FlowPad

a generic microfluidics platform for a wide range of applications

Remco den Dulk

Department of Technologies for Biology and Healthcare

INTRODUCTION

CEA

SCIENCE

FUNDAMENTAL RESEARCH (PHYSICAL AND LIFE SCIENCES)

TECHNOLOGIES

DEFENSE SECURITY

NUCLEAR ENERGY

KEY ENABLING TECHNOLOGIES

- **16,100 people**
- **10 Research Centers**
- **4400 M€ budget**
- **5844 patents**

REUTERS **«THE WORLD'S MOST INNOVATIVE RESEARCH INSTITUTIONS »**

TOP INSTITUTIONS | 2017 RANKINGS

1	Health & Human Services Laboratories	USA
2	**Alternative Energies and Atomic Energy Commission**	France
3	Fraunhofer Society	Germany
4	Japan Science & Technology Agency	Japan
5	National Institute of Advanced Industrial Science & Technology	Japan

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CEA-TECH

LETI


MICRO-NANO TECHNOLOGIES AND INTEGRATION IN SYSTEMS

LITEN


NEW ENERGY TECHNOLOGIES AND NANO MATERIALS

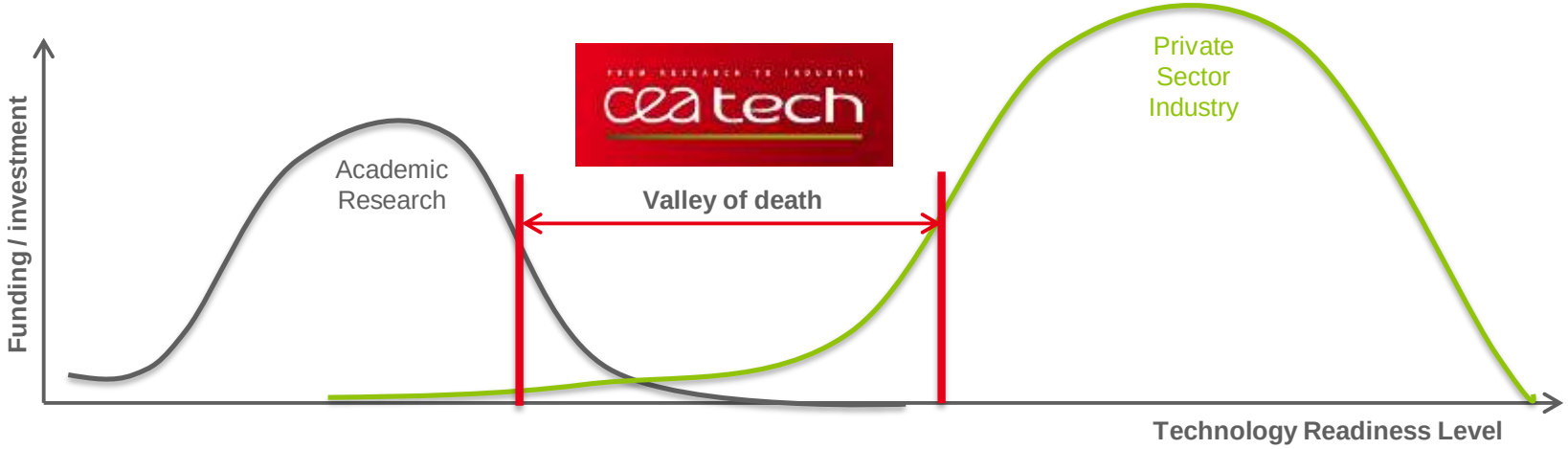
LIST


SOFTWARE-INTENSIVE SYSTEMS

PRTT


DISSEMINATE THE KETS DEVELOPED

- **4,500 people**
- **8 Local Centers**
- **650 M€ budget**
- **4299 patents**



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CEA-TECH

LETI — LITEN — LIST — PRTT


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SOFTWARE-INTENSIVE SYSTEMS


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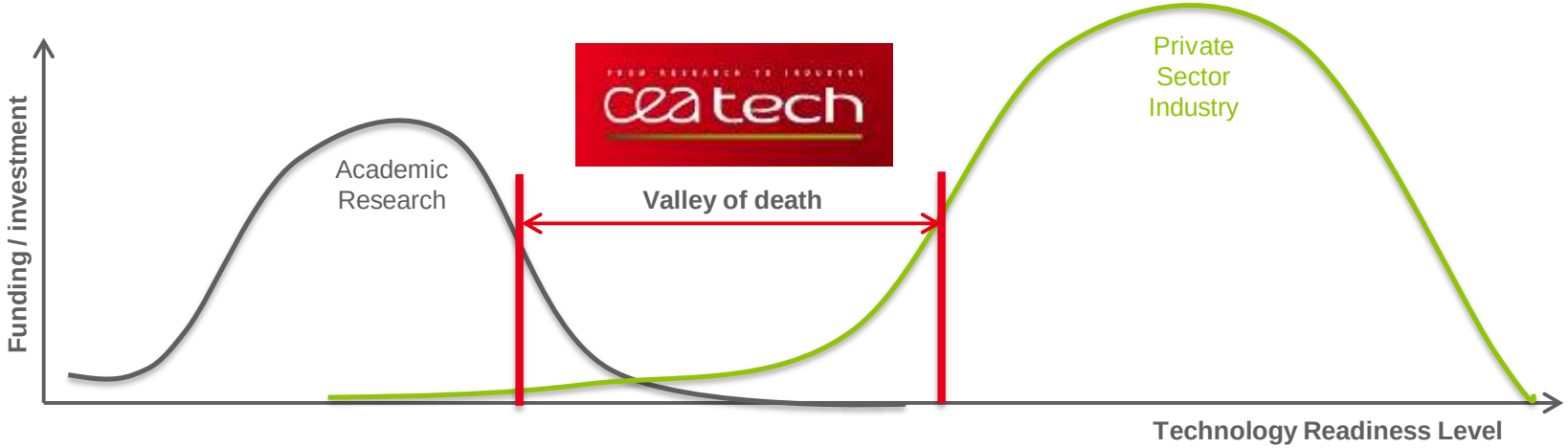
LETI

8 DEPARTMENTS WORKING ON MICRO / NANO TECHNOLOGIES AND SYSTEM INTEGRATION

- 1,900 people
- 2 International Leti Offices
- 315 M€ budget
- 2572 patents



INTRODUCTION

PREVENTION

DIAGNOSIS

LETI HEALTH

MONITORING

THERAPY

LETI

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- **Department of Technologies for Biology and Healthcare**
  - Detection technologies → electrical, optical, gamma & x-ray
  - Nanomedicine → drug delivery, nanoparticles
  - Lab-on-chip → microfluidics, (bio)chemistry, microfabrication

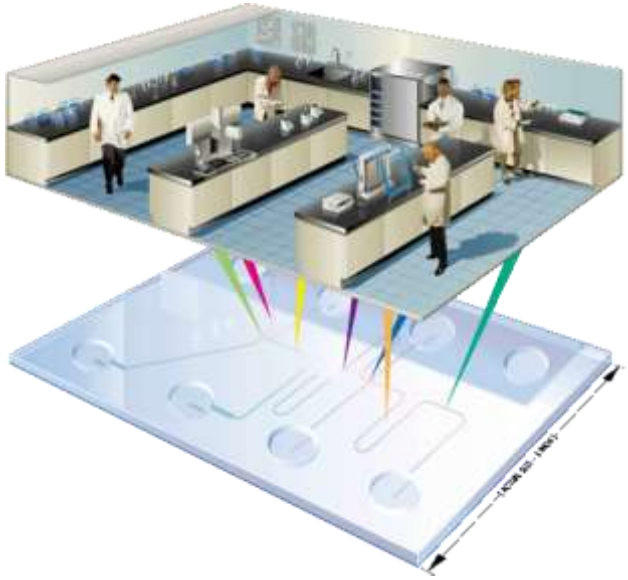
Multidisciplinary teams → One-stop shop for completely integrated systems

MICROFLUIDICS, AN ENABLING TECHNOLOGY

- **Miniaturization**
  - (trans)portable devices for use outside the lab
- **Automation**
  - easy to use for non-expert users
- **Time to result**
  - rapid relevant result

Sample-to-Answer

client with lab protocol



Lab-on-a-chip

CEA develops automation and integration in microfluidics

MICROFLUIDICS, AN ENABLING TECHNOLOGY

- **Miniaturization**
  - (trans)portable devices for use outside the lab
- **Automation**
  - easy to use for non-expert users
- **Time to result**
  - rapid relevant result

- **Challenges**
  - biological protocols can be very complex
  - applications are extremely diverse
  - many functions are required, hence very multidisciplinary

sample-to-answer sample-to-answer sample-to-answer sample-to-answer sample-to-answer sample-to-answer sample-to-answer sample-to-answer

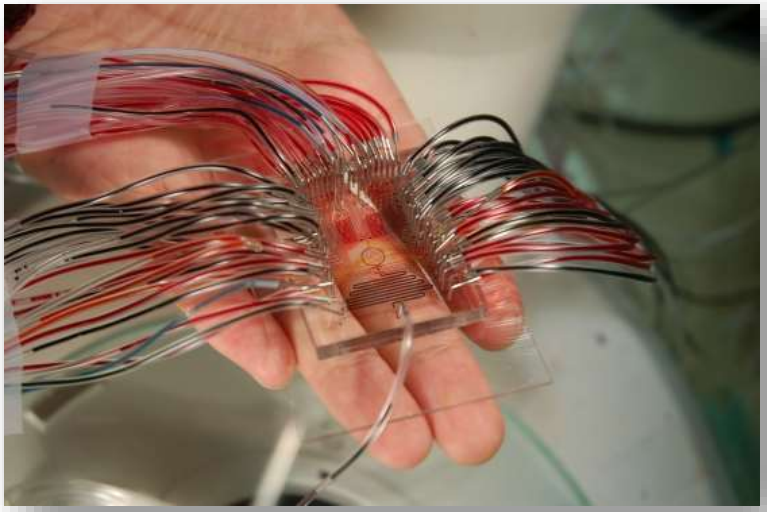
FLOWPAD – GENERIC MICROFLUIDICS PLATFORM

- **One instrument**
- **Various consumables**

enabling integration and automation of biological assays with a reduced development time

FLOWPAD – GENERIC MICROFLUIDICS PLATFORM

Avoid “chip-in-a-lab”



- **Connections:**
  - *220V*
  - *USB*
  - *compressed air*

FLOWPAD – GENERIC MICROFLUIDICS PLATFORM

- **Consumable:**
  - *fluidic channels*
  - *reaction chambers*
  - *integrated pneumatic valves and pumps*
  - *embedded reagents / particles*
  - *...*

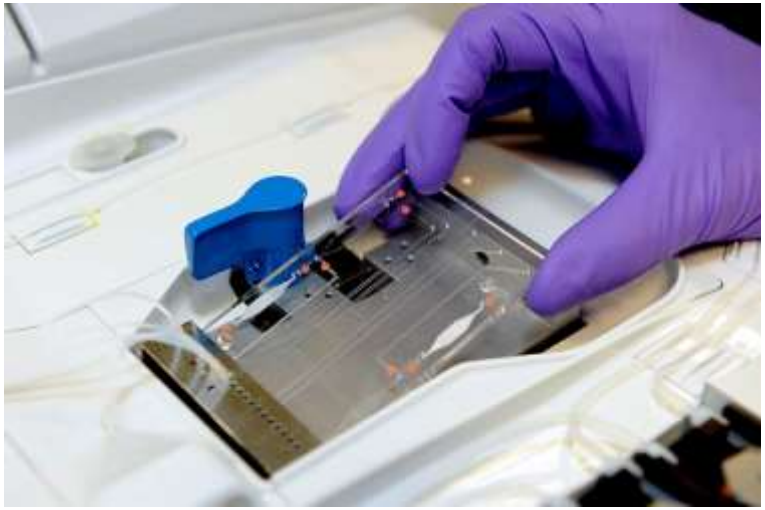


photo credit : L.Godart / CEA-Leti

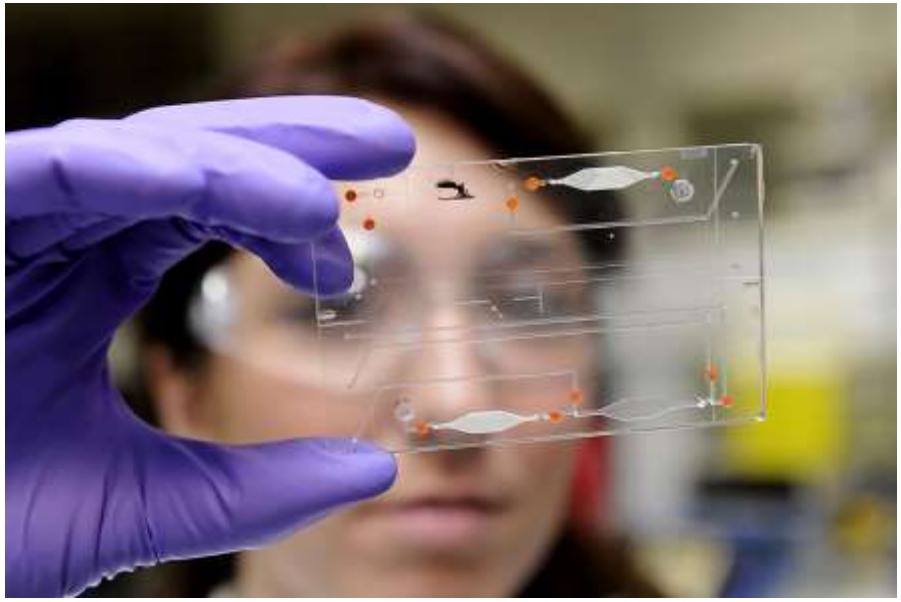


photo credit : L.Godart / CEA-Leti

- *standardized format (credit card)*
- *rapid prototyping by high-precision machining*
- *low-cost manufacturing by injection moulding*

FLOWPAD – GENERIC MICROFLUIDICS PLATFORM

- **Consumable:**
  - *fluidic channels*
  - *reaction chambers*
  - *integrated pneumatic valves and pumps*
  - *embedded reagents / particles*
  - *...*

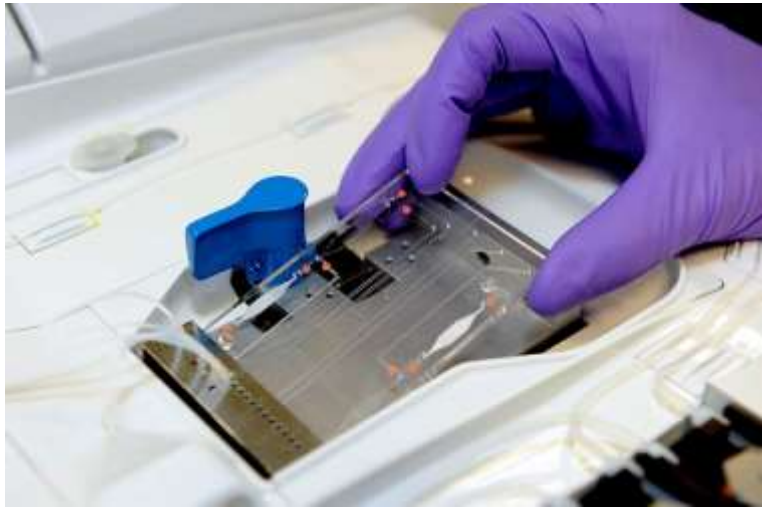
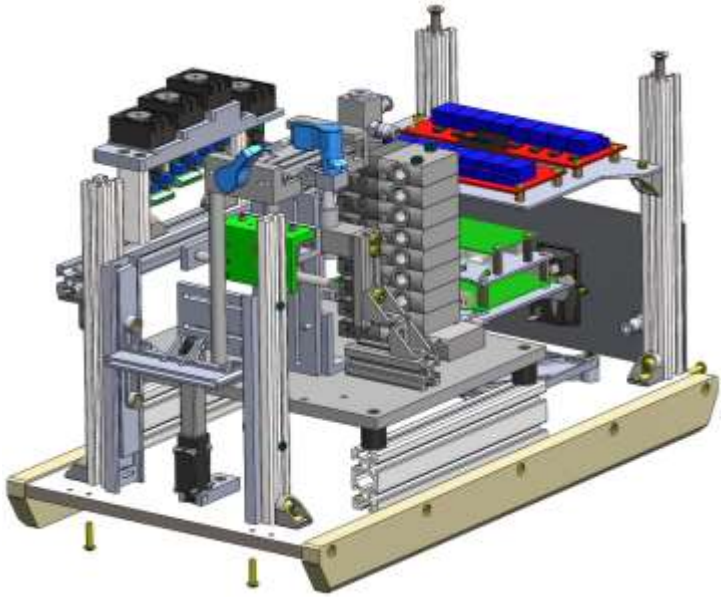


photo credit : L.Godart / CEA-Leti

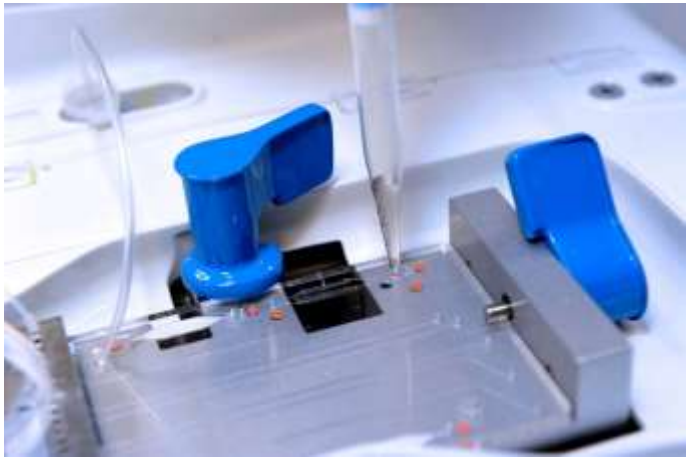
- **Instrument:**
  - *fluidic connections*
  - *pneumatic connections*
  - *fluid driving*
  - *magnetic manipulation*
  - *bead-beating*
  - *localized heating*
  - *optical detection*
  - *...*
  - *software controlled*



FLOWPAD – A WIDE RANGE OF APPLICATIONS

MicroPrep

sample preparation for pathogen detection



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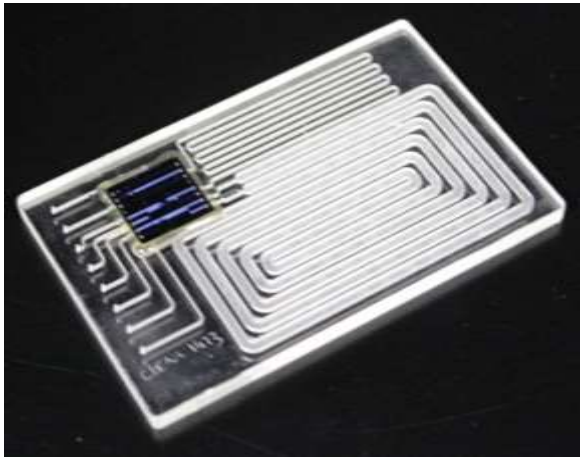
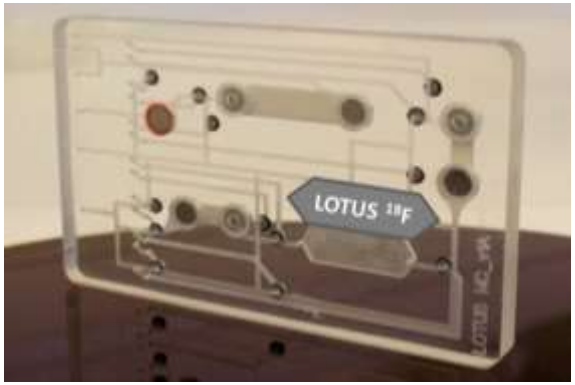
BioCapan

encapsulation of pancreatic cells



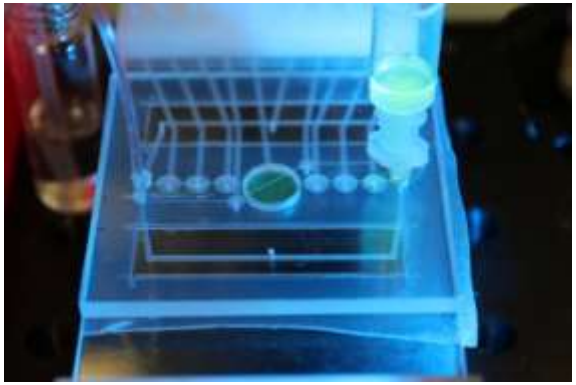
MicroFactory

synthesis of radiotracers close to the patient



Companion Diagnostics

monitoring of personalized medecine

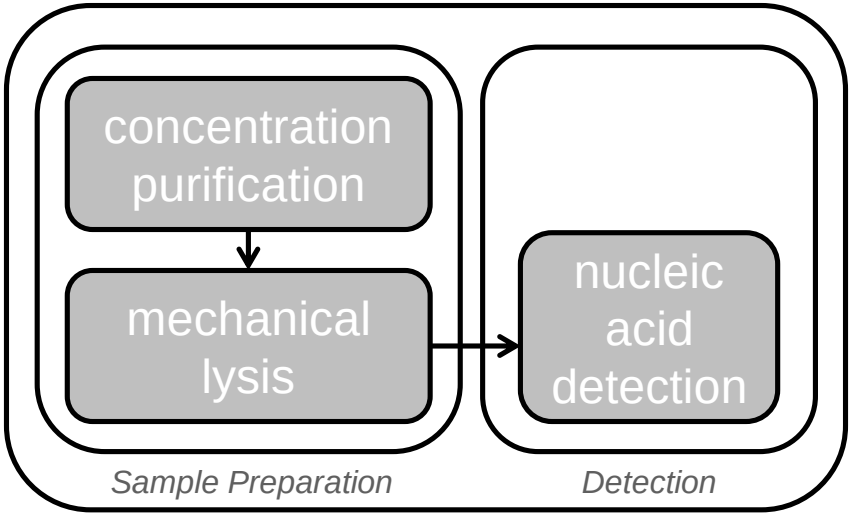


AUTOMATED SAMPLE PREPARATION AND DETECTION

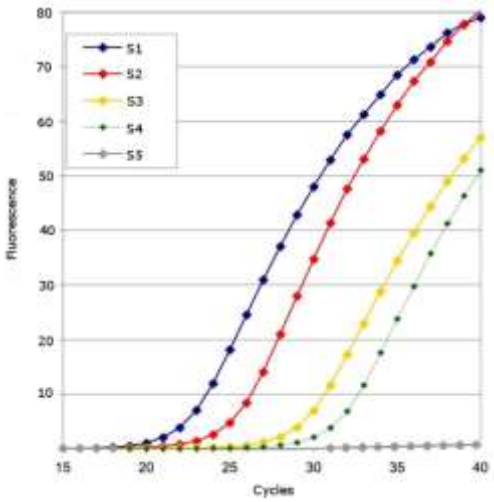
Sample-to-Answer

mL → µL

increase sensitivity



liberate DNA

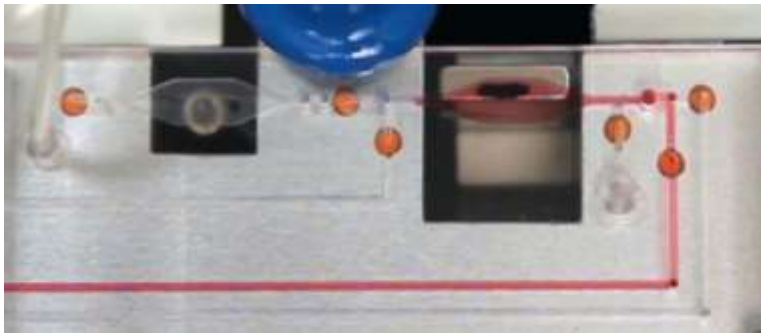


MODULAR APPROACH → BUILDING BLOCKS

mL → µL

increase sensitivity

concentration
purification



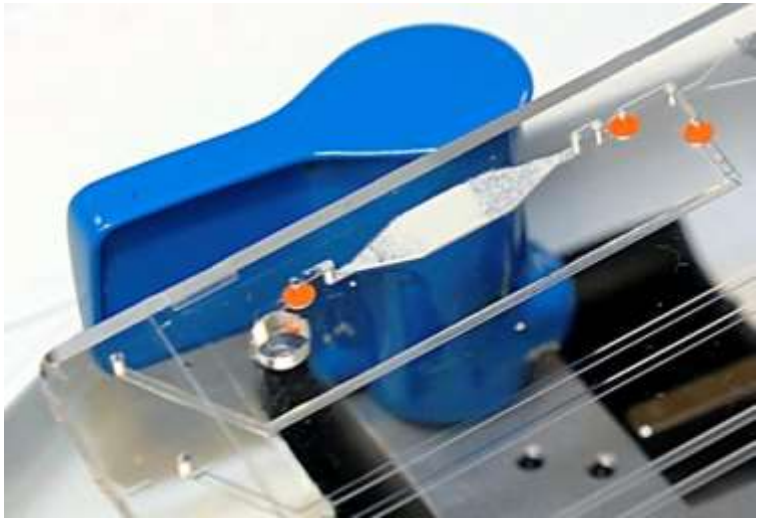
- **Manipulation of magnetic particles**
  - specific or non-specific capture
    - functionalized magnetic particles
    - based on electrostatic interaction (generic)
    - or molecular recognition (specific)
  - 50 times concentrated
    - input sample volume ~ 1 mL
    - concentrated sample volume ~ 20 µL
  - active washing and elution
- **Targets**
  - bacteria
  - white blood cells
  - DNA / RNA / miRNA
  - proteins

MODULAR APPROACH → BUILDING BLOCKS

- **Mechanical lysis (principle of bead-beating)**
  - glass beads embedded in consumable
  - lysis of highly resistant pathogens (bacterial spores)
    - not possible with chemical lysis
    - performance comparable to Precellys (Bertin Technologies)
  - duration of about 30 seconds

mechanical lysis

liberate DNA



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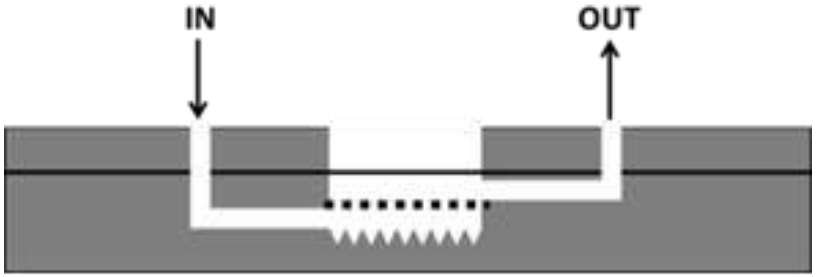
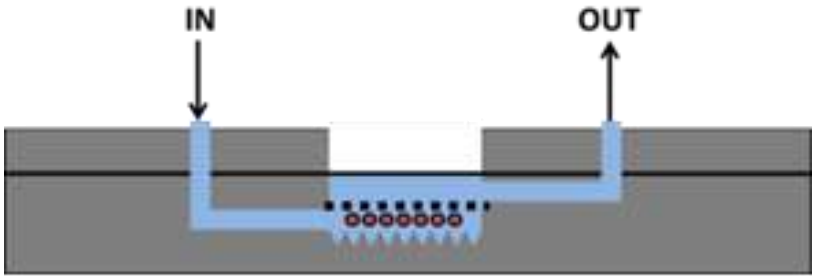
MODULAR APPROACH → BUILDING BLOCKS

mL → µL

increase sensitivity

concentration purification

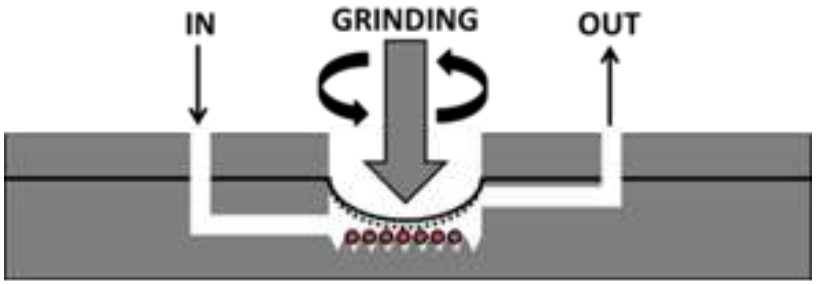


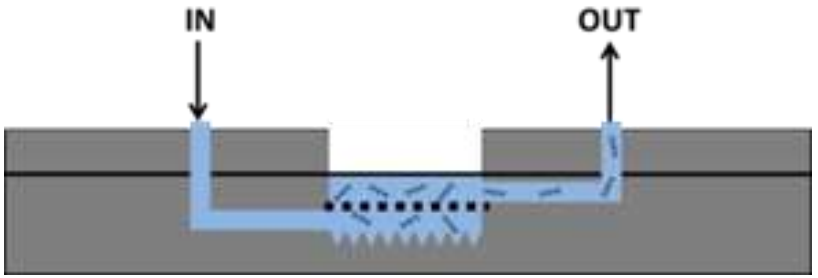


GRINDING LYSIS

mechanical lysis

liberate DNA



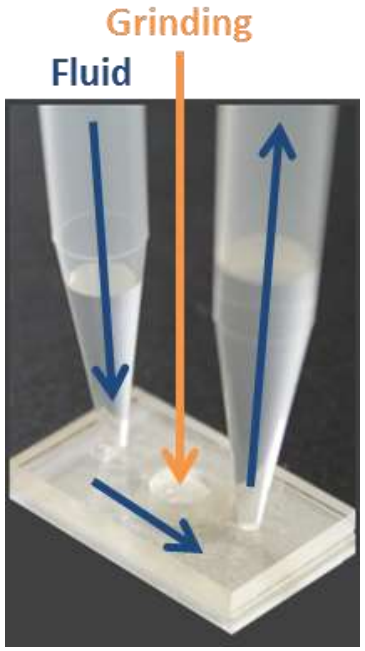


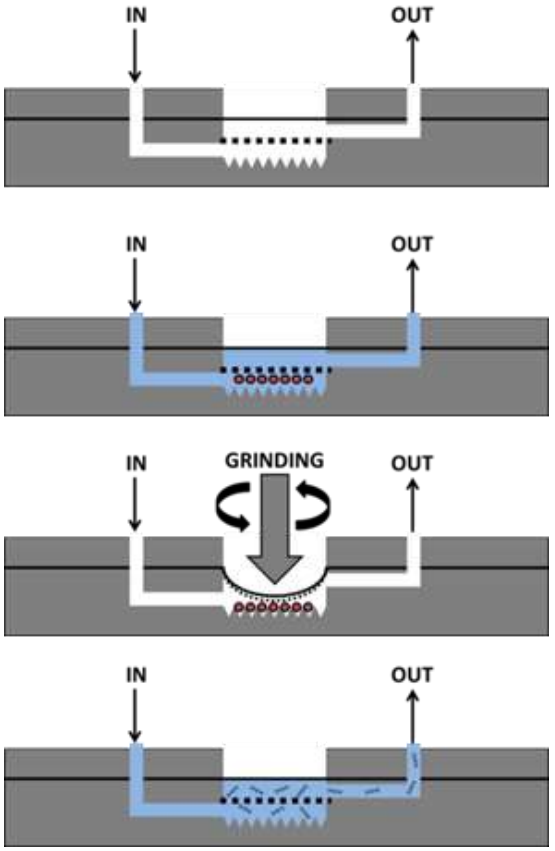
GRINDING LYSIS

mL → µL

increase sensitivity

concentration purification



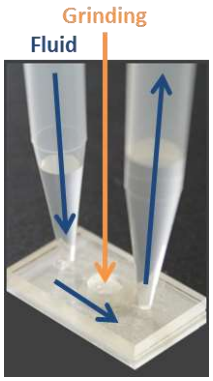


- **Grinding Lysis**
  - analyte concentration/purification + mechanical lysis in one device
  - based on filtration and grinding
  - duration of about 1 minute
  - performance comparable to or better than commercial bead-beater (Precellys, Bertin Technologies)
  - possible in both manual and automated format
  - technology patented and publication submitted

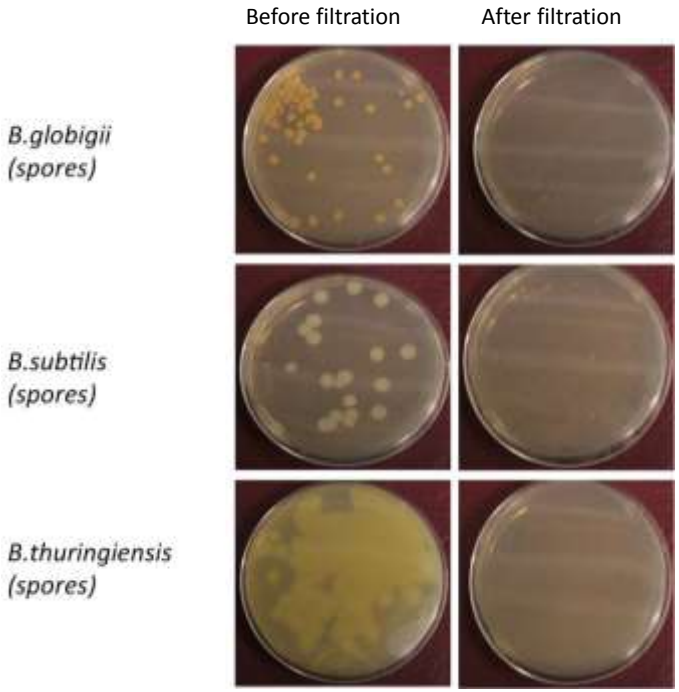
mechanical lysis

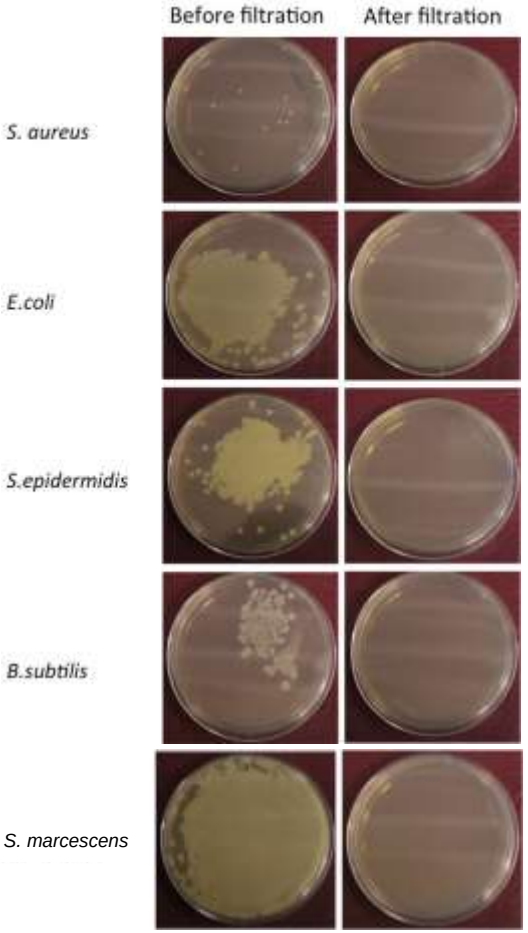
liberate DNA

GRINDING LYSIS ON MANUAL DEVICES

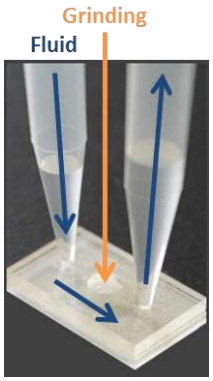


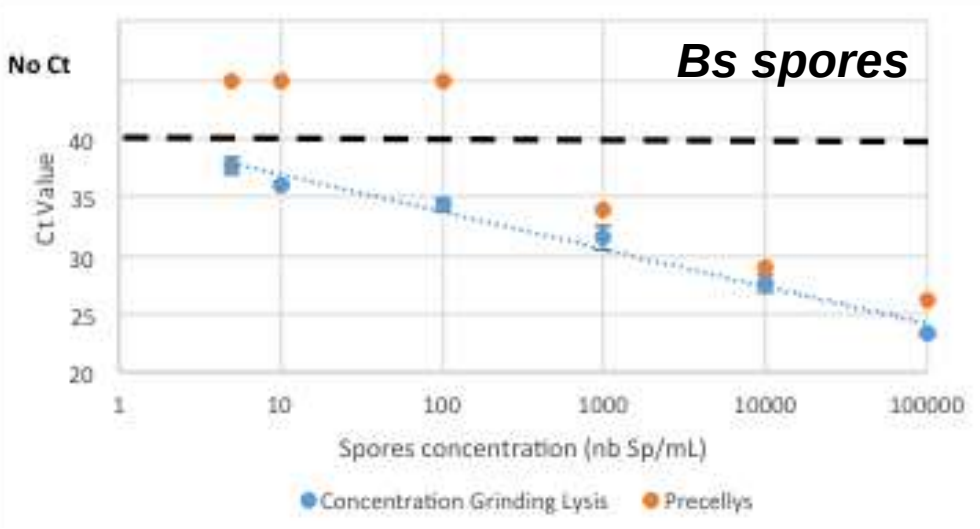
- **Compatibility with a wide range of pathogens**
  - Efficiency of filtration is 100% for all bacteria and spores tested
    → *no colonies visible after one night of culture of the filtered sample*



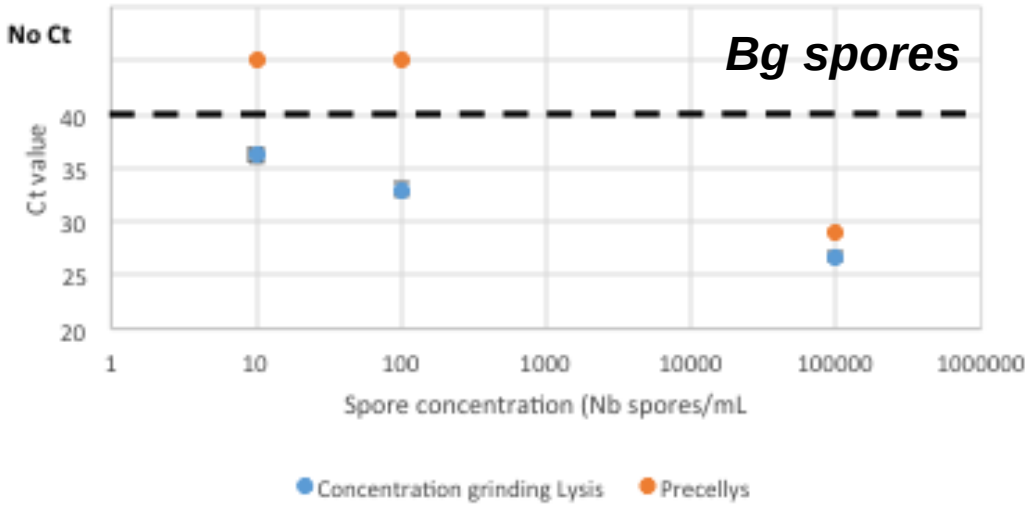


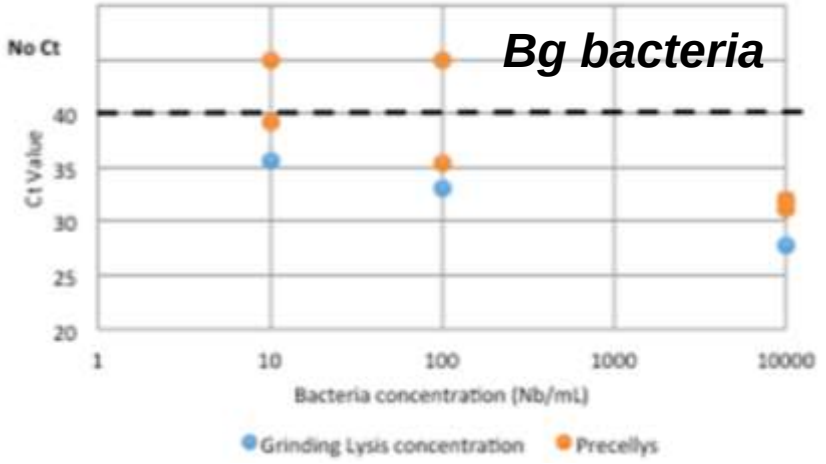
GRINDING LYSIS ON MANUAL DEVICES





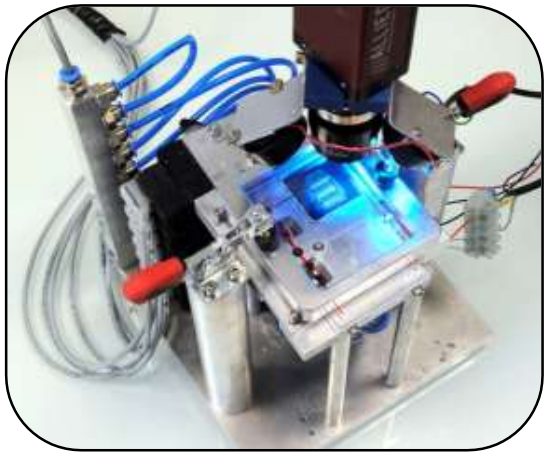
- **Manual Grinding Lysis followed by qPCR**
  - Excellent results, especially for the lower concentrations

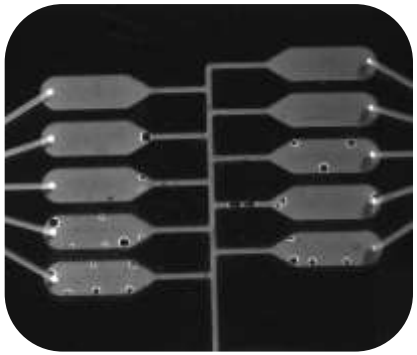




MODULAR APPROACH → BUILDING BLOCKS

- **Integrated amplification of nucleic acids**
  - *sample-to-answer* → integrated on the same consumable together with sample preparation
  - *multiplexing* → multiple chambers, each detecting a different target
  - *on-board reagents* → dried and/or lyophilized
  - *versatile* → compatible with many different types of NA amplification
    - qPCR or isothermal methods (LAMP, RPA, ...)
    - RNA amplification possible using reverse transcription (RT-qPCR)
    - fluorescent detection possible with probes or intercalant dyes (FAM, EvaGreen, SybrGreen, …)



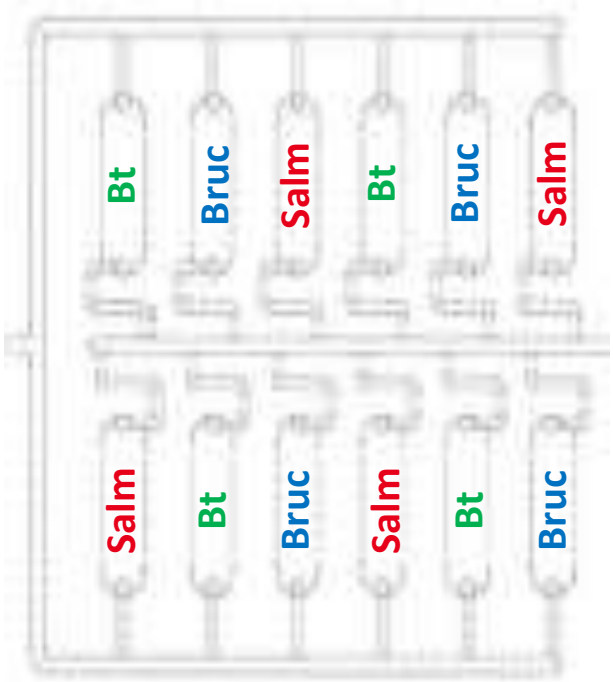


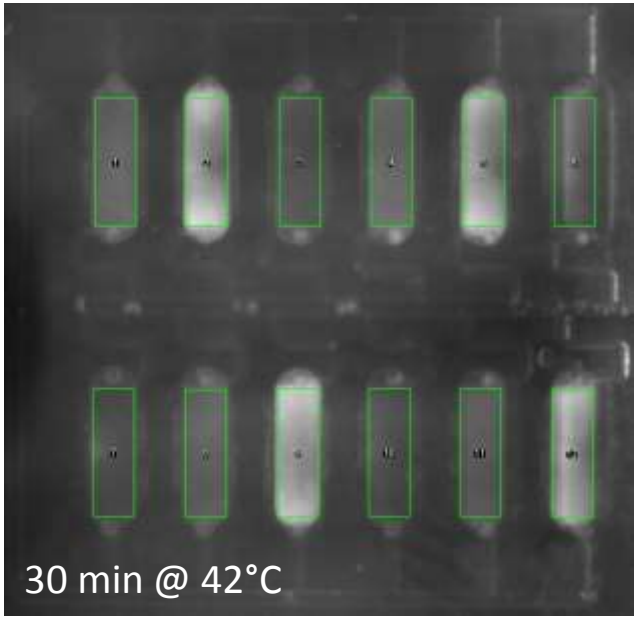
RPA (*Recombinase Polymerase Amplification*)

- isothermal (40°C)
- rapid (10 min)
- robust and specific
- not quantative

MULTI-TARGET RPA IN MICROFLUIDIC DEVICE

- **Detection of multiple targets in the same sample by using target-specific dried reagents in each chamber**



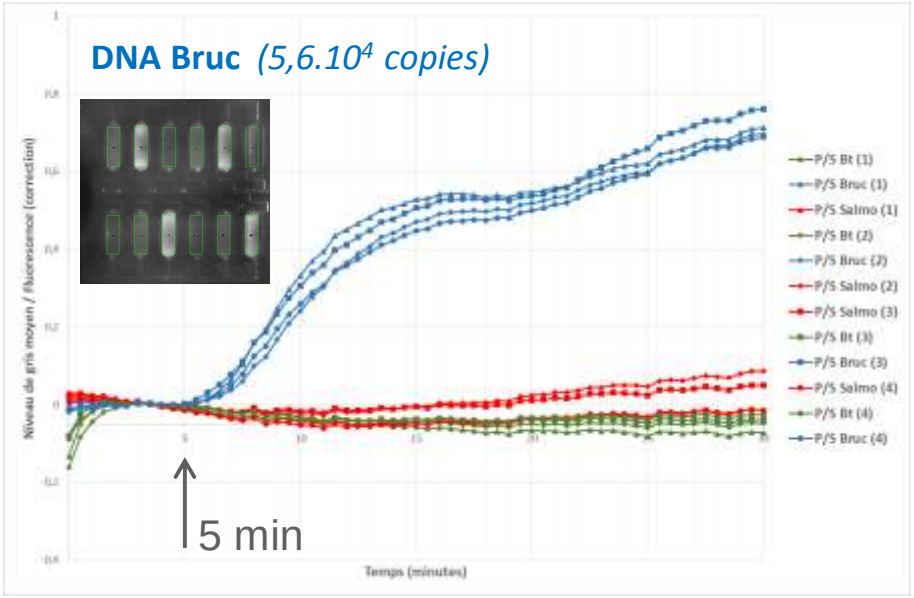


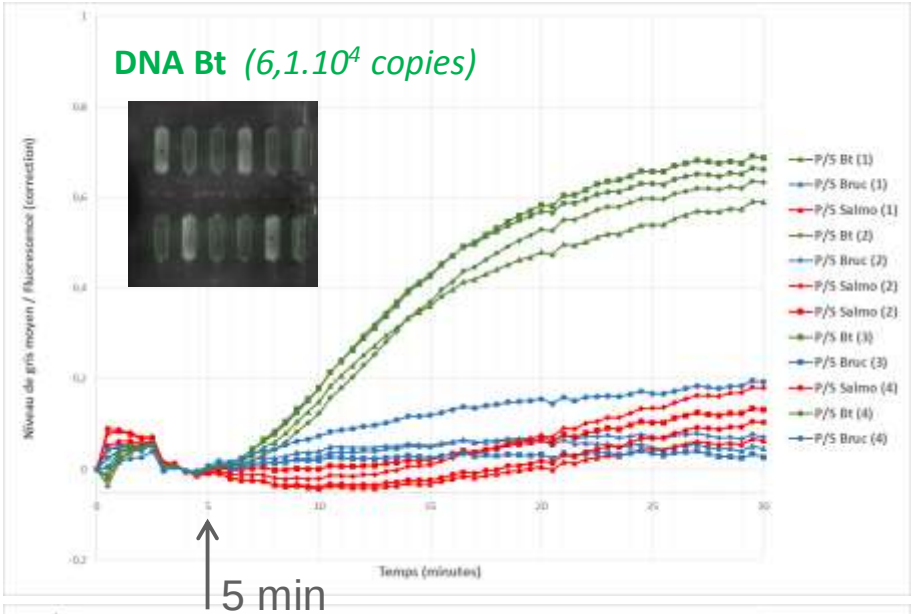
Bacillus thuringiensis

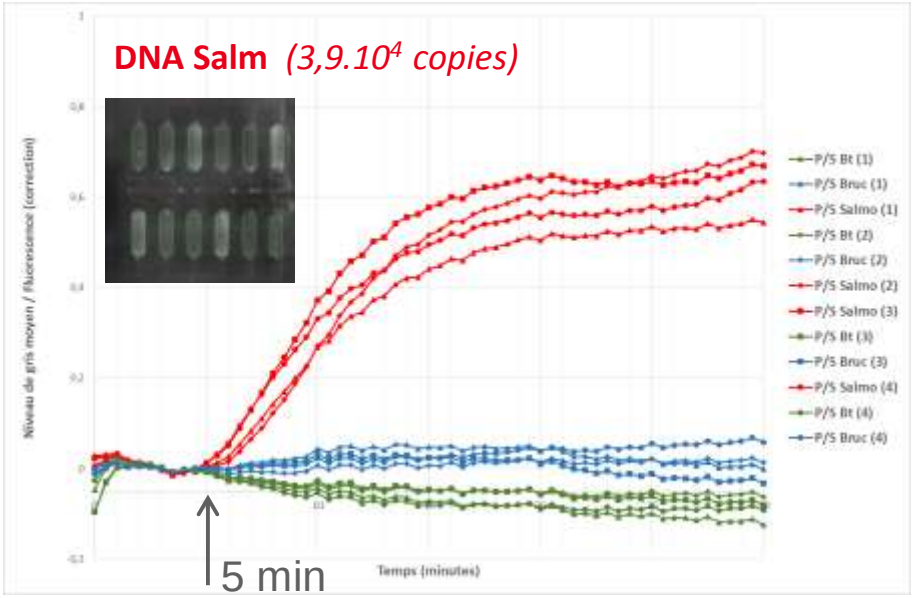
Brucella neotomae

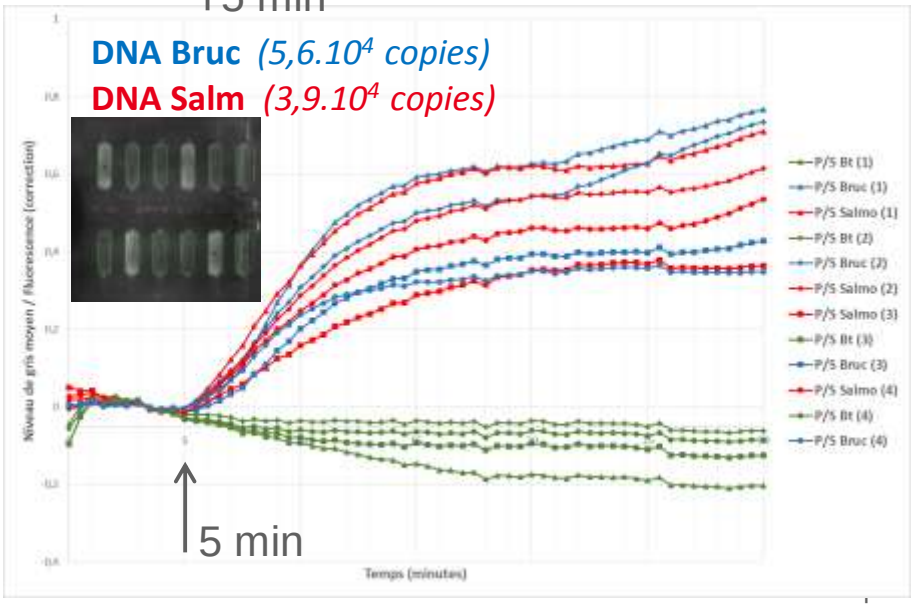
Salmonella typhi

MULTI-TARGET RPA IN MICROFLUIDIC DEVICE

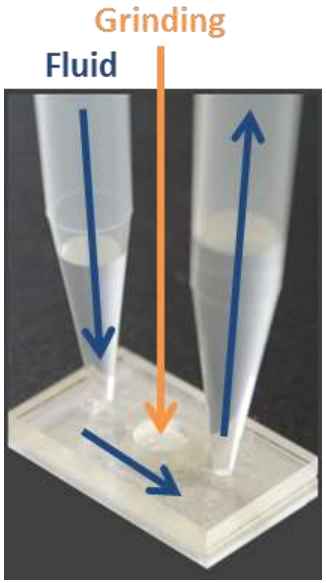


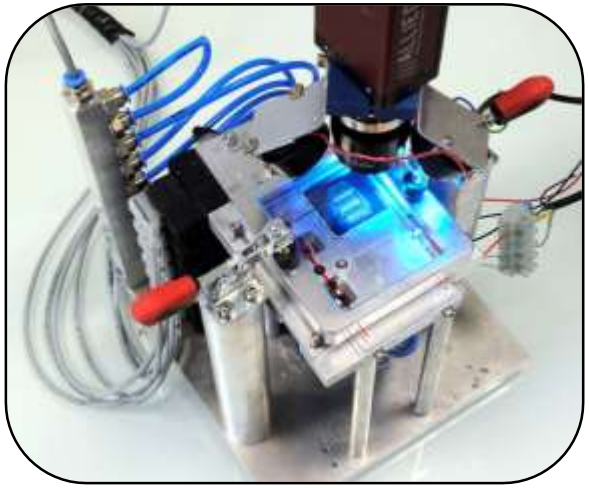


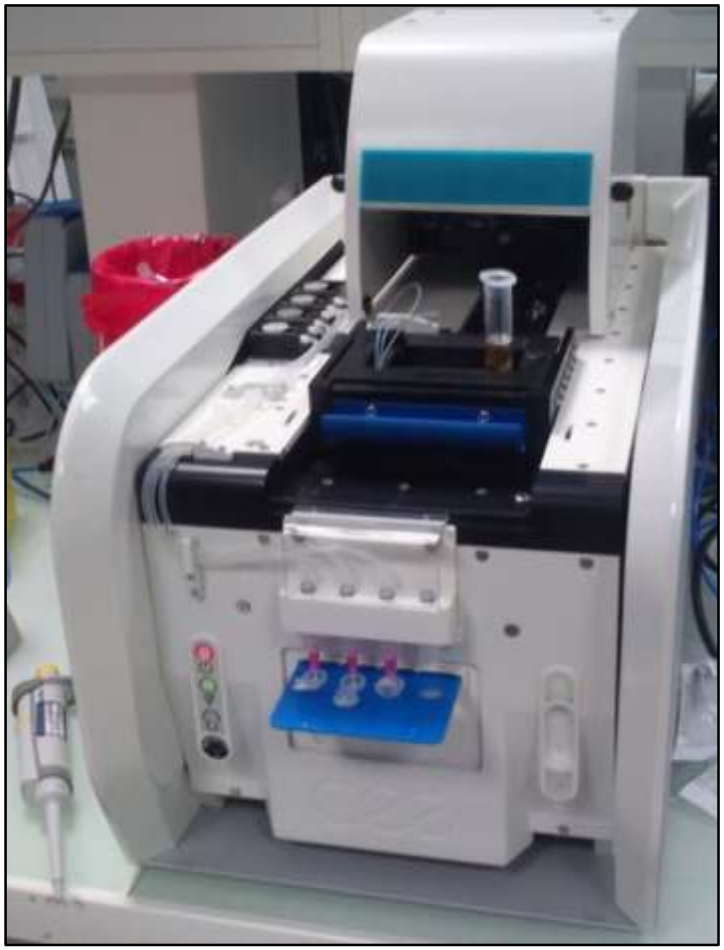


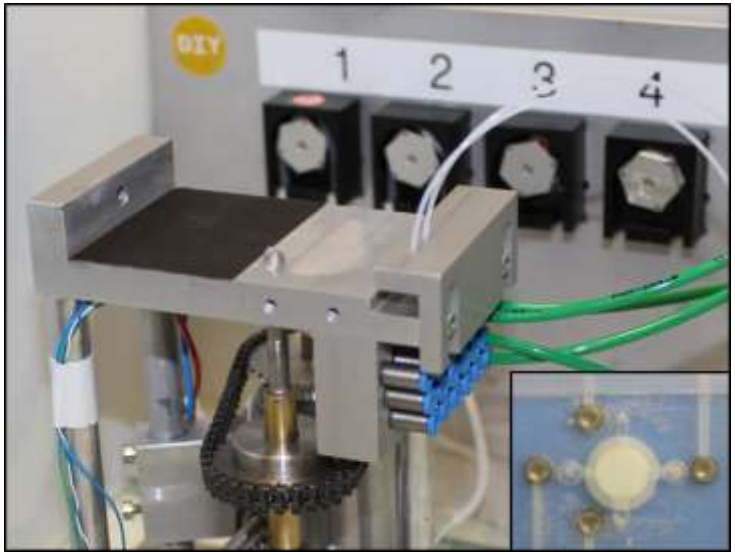


FROM MANUAL OPERATION TO AUTOMATED / INTEGRATED SYSTEM





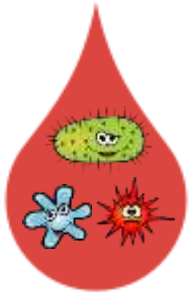




CHILDSPLAY

- **Diagnostics of bacteremia in sub-saharan Africa**
  - manipulation of magnetic particles
  - integrated NA detection (LAMP)

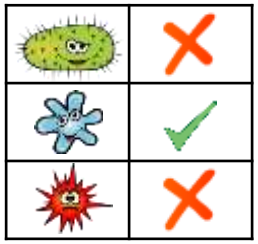
sample → lysis → microfluidic consumable + instrument → answer



1 mL blood

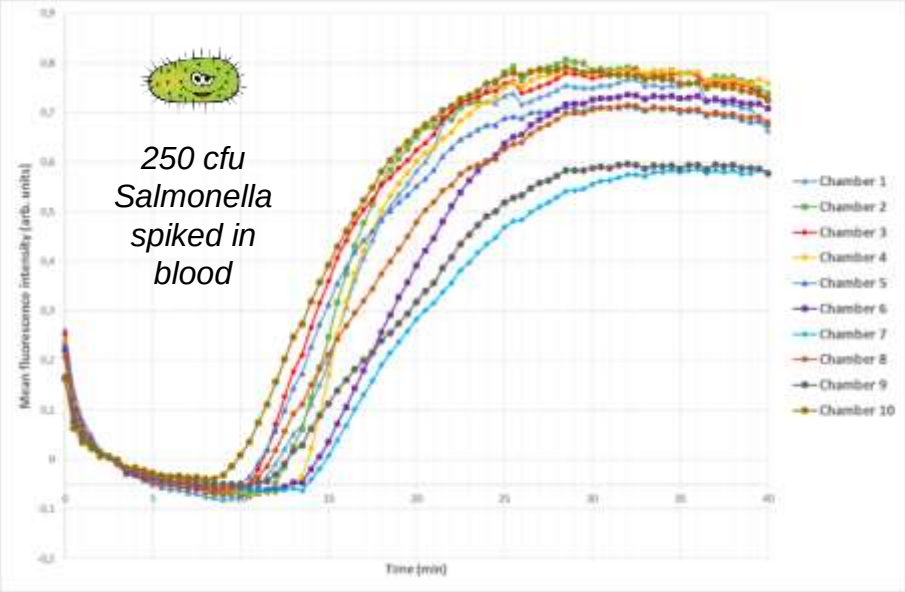
+ 2 mL lysis/binding buffer (and magnetic particles)

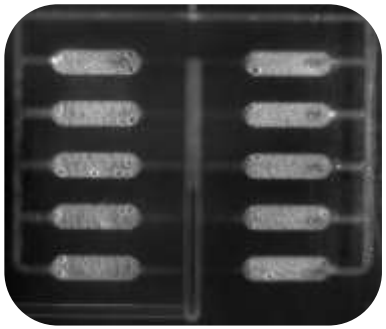
- Concentration & purification of DNA
- Multi-target LAMP

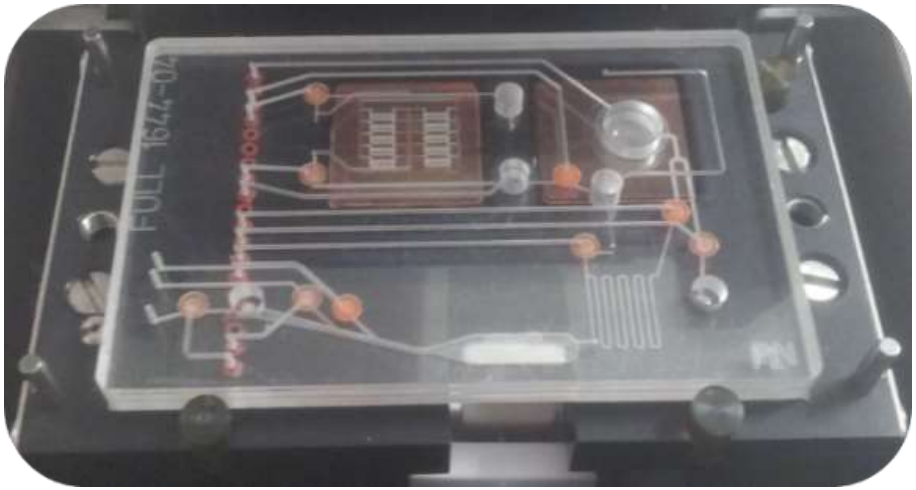


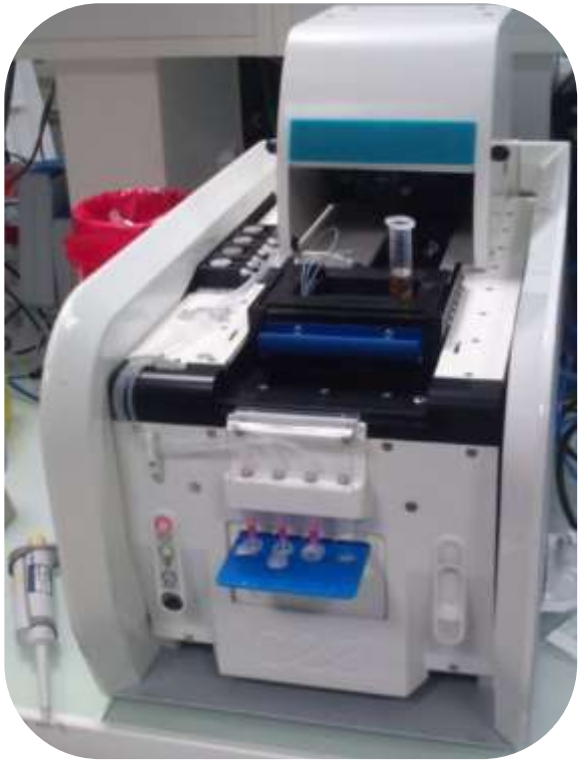
POS/NEG
multiple species
+ controls

CHILDSPLAY


250 cfu
Salmonella
spiked in
blood
Mean fluorescence intensity (arb. units)
Time (min)
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5
Chamber 6
Chamber 7
Chamber 8
Chamber 9
Chamber 10





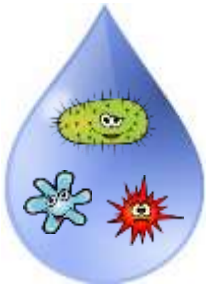


Financed by the French joint-ministerial CBRNE R&D program

MICROPCR

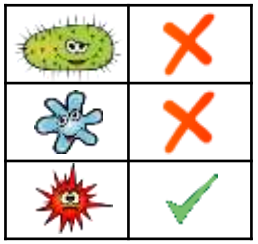
- **Identification of biological threats (CBRN)**
  - concentration + mechanical lysis (Grinding Lysis)
  - integrated NA detection (RPA)

sample → microfluidic consumable + instrument → answer



1 mL environmental sample

- Concentration & mechanical lysis of bacterial spores
- Multi-target RPA



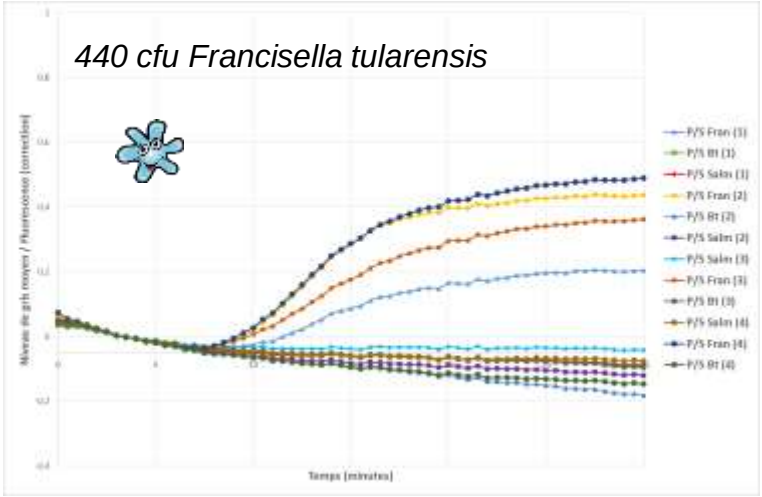
POS/NEG
multiple species
+ controls

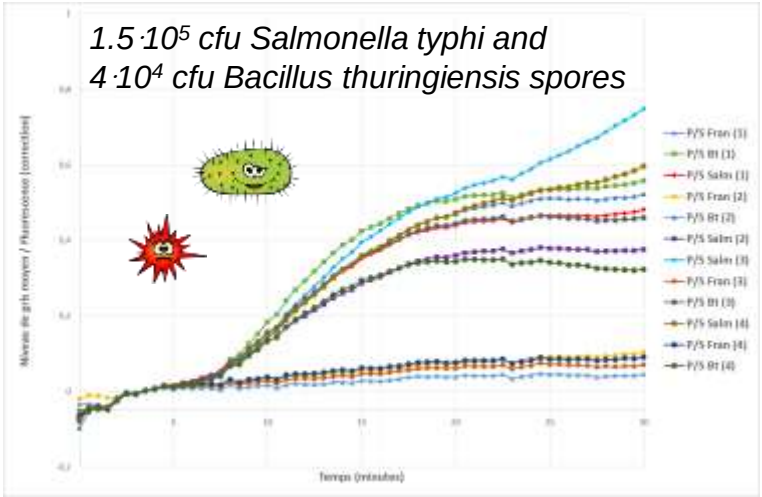
Financed by the French joint-ministerial CBRNE R&D program

MICROPCR

- **Main results**
  - Grinding Lysis sample preparation
    - concentration of bacteria from 1 mL
    - mechanical lysis of bacterial spores
    - DNA ready in less than 10 min
  - RPA identification
    - multi-target identification
    - embedded reagents (dried / lyophilized)
    - positive result in less than 10 min
  - Analytical performance
    - *Bacillus thuringiensis* spores and *Salmonella typhi* bacteria down to about 10^4 bacteria/mL
    - *Francisella tularensis* down to about 10^2 bacteria/mL

Sample-to-Answer integrated and automated





CONCLUSION

- **Flowpad is a versatile microfluidic platform, for many different sample types and applications, that enables rapid integration of biological assays into an automated microfluidic format**

MicroPrep

sample preparation for pathogen detection

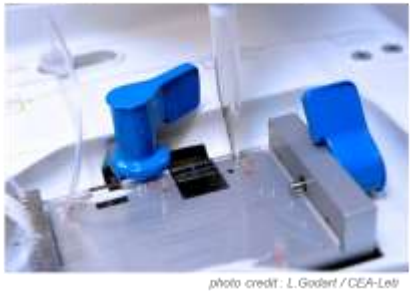
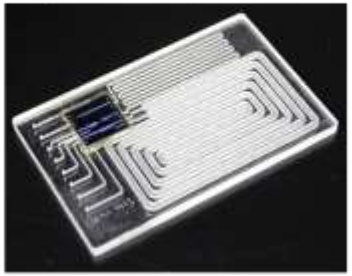


photo credit : L.Godart / CEA-Leti

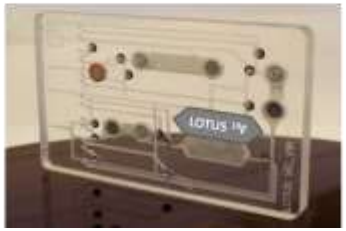
BioCapan

encapsulation of pancreatic cells



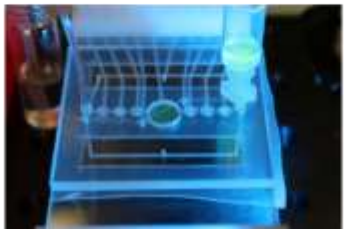
MicroFactory

synthesis of radiotracers close to the patient



Companion Diagnostics

monitoring of personalized medecine



Project CollAir

anses

agence nationale de sécurité sanitaire alimentation, environnement, travail

THANK YOU FOR YOUR ATTENTION

Thank you
for your attention

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