10-2023	Ignacio Ochoa (UNIZAR)	Final draft sent	
1.0	31-10-2023	Carlos Sánchez Somolinos (CSIC)	Submitted to Commission	

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

2. LIST OF ABBREVIATIONS

CEA: Carcinoembryonic Antigen

ELISA: Enzyme-Linked Immunosorbent Assay

IR: Infrared

IVD: In-Vitro Diagnosis

POC: Point-of-care

WP: Work Package

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

3. DELIVERABLE DESCRIPTION AND SUMMARY

This document delineates the endeavors of the consortium in substantiating the efficacy of valves produced in the context of Work Package 2 (WP2) when employed in a real operational milieu tailored to the in vitro diagnostic (IVD) application for the detection of Carcinoembryonic Antigen (CEA) inside our integrated fluidic platform.

.

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

4. INTRODUCTION

4.1 General introduction of IVD in PRIME

In vitro diagnostics (IVD) constitute a category of medical devices utilized for the analysis of human specimens, such as blood, urine, tissue, and other bodily fluids, outside of the living organism. In vitro diagnostics encompass a diverse array of tests and procedures specifically engineered for the detection, diagnosis, and monitoring of various medical conditions and diseases. These products may be employed by qualified healthcare personnel within healthcare facilities, or, owing to their user-friendliness and ease of operation, they can also be used by the general public. Those lasts ones are known as point of care (POC) devices.

Point-of-care (POC) devices refer to diagnostic tests performed in close proximity to the patient, often situated outside the conventional clinical laboratory environment. These tests are designed to yield prompt results, facilitating immediate clinical decision-making. Notable examples of such tests include pregnancy tests and assays for the detection of COVID-19 antigens. Most of these tests are founded upon lateral flow technology, wherein a sample is applied to a paper-based test device, and results are displayed within a matter of minutes.

Despite the numerous advantages associated with lateral flow technology, certain analyses remain unfeasible with this approach. One of the primary limitations arises when assays necessitate the inclusion of more than one liquid.

In our particular case, we have developed an IVD test integrated into our fluidic control platform, incorporating valves developed within Work Package 2 (WP2), for the detection of Carcinoembryonic Antigen (CEA). This test involves the use of three distinct liquids and serves as an illustrative example of future, more advanced developments while upholding the technique's portability.

4.2 Objectives

The principal objective of this project deliverable is to provide an account of the validation procedures that have been conducted to demonstrate the functionality and efficacy of the microfluidic platform developed within the PRIME project. This platform has been designed with the specific intent of detecting Carcinoembryonic Antigen (CEA) antigens through the integration of an Enzyme-Linked Immunosorbent Assay (ELISA) test with a microfluidic device equipped with fluidic control elements.

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

5. VALIDATION OF THE TECHNOLOGY IN A REAL APPLICATION

5.1 Definition of specifications, design and fabrication of the final IVD integrated platform

Although a modular solution was initially chosen, the final objective of PRIME has been the integration into a platform of both the control module (valve) and the IVD module. After defining the specifications, the final platform design has been created and developed. It is composed of different layers of materials that allow us to define both the fluidic inlets/outlets and the channels and the treated channel of the sensing unit, in addition to the integration of the active valve developed on this same platform.

6. RESULTS

As previously indicated in the preceding reporting period, the new channel models demonstrated promising outcomes, including enhanced reproducibility. Nonetheless, the concentrations of the analyte detected still did not approach the clinically relevant range established for our model analyte, Carcinoembryonic Antigen (CEA).

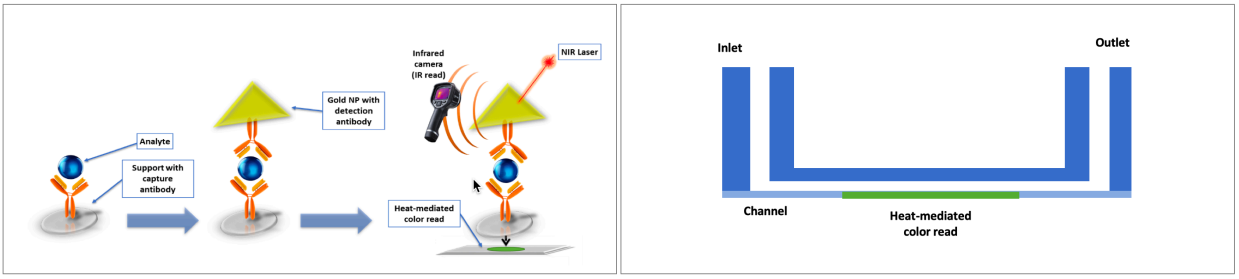

Figure 1, (left) PRIME nanoparticle-based sensor with a optical thermal transducer and (right) channel-like sensor configuration.

During the last part of the project, we have optimized the blocking methods and flow assays to enable parallelization of experiments on the 3-channel microfluidic chips. Additionally, we have explored the limits of detection for the bioassay employing the first-generation 3-channel chips.

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010

Upon the successful optimization of these steps, the experiment was transitioned to the integrated microfluidic platform, which incorporates control modules and sensing units. This novel design has the advantage of reducing the required reagent volumes, thereby allowing an increase in reagent concentrations without increasing overall costs.

Final results in the integrated platforms revealed, despite the reduction in irradiation power and exposure time, a substantial signal captured by the infrared (IR) camera, with discernible marks on the thermographic paper. No false positives were observed in the negative control samples, as neither the IR camera nor the thermographic paper detected any heat surges. Control sample distribution consistently exhibited minimal variability, indicative of a robust measurement process. As anticipated, the prototypes loaded with CEA solutions exhibited higher signals corresponding to increased analyte concentrations.

7. CONCLUSIONS

This project has made significant advancements in the integration of new fabricated light control valves in microfluidic platforms capable to perform complex bioassays with 3 different liquids.

PRIME has received Funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 829010