1. Publishable summary

The aim of the NANO-MUBIOP project is to develop a quantitative, high sensitivity, multiplexing bioassay method to detect a target biological system (as for example HPV, test-bed system for the project) by means of the use of non biological particles in the nanometre size range. The interaction between non biological nanoparticles and the target biological system allows the detection of the target without any DNA amplification stage, thus leading to enhance diagnostic capabilities and overcoming many of the limitations of the existing methods (Fig. 1).

In order to start the development of the new device, the NANO-MUBIOP partners firstly defined the technical requirements and specification of the method. In order to support the future industrialization and commercialization of the new NANO-MUBIOP device, the project partners also reviewed the FDA regulatory procedures. An *in silico* analysis of HPV genome for the identification of the conservative and variable regions of the DNA sequences of each of the 120 different HPV genomes was carried out. Subsequently, a collection of designed specific and generic bio-probes for the HPV virus genotypes has been made (Fig. 2). The non-bio substrate (Zeonor) where probes should be printed was also defined and optimized, as well as the array (Fig. 3) and the micro-disposing system (Fig. 4) and the related jettable fluid. Silica nanoparticles were chosen as the most suitable particles for the project. Silica nanoparticles with a diameter range of 80 to 120 nm for the size determination were then produced, and they were then successfully functionalised with carboxyl (COOH) groups to allow conjugation to DNA sequences.
The complete design, construction and characterization of a laboratory prototype and a miniature-scale prototype of the optical system for nanoparticles detection were performed, as well as the robotic components and fluidics (Fig. 5). During the first half of the project a mathematical model has been developed in the mesodynamic range of docking involving long DNA sequences and bio-targets. Parameters resulting from such calculations have been used for choosing the right parameters in the experimental nanoparticle and substrate functionalisation (Fig. 6-7).
An *in vitro* testing activity has been also made on synthetic targets aiming to improve sample preparation and test conditions on biological samples. Different nanoparticles (in terms of size, materials, density, and charge) and also different oligonucleotides (in terms of length and also presence of poli-N) have been also tested. During the first 18 months of the project a technique based on magnetic particles as the most suitable DNA extraction methodology to be applied in the preparation of the samples for the NANO-MUBIOP platform has been also defined (Fig. 8). During the first period of project, several dissemination activities were developed. A project website was created (www.nanomubiop.eu) and updated regularly. During the first period of the project an Ethical and Medical Board was set up with 3 external ethical experts with the scope of provide ethical and medical evaluation of the results achieved as well as of the activity performed.
The first expected impact of the NANO-MUBIOP project is to introduce substantial innovations in biochemical analysis for the medical sector, with a new multiplexing methodology that will pave the way for future diagnostics applications. Regarding more specifically HPV, the NANO-MUBIOP method will indeed offer a cost-effective solution for vaccine validation community. In terms of economic impact, a strong economic impact for the NANO-MUBIOP tool manufacturer is expected, as well as for the public/private national or local health service providers: The NANO-MUBIOP tool will also allow the patients to get genotyping at much lower prices, even lower than for the current combination of liquid PAP+HPV DNA-HC2. Moreover, the time to genotyping the sample will be the same as for a PAP test.

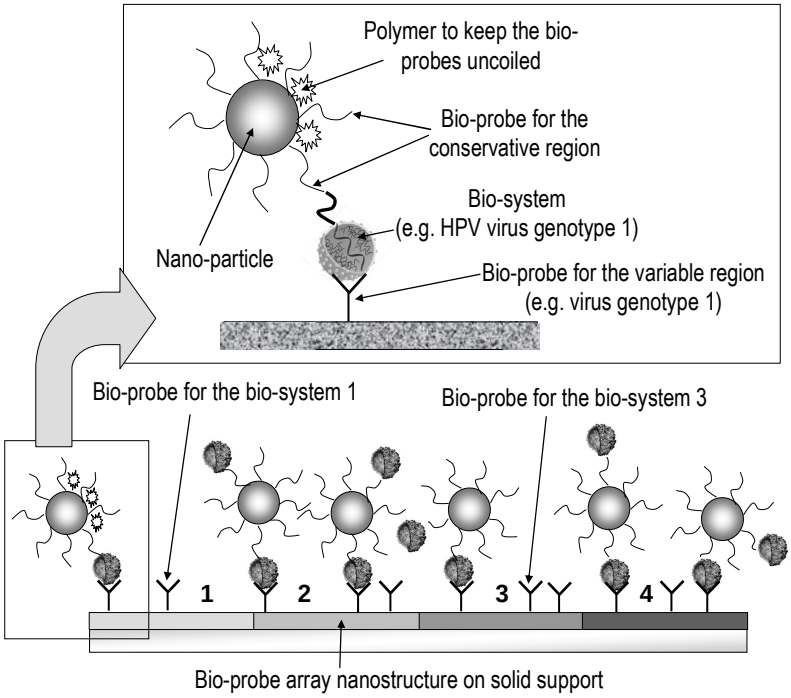


Figure 1: Schematic representation of the NANO-MUBIOP method

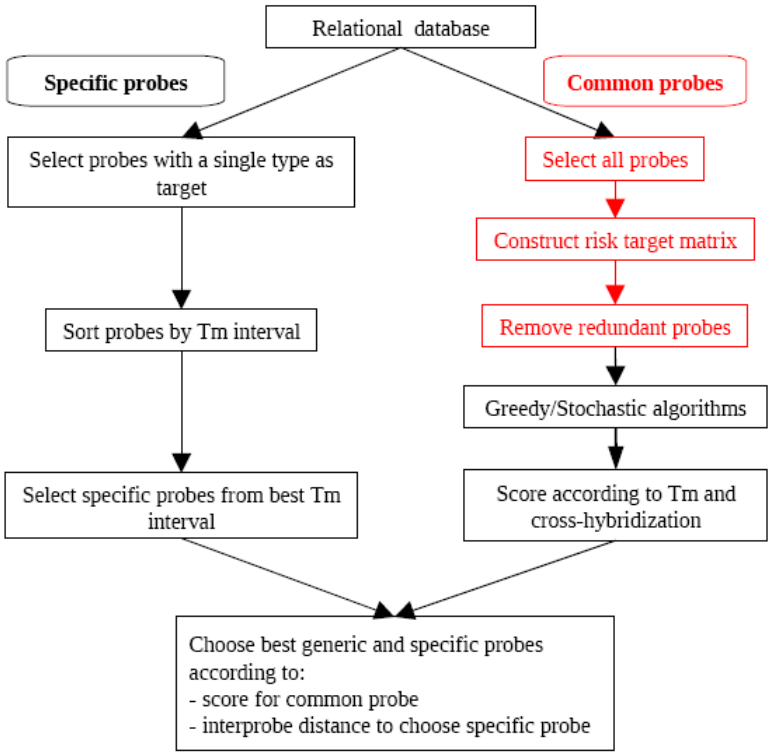


Figure 2: General procedure for the design of the specific and common probes

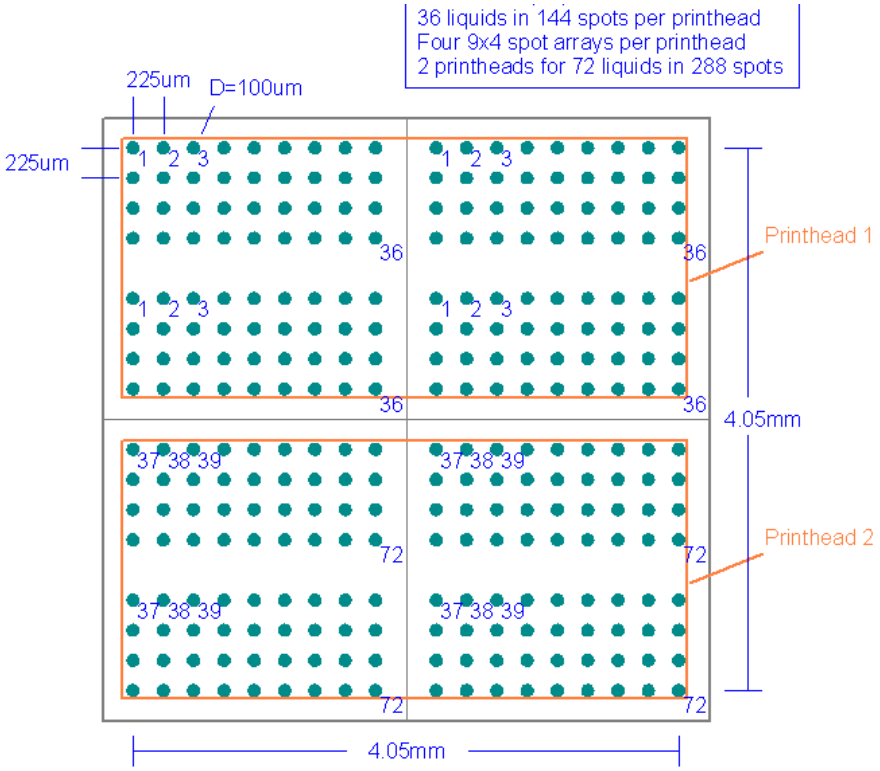


Figure 3: The final design of the array considered to print 288 spots in an array of 4 X 4 mm

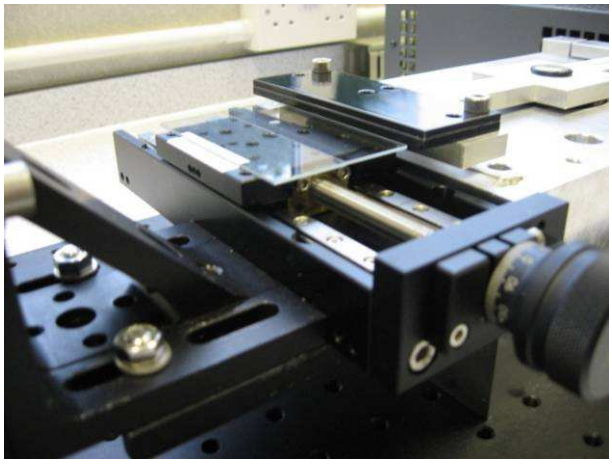


Figure 4: The micro-disposing system

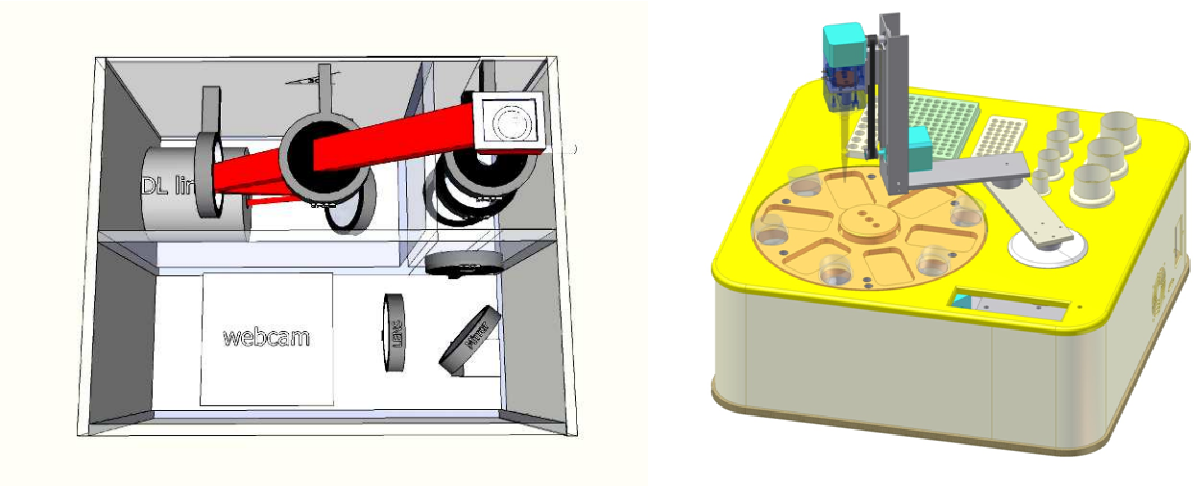


Figure 5: The design of the miniature-scale prototype for optical detection and the robotic liquid handling

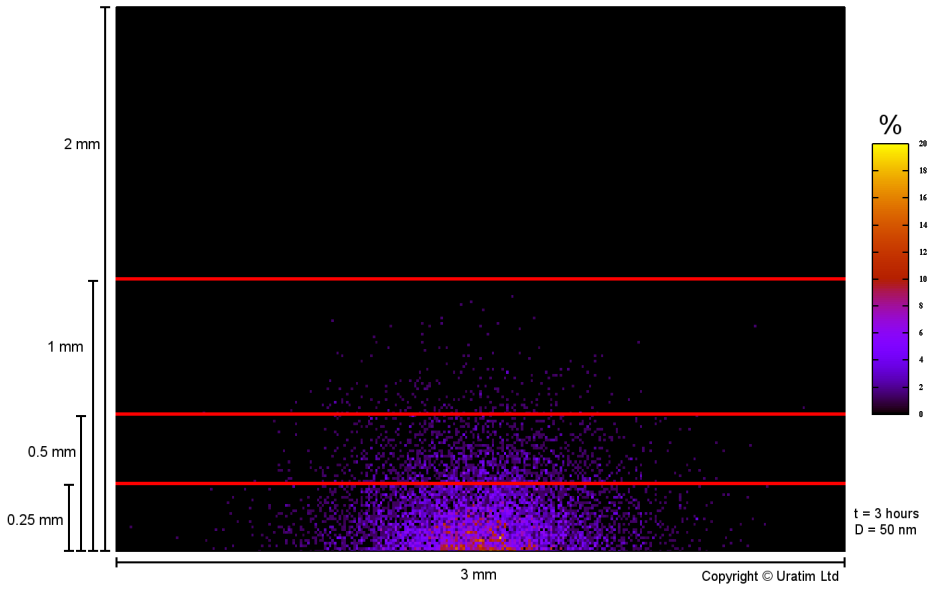


Figure 6: The incubation time dependence on the cuvette height. Timeframe is 3 hours, NP diameter is 50 nm, the colors correspond to the probability, that a particle at a starting position would dock to the target spot within the timeframe.

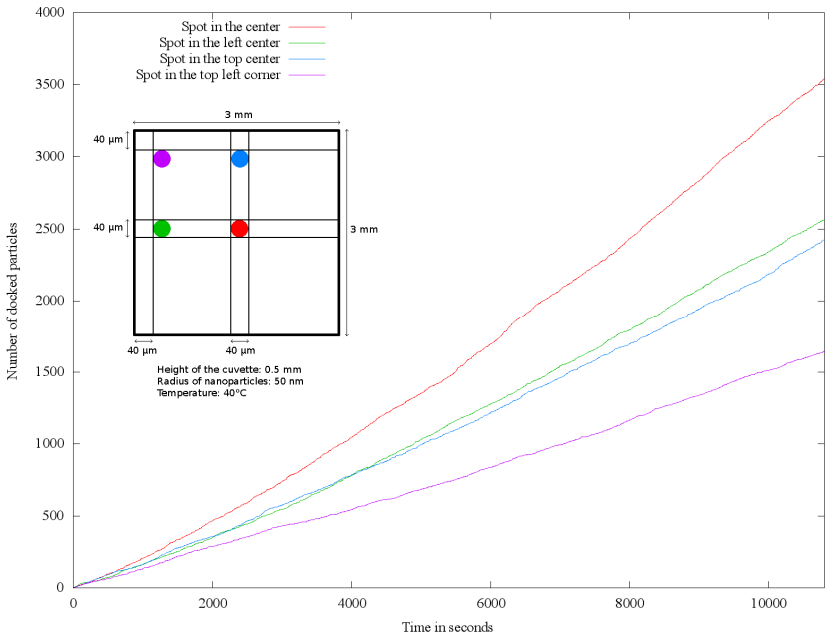
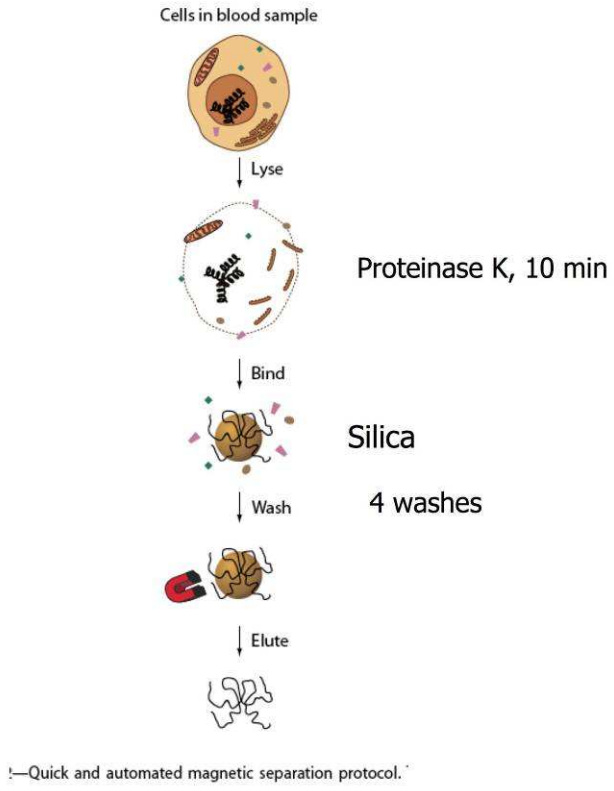


Figure 7: The effect of different geometric distribution of the spots to the incubation time



!—Quick and automated magnetic separation protocol.

Figure 8: DNA extraction methodology based on magnetic particles

Figure 9: NANO-MUBIOP Project logo