Stand-alone platform for time-effective early detection of sepsis

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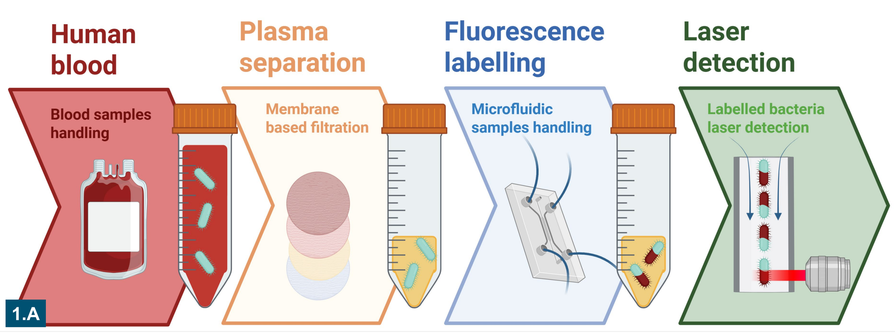
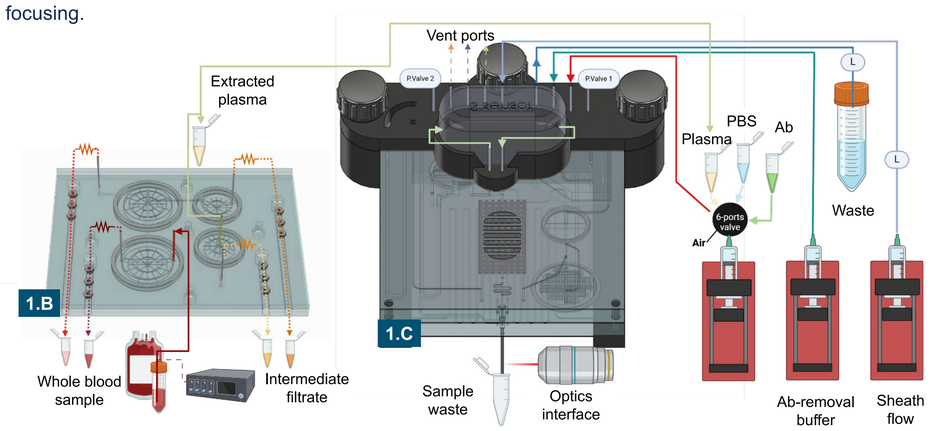
Sepsis is a fast-progressing and deadly condition that affects millions of people each year, making early detection essential. Traditional diagnostic methods are often slow and complex. To address this, we developed a stand-alone, automatized, cost-effective, and open-source platform for fast sepsis patient screening. This device features two microfluidic cartridges designed for liquid biopsy manipulation and filtration (cartridge 1), and antibody labelling and real-time detection of sepsis-related bacteria (cartridge 2) through a fluorescence reader.
The disposable microfluidic cartridges separate pathogens from whole human blood (4 mL), add labelling antibodies, remove air bubbles and unbound antibodies from the solution. Subsequently, labelled bacteria are focused into a 10 µm diameter flow stream for optical analysis. The fully automated process takes a total time of 40 minutes. This compact, low-cost microfluidic platform minimizes the required sample volume, and is targeted at a detection of bacteria at a concentration of 100 CFU/mL in whole blood. Moreover, the device can be interfaced with modern laser-equipped microscopes, accelerating detection and making it accessible to a wide range of optical instruments. In the future, we plan to use the platform for the real-time detection and quantification of cancer-released extracellular vesicles from liquid biopsy samples by implementing a dedicated CMOS photonic chip.

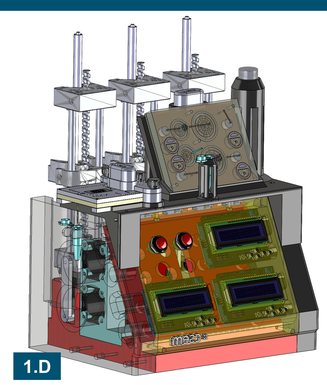
1. The goal: rapid separation and detection of bacteria, at low concentration, directly from whole human blood

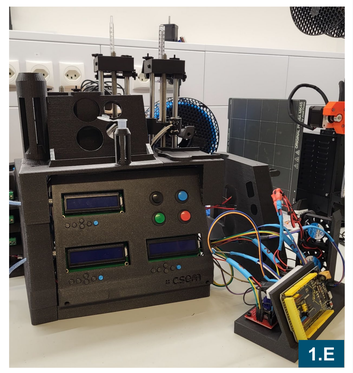


Process flow to isolate and quantify bacteria from whole blood samples (1.A). Bacteria are isolated through a cascade of membranes with controlled pore sizes. Then, bacteria are labeled with specific fluorescent probes and quantified using laser-based detection techniques.

The first microfluidic chip (1.B) extracts the plasma containing bacteria from whole blood samples. The second device (1.C) handles the sample dilution, antibody labelling, and samples' hydrodynamic flow focusing.



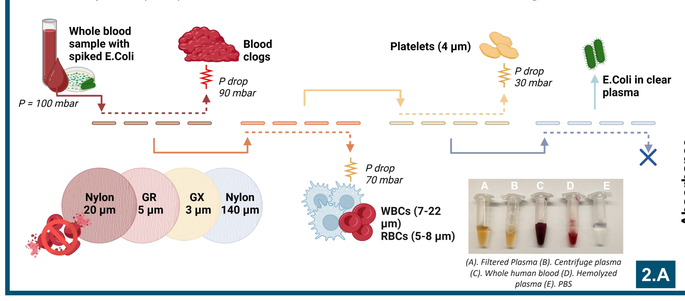


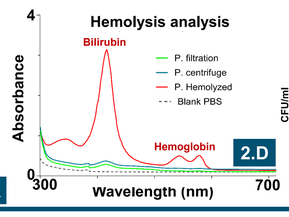


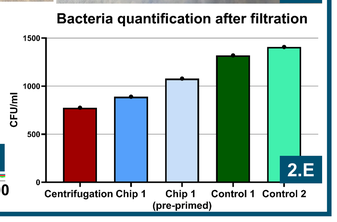
Stand-alone, programmable sample control platform housing both microfluidic cartridges (1.D). 3 syringe pumps and integrated servomotors control the flow rate and valves. The system is powered by 3 ATmega328P microcontroller boards, 3 TMC2208s, and a main motherboard (1.E).

2. Isolation of *E.coli*

Membrane-based bacteria isolation from whole blood sample (2.B, 2.C). Isolation is based on tangential flow filtration (TFF) limiting shear stress (2.A) and hemolysis (2.D). A specific pressure drop is set for each stage. A cascade of membranes enables precise particle size selection with high bacteria yield (2.E). Results are benchmarked with centrifugation.

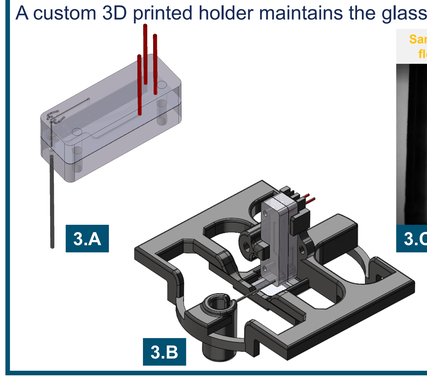


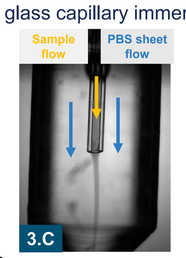


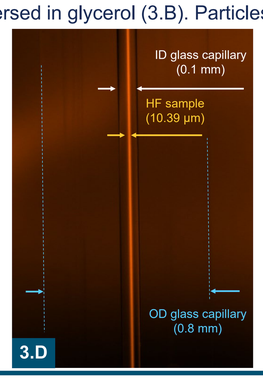


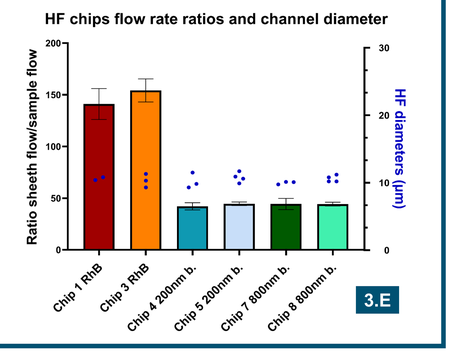
3. Sample hydrodynamic flow focusing

Microfluidic chip for sample hydrodynamic focusing (HF) (3.A). The cartridge implements a three-dimensional (3.C), sheath flow to focus the sample in the center of a glass capillary with a diameter of 10 µm. The focused sample is analyzed with a fluorescence detection system (3.D). A custom 3D printed holder maintains the glass capillary immersed in glycerol (3.B). Particles of different sizes can be effectively focused (3.E).



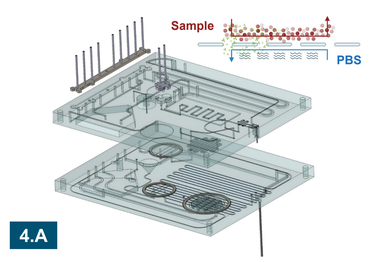


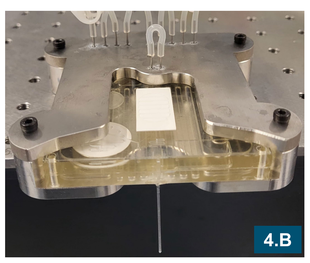


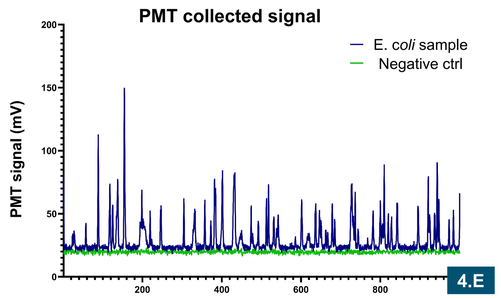


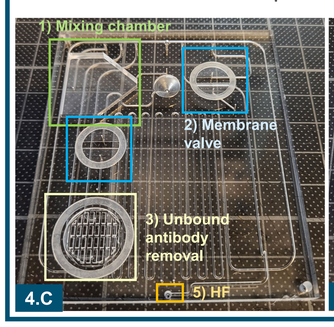
4. Bacteria quantification in whole human blood

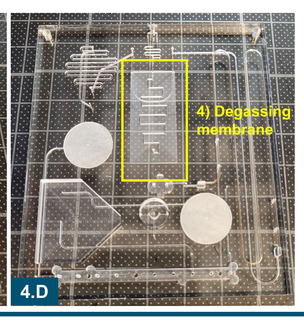
Quantification of isolated bacteria in filtered plasma samples using a microfluidic chip and laser detection. The cartridge (4.A, 4.B) is composed of five functional sub-modules. 1) bacteria are mixed with fluorescent probes. 2) a series of valves control the flow of the sample through the system. 3) removal of unbound labels, 4) removal of air bubbles that could interfere with detection. 5) the sample is focused into a glass capillary (4.C, 4.D). As bacteria pass through the laser beam, the fluorescent signal is captured by a photomultiplier (4.E). Signal peaks are counted and correlated to the bacteria over time (4.F).

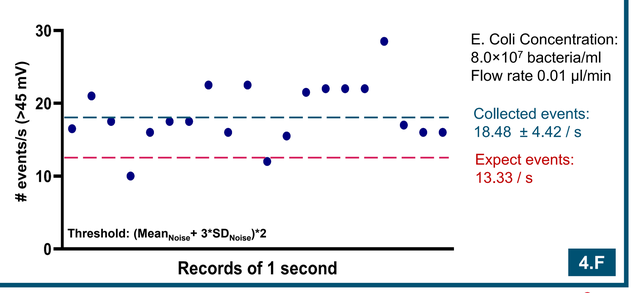












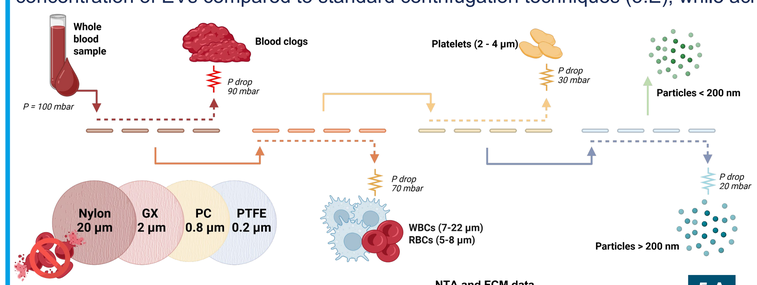
E. Coli Concentration:
8.0×10^7 bacteria/ml
Flow rate 0.01 µl/min

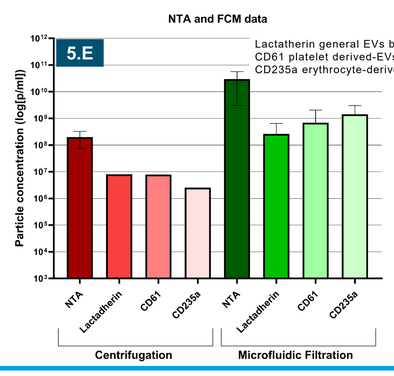
Collected events:
18.48 ± 4.42 / s

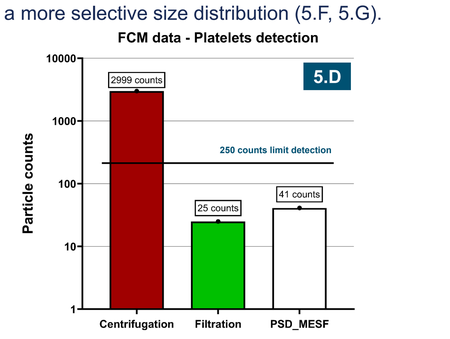
Expect events:
13.33 / s

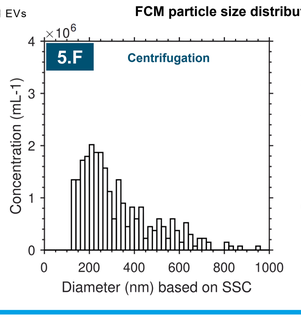
5. Future application: extracellular vesicle separation and detection

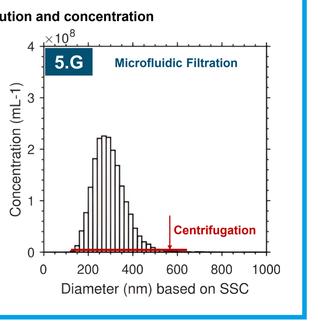
Application of the blood filtration module for extracellular vesicle (EV) separation from human blood aimed at isolating particles smaller than 200 nm from plasma samples (5.A, 5.B, 5.C), without hemolysis and platelet-free conditions (5.D). This method aims to maintain a higher concentration of EVs compared to standard centrifugation techniques (5.E), while achieving a more selective size distribution (5.F, 5.G).











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