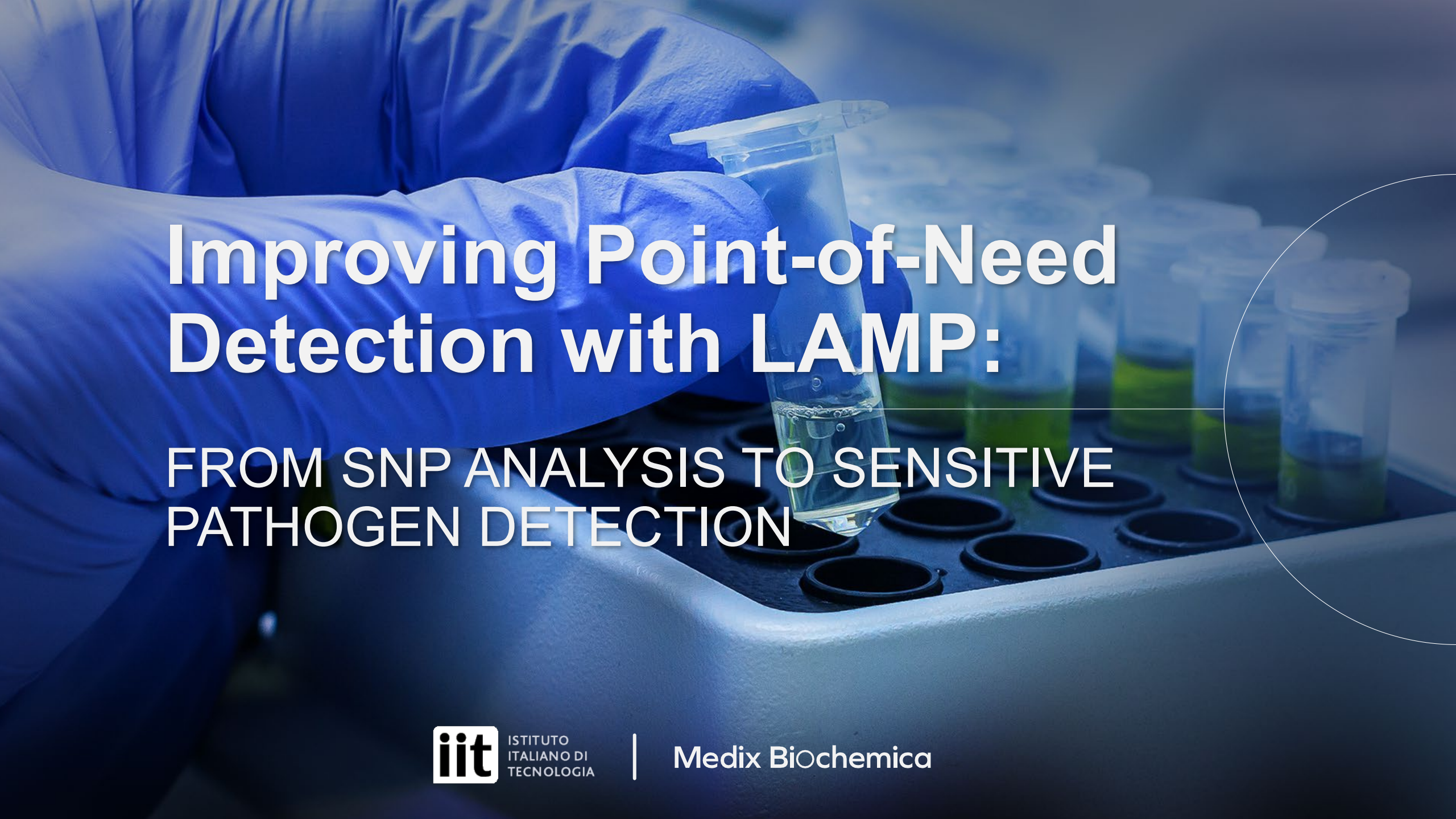

Improving Point-of-Need Detection with LAMP:

FROM SNP ANALYSIS TO SENSITIVE PATHOGEN DETECTION

ISTITUTO ITALIANO DI TECNOLOGIA | Medix Biochemica

Before we get started

Please use the Q&A button at the bottom of your screen to submit any questions.

There will be a Q&A session at the end of this webinar. If we do not have time to answer your questions, we will reach out by email following the webinar.

This webinar is being recorded. The recording as well as on-demand link will be released following the webinar.

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Independent, International, and Industry-Leading Raw Materials Supplier

- Provider of high quality antibodies, antigens, proteins, enzymes
- Expertise in immunoassays, clinical chemistry
- Engineered Polymerases for Molecular Diagnostics
  - Unique Polymerases for DNA/RNA
  - ISO:13485 Conformity
  - Assay Development Services
  - Lyophilization Services
  - Lyo-Ready PCR Products

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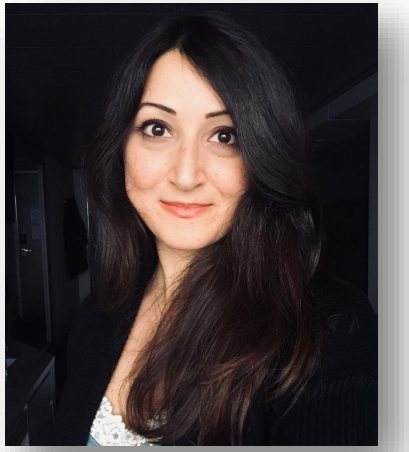
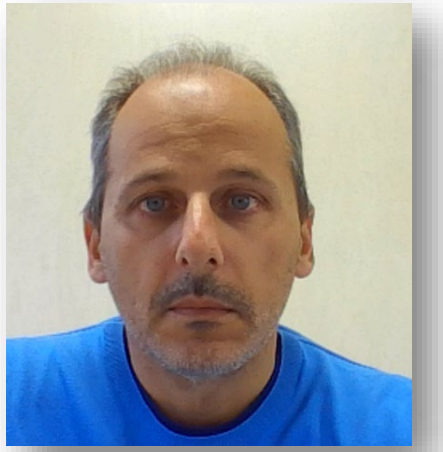
Featured Speakers and Panelists



Dr. Paola Cecere

Postdoctoral Researcher

Istituto Italiano Di Tecnologia



Dr. Pier Paolo Pompa

Research Director

Istituto Italiano Di Tecnologia

Dr. Giuseppina Sannino

Global Business Development Manager, MDx

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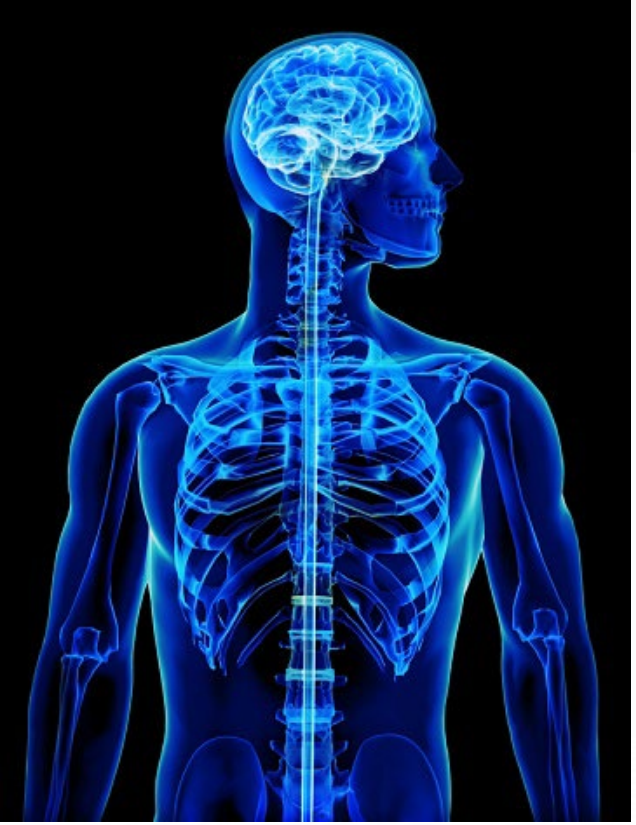
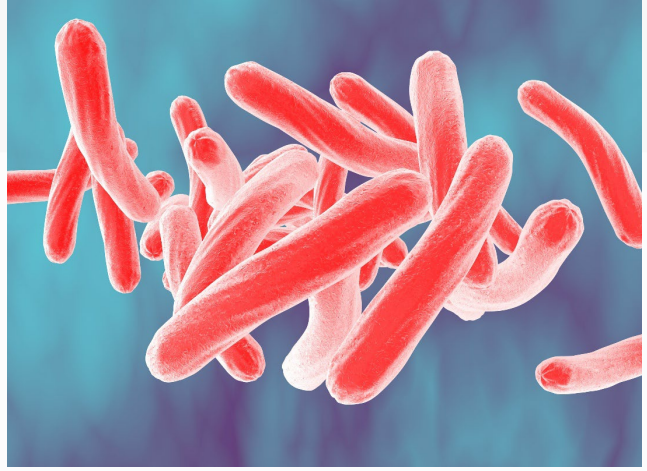
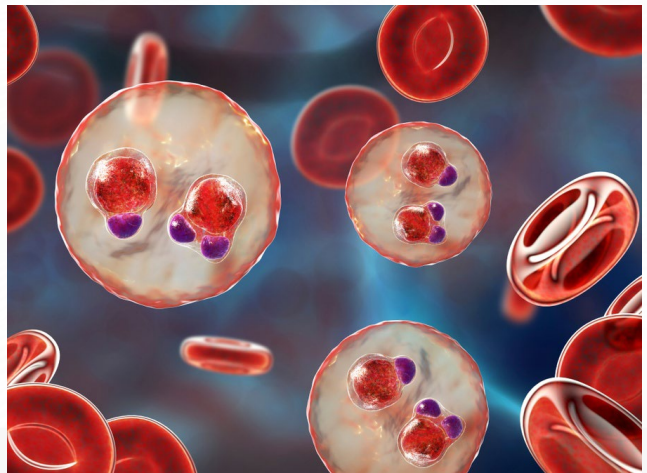






ISTITUTO ITALIANO DI TECNOLOGIA

Improving Point-of-Need Detection with LAMP: from SNP Analysis to Sensitive Pathogen Detection

Dr. Paola Cecere
Postdoctoral Researcher – Istituto Italiano Di Tecnologia

29th October 2024

ISTITUTO ITALIANO DI TECNOLOGIA

ISTITUTO ITALIANO DI TECNOLOGIA
NANOBIOINTERACTIONS & NANODIAGNOSTICS

Dr. Pier Paolo Pompa

Principal Investigator

Nanodiagnostic platform

Integrated, smart, low-cost, and rapid assays/sensors for on field and point-of-care diagnostics.

Specific areas of applications span from clinical diagnostics (cancer, genetic, and infectious diseases) to food safety and traceability, and environmental control (pathogens, pollutants and contaminants).

This research line is based on hybrid detection strategies:

- green and controlled synthesis of nanomaterials
- surface (bio)chemistry
- plasmon non-linear response
- nanozymes
- biotechnology and molecular biology

LAMP-based GENETIC TESTING

LAMP: Loop-mediated isothermal **AMP**lification

Nucleic Acids Research, 2000, Vol. 28, No. 12 e63

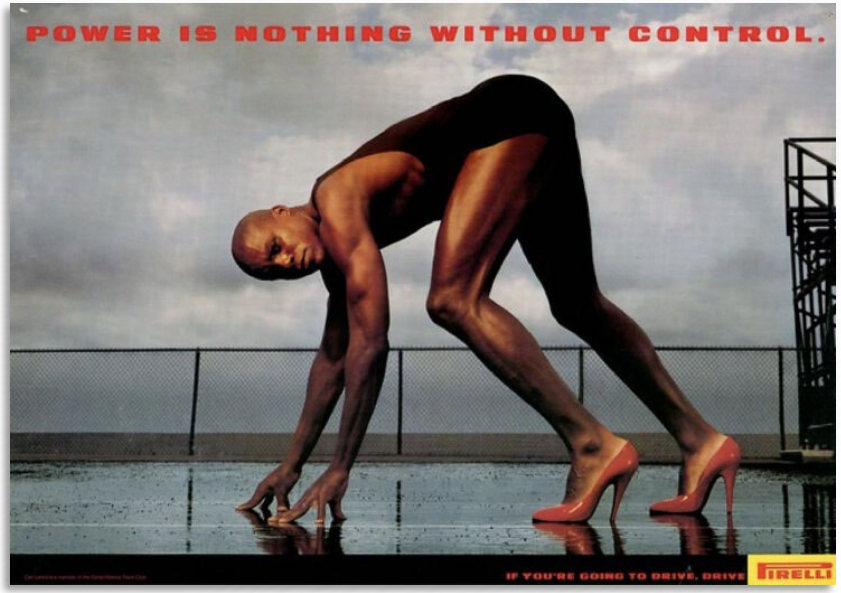
Loop-mediated isothermal amplification of DNA

Tsugunori Notomi[1,3,*], Hiroto Okayama[2], Harumi Masubuchi[1], Toshihiro Yonekawa[1], Keiko Watanabe[1], Nobuyuki Amino[3] and Tetsu Hase[1]

[1]Eiken Chemical Co. Ltd, 1381-3 Shimoishigami, Ohtawara, Tochigi 324-0036, Japan, [2]Department of Biochemistry and Molecular Biology, The University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan and [3]Department of Laboratory Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

- Outstanding reaction for DNA and RNA amplification, with broad and multifaceted capabilities
- Robustness and accessibility

Mastering design to maximize the elevated LAMP performances



Pirelli's iconic slogan

Our Solution:

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Medix Biochemica

"Lyo-LAMP"

Unleashing LAMP potential with our expertise!

Today's Topics

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Section 1

1. **How** does LAMP work?
2. **What** are LAMP's strengths?
3. **Which** challenges do we face with LAMP?
4. **Where** can LAMP be carried out?

Section 2

Our answer: "Lyo-LAMP"

1. Lyo-LAMP identity card
2. Deep dive into Lyo-LAMP
3. Applications and perspectives

How does LAMP work?

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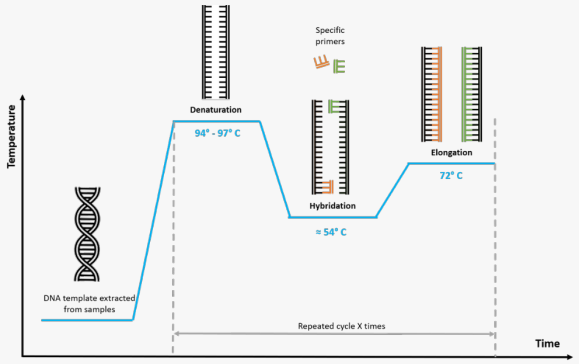
The answer is in the name

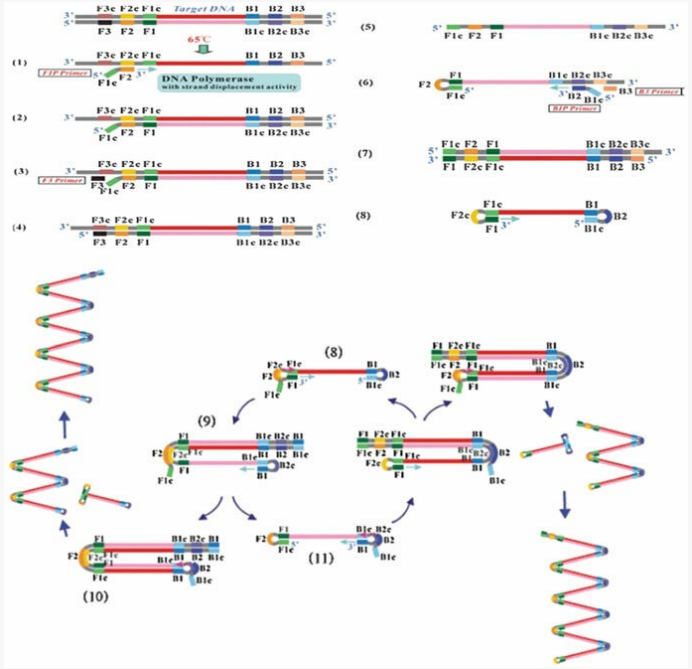
PCR

(Polymerase Chain Reaction, Kary Mullis, 1983)

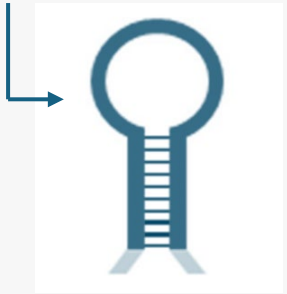


thermal cycles

LAMP



Loop-mediated



Loop: ssDNA

Stem: dsDNA

isothermal amplification

single reaction temperature (60-65 °C), no denaturation step

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Exploring the LAMP mechanism – things to keep in mind

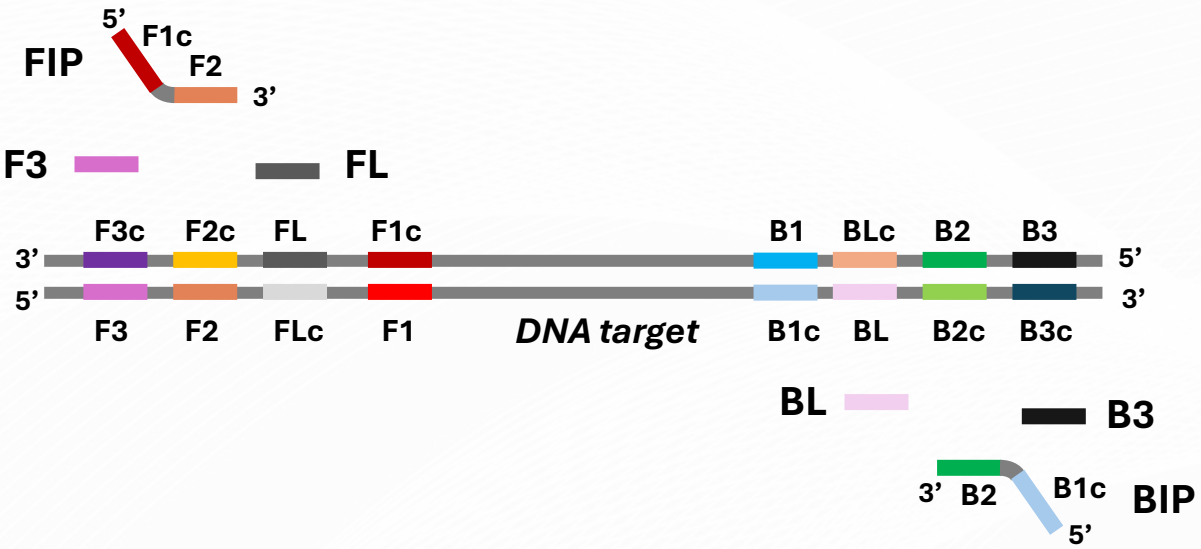
LAMP primers

set of 4-6 specially designed primers, specific for 6-8 target regions

FIP = forward inner primer
BIP = backward inner primer
→ Guide for the formation of the loop-stem structures

F3 = forward outer primer
B3 = backward outer primer
→ Work only in stage 1

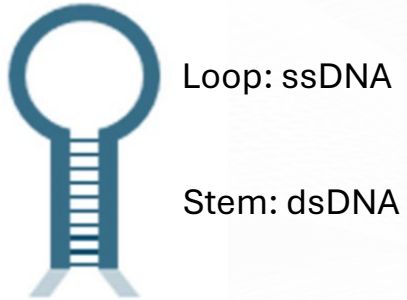
FL = forward loop primer
BL = backward loop primer
→ Accelerating primers, work only in stage 2

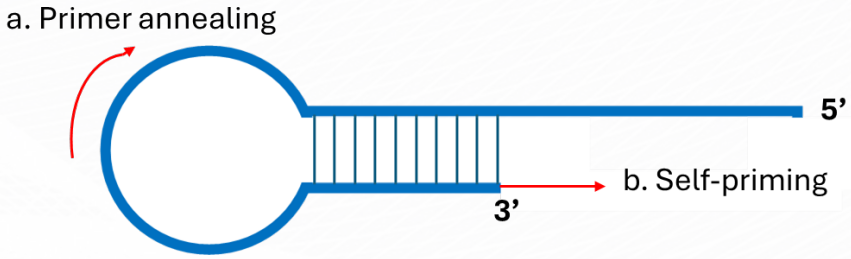


Exploring the LAMP mechanism – things to keep in mind

Alternative mechanisms to the denaturation stage in LAMP

1. dynamic equilibrium of the dsDNA target at 60-65 °C
2. DNA polymerase with high strand-displacement activity
3. two priming sites for each stem-loop structure:

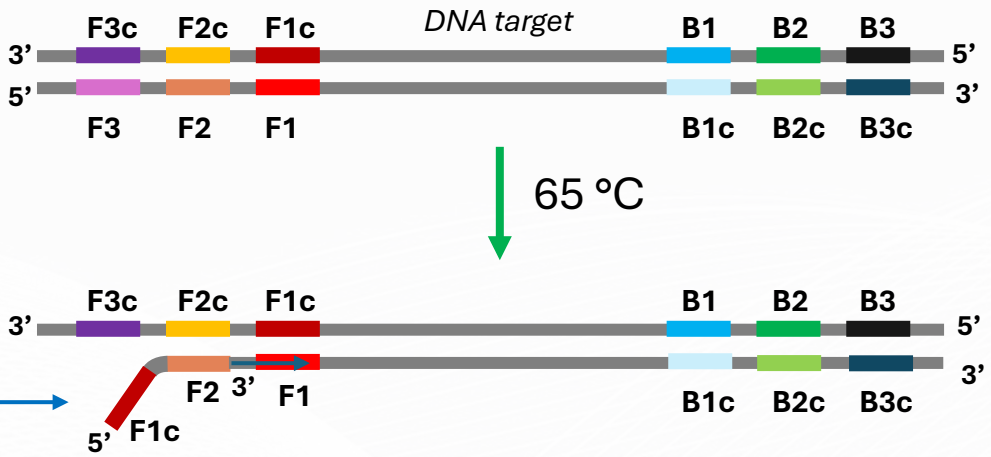




How does LAMP work?

STAGE 1: production of the starting material

STAGE 2: auto-cycling strand-displacement amplification/elongation step



1. partially denaturated template

2. strand invasion by FIP

3. FIP elongation

How does LAMP work?

STAGE 1: production of the starting material

4. F3 annealing

F3 (outer primer)

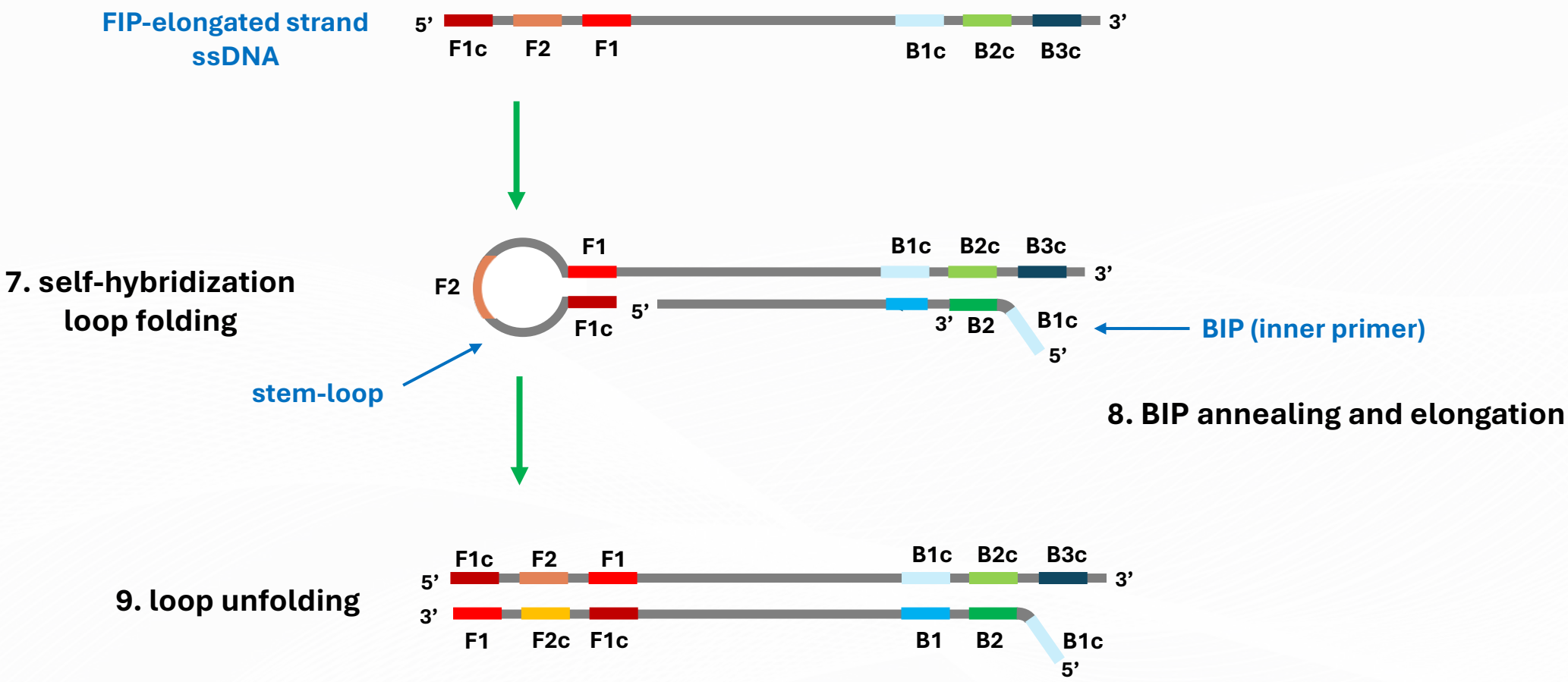
5. F3 elongation

FIP-elongated strand
ssDNA

6. polymerase strand displacement activity

How does LAMP work?

STAGE 1: production of the starting material



How does LAMP work?

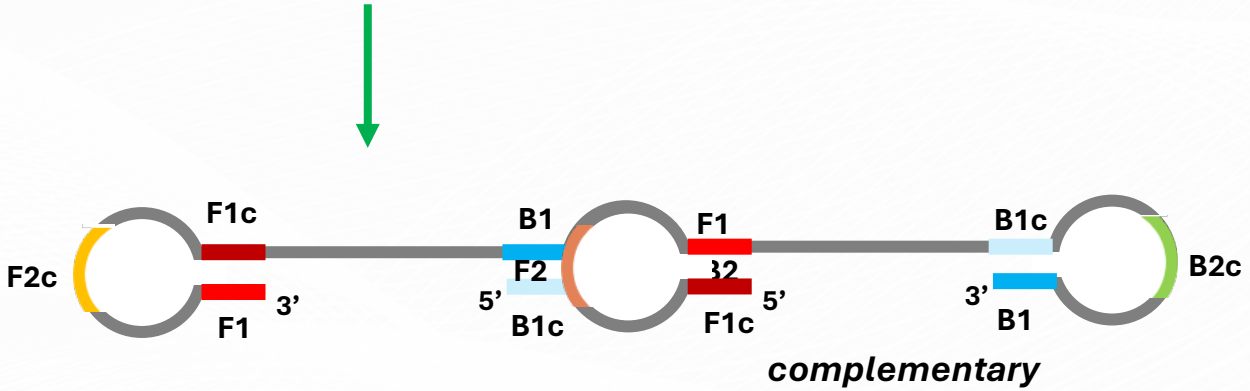
STAGE 1: production of the starting material

10. B3 annealing

11. elongation

12. BIP-elongated strand
ssDNA displacement

13. loop folding at both ends



DUMBELL-LIKE STRUCTURE

How does LAMP work?

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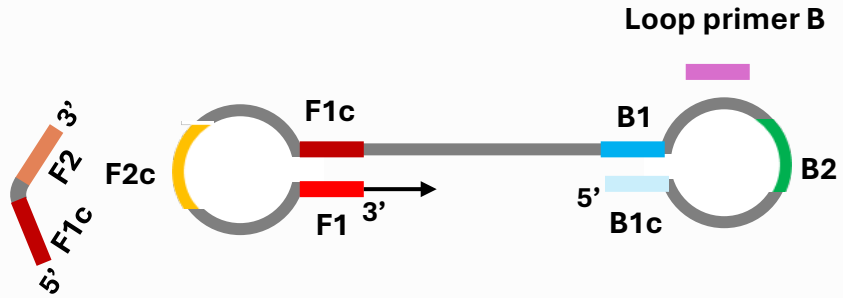
DUMBELL-LIKE STRUCTURE

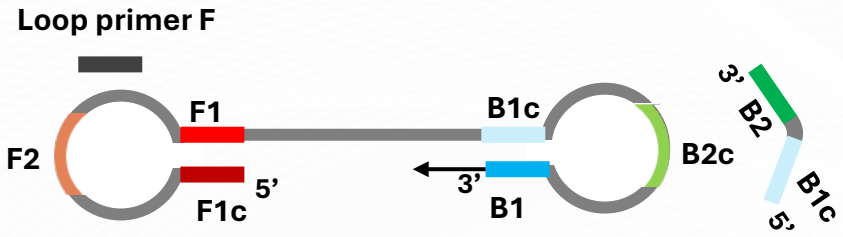
artificial ssDNA target

STAGE 2: auto-cycling strand-displacement amplification/elongation step

How does LAMP work?

STAGE 2: auto-cycling strand-displacement amplification/elongation step





MULTIPLE

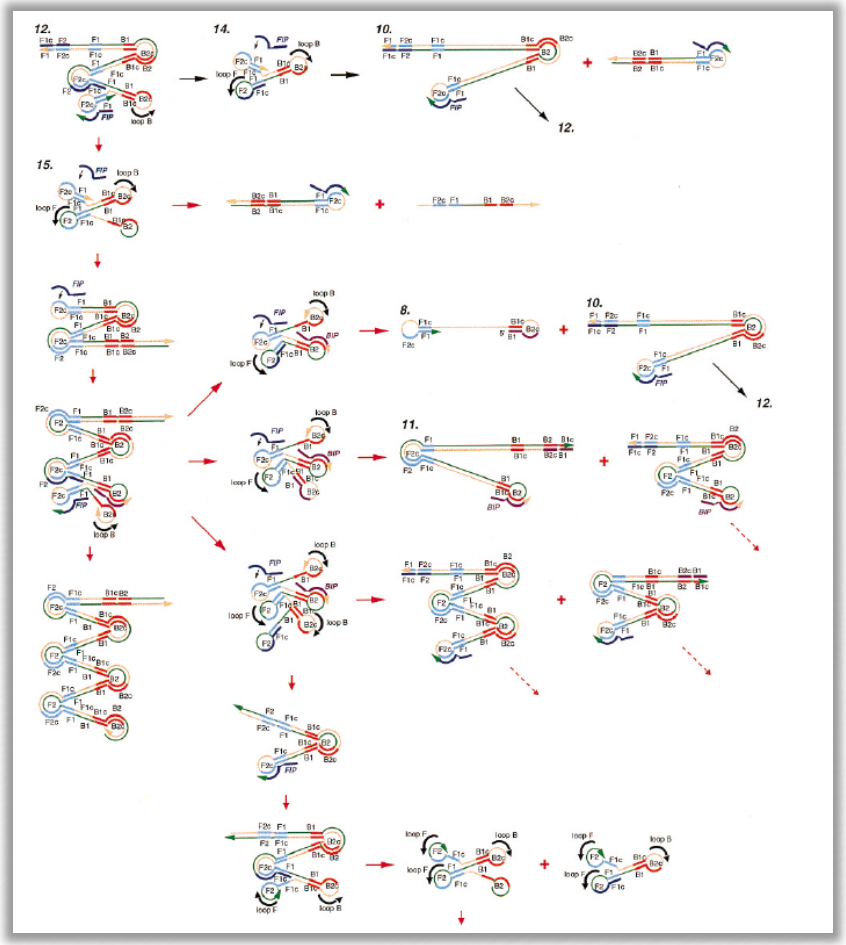
AMPLIFICATION STARTING POINTS

on the dumbell-like structures:

1. annealing inner primer/loop
2. self-priming (3' stem end)
3. annealing loop primer/loop

How does LAMP work?

STAGE 2: auto-cycling strand-displacement amplification/elongation step



Nagamine et al, Molecular and cellular probes, 2002

- Multiple amplification sites
- Loop unfolding-refolding
- Strand-displacement activity

Exponential

Auto-cycling

Amplification

- Alternatively inverted repetitions of the target
- Cauliflower-like structures

→ Agarose gel

What are LAMP's strengths?

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	PCR the precision powerhouse for lab-based quantification	**LAMP** the speedy and hassle-free tool for on-the-spot diagnostics	
Temperature	Thermal cycles	Isothermal	
Equipment	Expensive thermal cycler	Minimal instrumentation	→ Cost-effective
Robustness	Sensitive to inhibitor	Highly tolerant to inhibitors	→ Simplified extraction protocols
Amplification efficiency	10^6	10^9 – 10^10	→ Greater yield

SPEED

SENSITIVITY

SPECIFICITY

LAMP design-dependent parameters → **High-performing LAMP**

What are LAMP's strengths?

ISTITUTO ITALIANO DI TECNOLOGIA

	PCR the precision powerhouse for lab-based quantification	LAMP the speedy and hassle-free tool for on-the-spot diagnostics	
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Robustness	Sensitive to inhibitor	Highly tolerant to inhibitors →	Simplified extraction protocols
Amplification efficiency	10^6	10^9 – 10^10 →	Greater yield
		High-performing LAMP	
SPEED	> 1 hour	7 - 40 mins →	**Faster time-to-results**
SENSITIVITY	10-100 copies/reaction	1-3 copies/reaction →	**Greater sensitivity**
SPECIFICITY	2 primers x 2 target regions	4-6 primers x 6-8 target regions →	**Greater specificity**

Which challenges do we face with high-performing LAMP?

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1. Complex primer design

Overall assay performance:
- sensitivity (false negative %)
- specificity (false positive %)
- reaction rate
- reliability/reproducibility

Detection of challenging DNA markers (as SNPs)

- Chemical and thermodynamic requirements
- Specific detection
- Undetectable background
- Avoid non-specific binding and primer dimers

Need for extensive expertise in primer design

2. Mix composition

- Overall assay performance
- Efficient warm-start polymerase
- Lyo-compatible

3. Risk for carryover contaminations

The assay must include a system against cross-contaminations

4. Extraction protocol

Simple, fast and LAMP-compatible

Where can LAMP be carried out?

A **high-performing LAMP** can be particularly suitable for

Genetic testing

Point-of-care

Rapid testing at the point of need (centralized or decentralized settings)

- Fast turnaround time
- User-friendly
- Affordability
- Portability

High-throughput

Continuous and large-scale screenings

- Scalability
- High sample capacity
- Automation
- Reproducibility

Main requirements for POC and HT testing design:

- **Chemistry**: fast and reliable, no cross-contaminations, inhibitor tolerance
- **Workflow**: short and easy
- **Supply Chain**: low-cost components with high performance, scalability, room storage
- **Instrumentation**: wide equipment availability, cost-effectiveness

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DI TECNOLOGIA

Expertise in LAMP technology

Medix Biochemica

Expertise in enzyme engineering
and lyophilization

Our Answer:

“Lyo-LAMP”

High-performing LAMP

in Lyophilized format

for molecular diagnostic solutions

Smart

User-Friendly

Business-Friendly

“Lyo-LAMP” – Identity card

Smart

- All-inclusive solutions → DNA extraction & amplification
- High-performing LAMP →
  - Customized primer set
  - High sensitivity and specificity
  - Fast results
- Lyophilized format → RT-shipping and storage, with long-term stability

User-Friendly

- Highly tolerable test
- Easy-to-use
- Minimal instrumentation required

Business-Friendly

- Cost-effective
- Alternative solutions to the limits of traditional methods
- Scalable
- Custom on-demand

Deep dive into “Lyo-LAMP” – Unlocked Versatility

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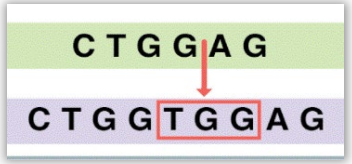
“Lyo-LAMP” is suitable for the detection of different **DNA markers**, such as:

- Species-specific genes

- SNP-genotyping
(Single nucleotide polymorphism)

- InDel-genotyping
(insertion-deletion)



Targets:

- Human genome
- Farm-animal and pet genome
- Microorganism genome (bacteria, parasites, virus)
- Food

Applications

- Infectious diseases
- Genetic predisposition and susceptibility
- Pharmacogenetics and nutrigenetics
- Food safety and traceability
- Environmental monitoring (water, biosolids, waste-water)
- Bio-terrorism

Deep dive into “Lyo-LAMP” – 3 simple POC-compliant steps

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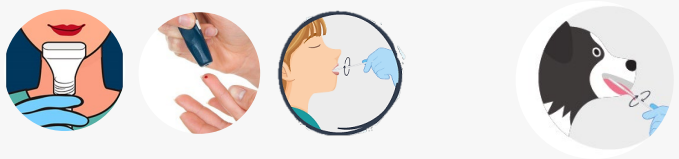
1 **Sampling**

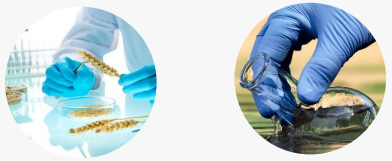
Different (target-depending) sample types, non-invasive procedures

Human and veterinarian samples



saliva – buccal/nasal swab - fingerprick

Food and beverages samples



Deep dive into “Lyo-LAMP” – 3 simple POC-compliant steps

ISTITUTO ITALIANO DI TECNOLOGIA

1 **Sampling**

2 **Streamlined protocol for DNA extraction**

Thermo-chemical (sample-depending) DNA extraction

- Ready-to-use extraction solution with RT storage
- Simple heater required
- A few simple operating steps

No DNA purification!

Total processing time: 5-10 mins!

Deep dive into “Lyo-LAMP” – 3 simple POC-compliant steps

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1. **Sampling**
2. **Streamlined protocol for DNA extraction**
3. **LAMP amplification step**

Total reaction time from 7 to 40 mins!

- High-performing LAMP with specific primers
- LyoBeads technology
- Fluorescence-based detection (real-time)
- Open test (no test-associated instrument)
- Simple instrumentation required

The test design prevents **LAMP carry-over cross-contaminations**:

- ✓ Using a one-pot closed-tube format for amplification and detection;
- ✓ Integrating an enzymatic system for contamination degradation.

“save-time” test for fast screening

Deep dive into “Lyo-LAMP” – Customized lyo-bead technology

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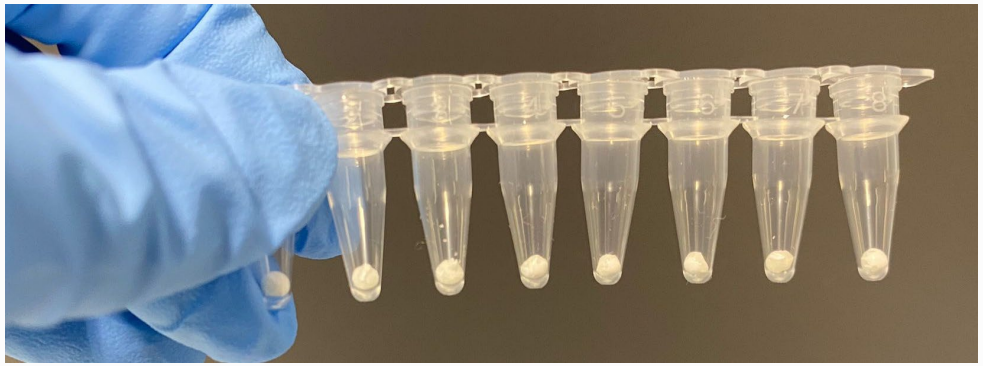


- Pre-dispensed LAMP mix in freeze-dried spheres (1 bead = 1 reaction)
- Specific customized primer set design

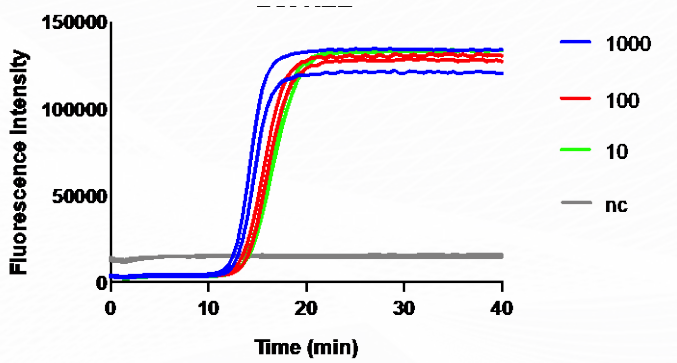
Simple handling

Lyo-bead + buffer + DNA extract

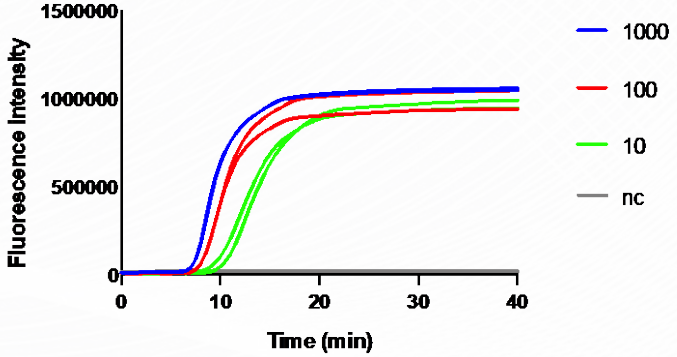
- Ready-to-use
- Customized for our tests (optimized bead composition)
- Long-term stability, room temperature shipping and storage
- Scale-up production

Salmonella enterica

Competitor in solution



Lyo-LAMP



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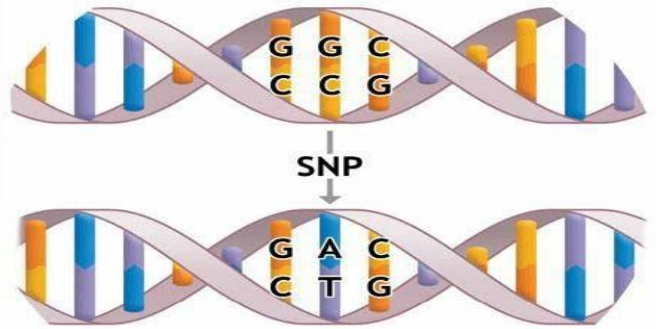
Real-Time SNP and InDel-Genotyping

“Lyo-LAMP” applications: Real-Time SNP and InDel-Genotyping



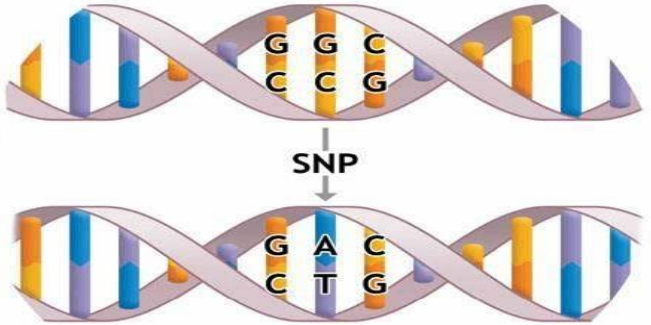
SNPs (single nucleotide polymorphism)

genetic point mutations, that recur with a frequency of at least 1% in a species population

InDels (insertion-deletion polymorphism)

indels can be small, involving just a few base pairs, or larger, spanning several base pairs

“Lyo-LAMP” applications: Real-Time SNP and InDel-Genotyping



Ideal marker

SNPs are involved in development and progression of different diseases, in the genetic susceptibility and predisposition to specific conditions, in pharmacogenetics, in varietal discrimination, etc.

Challenging marker

- Complex and expensive procedures
- High background signal
- High-quality DNA samples

Traditional methods
e.g. microarrays, PCR-RFLP, TaqMan assays, Next-generation sequencing…

→ ~~POC testing~~

Real-Time SNP-Genotyping with “Lyo-LAMP”: The Detection Strategy

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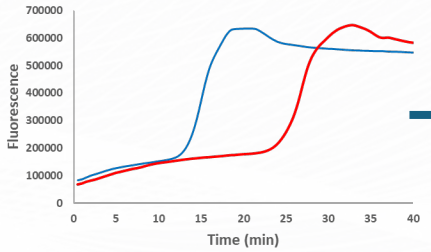
Double-tube test	TARGET GENOTYPE: SNP-mutated	TARGET GENOTYPE: SNP-wild type	TARGET GENOTYPE: SNP-heterozygous
tube 1 **mut LAMP-probe**	Perfect match ✓	single-base mismatch ✗	Perfect match ✓
tube 2 **wt LAMP-probe**	single-base mismatch ✗	Perfect match ✓	Perfect match ✓

Lyo-LAMP design → **amplification efficiency** → perfect match > single-base mismatch

- perfect match → Fast LAMP
- single-base mismatch → Slow LAMP



Time gap reading for the test result

↓

Single-base discrimination

Real-Time SNP-Genotyping with “Lyo-LAMP”: The Detection Strategy

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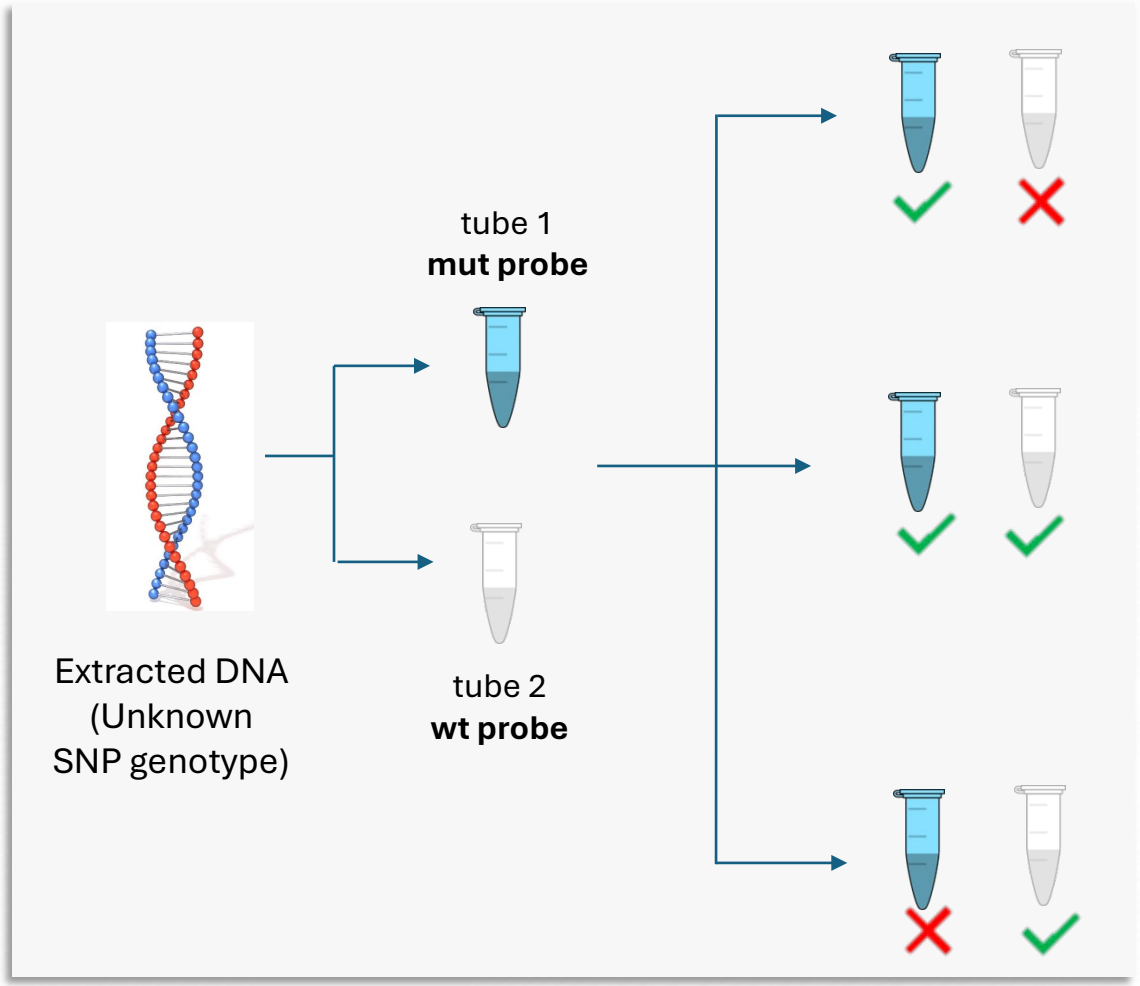
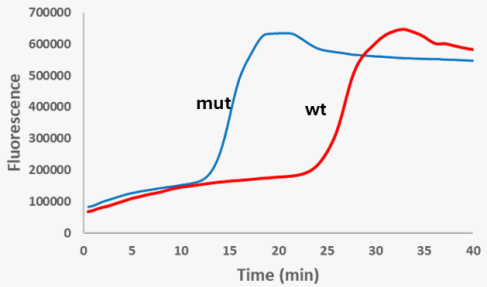
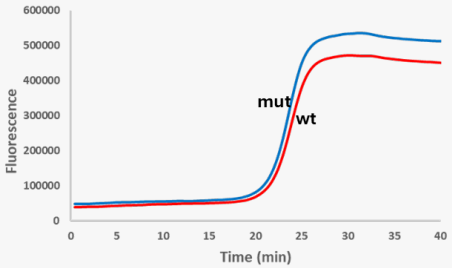


TEST READOUT

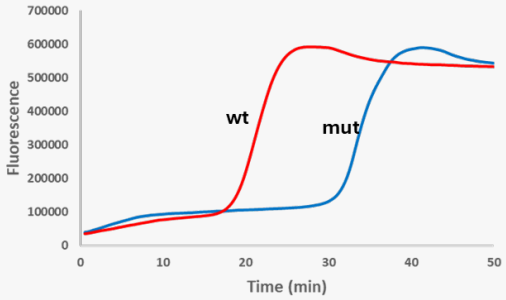
TEST RESULT



mut
homozygous



mut-wt
heterozygous



wt
homozygous

Real-Time SNP-Genotyping with “Lyo-LAMP”: Illustrative Workflow

ISTITUTO ITALIANO DI TECNOLOGIA

ALL-INCLUSIVE TEST

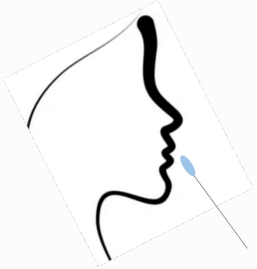
Components:

- Ready-to-use extraction solution
- mut lyo-bead tube
- wt lyo-bead tube

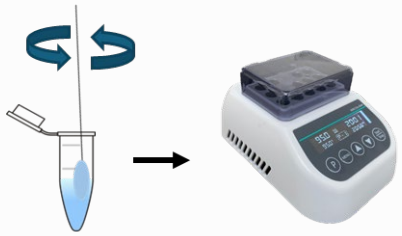
Test results:

- homozygous mutation
- heterozygous
- no mutation

3 simple steps!



Collect the buccal swab

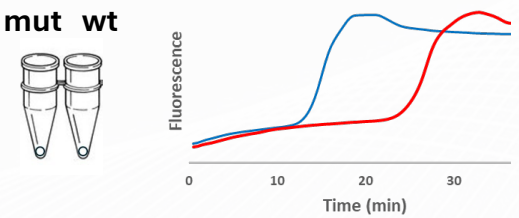


95 °C, 5 min

Place and stir the swab into the extraction solution

Place the tube into a heater set at 95 °C, for 5 minutes

Purification-free



Resuspend the wt and mut lyobeads and add the extract into each tube

Place the 2 reaction tubes into the instrument

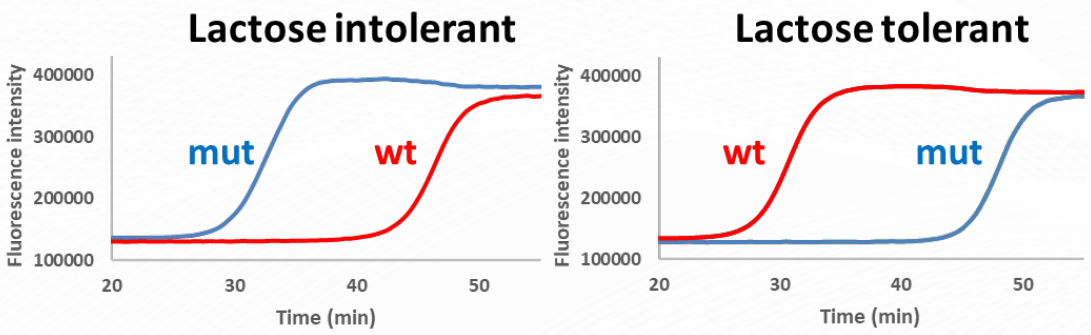
The test is completed within 30-35 minutes

1. Sample Collection **20 seconds**
2. Sample Prep **5 minutes**
3. Test Execution **35 minutes**

Real-Time SNP and InDel-Genotyping with “Lyo-LAMP”: Test Panel

ISTITUTO ITALIANO DI TECNOLOGIA

Target	Marker type	Sample	Application
MCM6 gene	13910 C>T (SNP)	buccal swab	Lactose intolerance
MTHFR gene	677 C>T (SNP)	buccal swab	Hyperhomocysteinemia and folate level
*CYP2C19*2*	681 G>A (SNP)	buccal swab	Antiplatelet drug (clopidogrel) metabolism
*CYP2C19*3*	636 G>A (SNP)	buccal swab	Antiplatelet drug (clopidogrel) metabolism
ACE gene	rs4340 in287 (InDel)	buccal swab	Salt sensitivity
MDR1 gene (dog)	nt230 del4 (InDel)	buccal swab	P-gp dependent drugs toxicity



Genetic Predisposition to Lactose Intolerance

Opportunity for customizable targets!

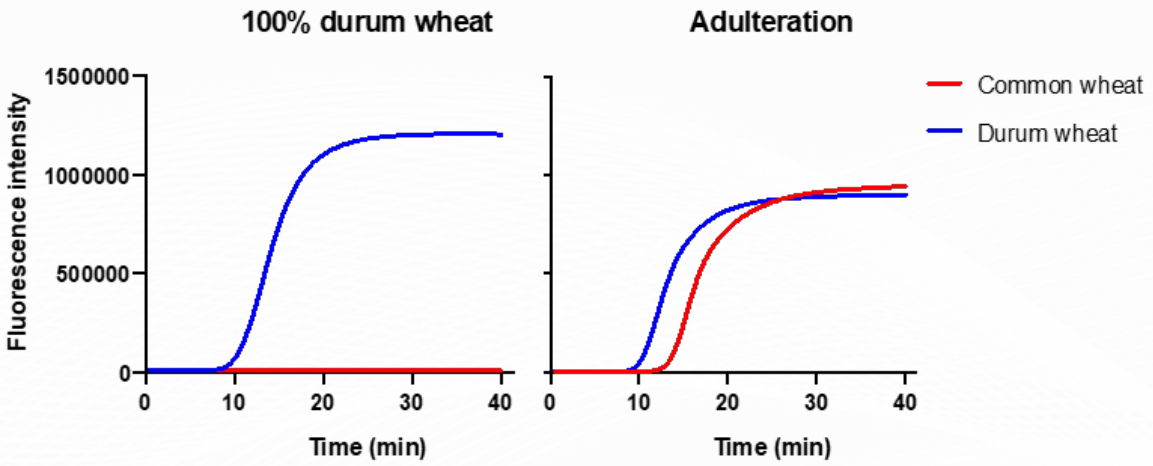
2

Food Fraud and Traceability

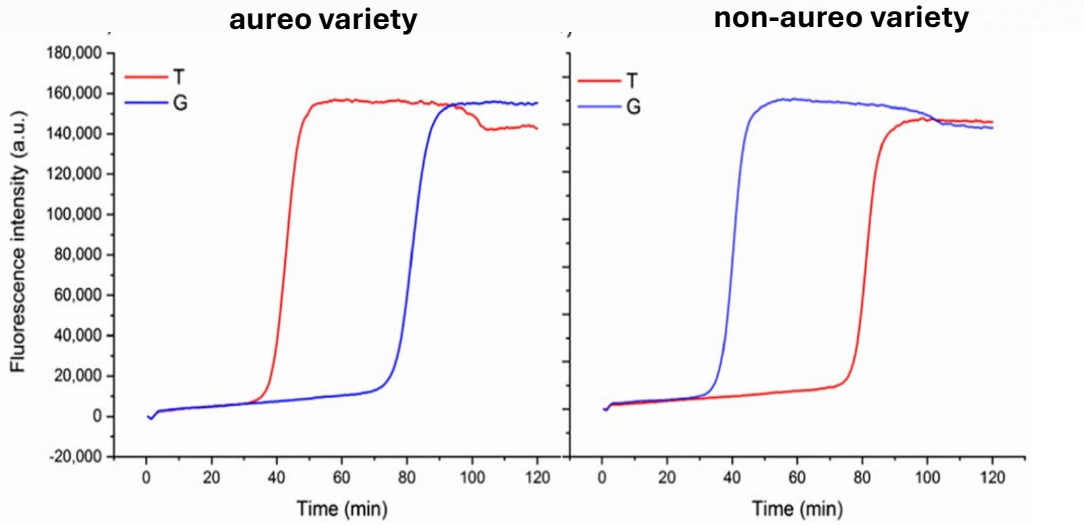
Food Fraud and Traceability with “Lyo-LAMP”: Test Panel

ISTITUTO ITALIANO DI TECNOLOGIA

Target	DNA marker	Matrix	Sensitivity	Application
Triticum aestivum (common wheat) vs Triricum durum (durum wheat)	species-specific gene	wheat	0.3% adulteration	species discrimination
Durum wheat «aureo» variety vs durum wheat «non aureo» varieties	SNP on Chr7A	wheat	10% adulteration	varietal discrimination



Species Discrimination



Varietal Discrimination

Barilla

Routine Quality Controls

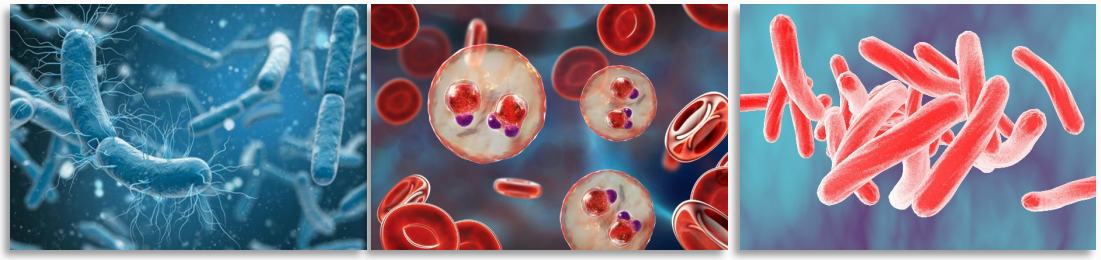
3

Microorganism Detection

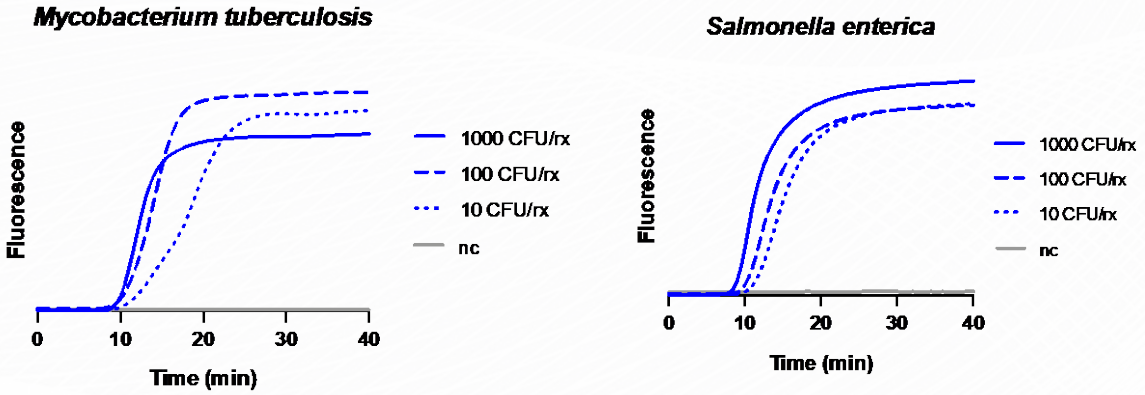
Microorganism detection with “Lyo-LAMP”: Test Panel

ISTITUTO ITALIANO DI TECNOLOGIA

Target	Matrix/sample	Application
Escherichia coli	tap water milk	water contamination bovine mastitis
Legionella pneumophila	tap water	water contamination
Aeromonas hydrophila	tap water	water contamination
Salmonella enterica	biosolids	biosolids contamination
Enterococcus faecium	tap/waste water	water contamination
Pseudomonas aeruginosa	milk	bovine mastitis
Streptococcus pneumoniae	nasal swab	sinusitis
Staphylococcus aureus (MRSA vs. MSSA)	nasal swab	sinusitis
Clostridium tyrobutyricum (spores)	milk	cheese spoilage
Mycobacterium tuberculosis	saliva	tuberculosis
Plasmodium spp.	finger prick	malaria



Reaction time: 10-20 mins
Sensitivity: 2-10 copies/reaction



Opportunity for customizable targets!

Diagnosis of *Mycobacterium tuberculosis* – Alternative Methods

Traditional Methods Include:

SMEAR microscopy

Culture

PCR based methods

Antigen detection

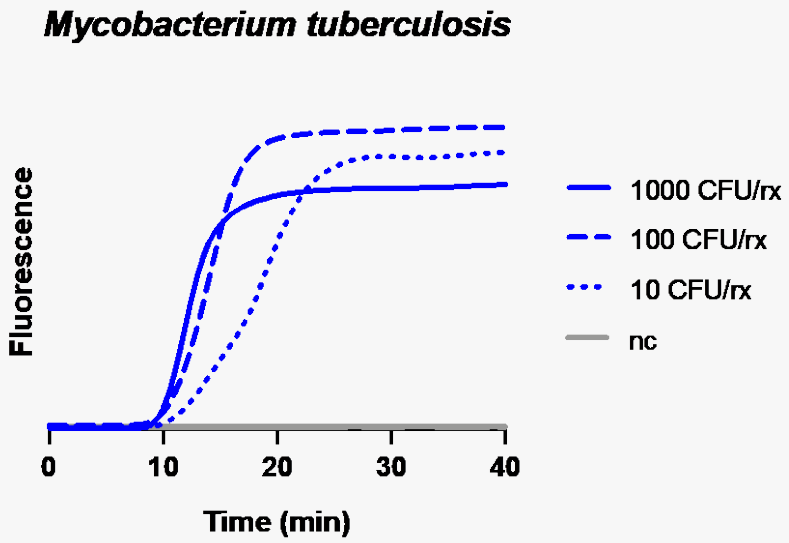
Phage based assays

Tuberculin skin testing

Gold-standard POC competitor (example)

- PCR based
- ~77 minutes TTR
- Device-associated test

"Lyo-LAMP"



Infectious Disease Detection with “Lyo-LAMP”: Illustrative Workflow

ISTITUTO ITALIANO DI TECNOLOGIA

ALL-INCLUSIVE TEST

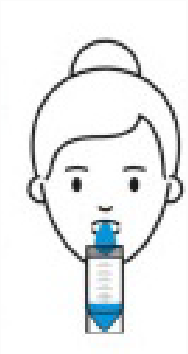
Components:

- ready-to-use extraction solution;
- reaction tube with the LAMP-lyobead

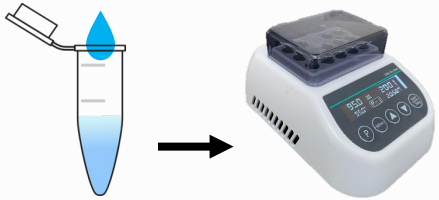
Test results:

- Positive or Negative

3 simple steps!



Collect the saliva sample

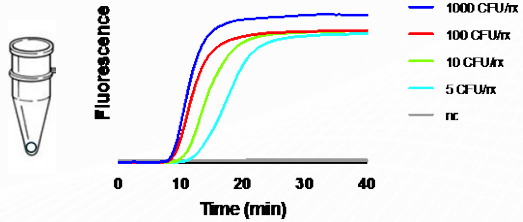


95 °C, 5 min

Add saliva into the extraction solution;

Place the tube into a heater set at 95 °C, for 5 minutes.

Purification-free



Resuspend the lyobead and add the extract

Place the reaction tube into the instrument

The test is completed within 10-20 minutes

1. Sample Collection
20 seconds

2. Sample Prep
5 minutes

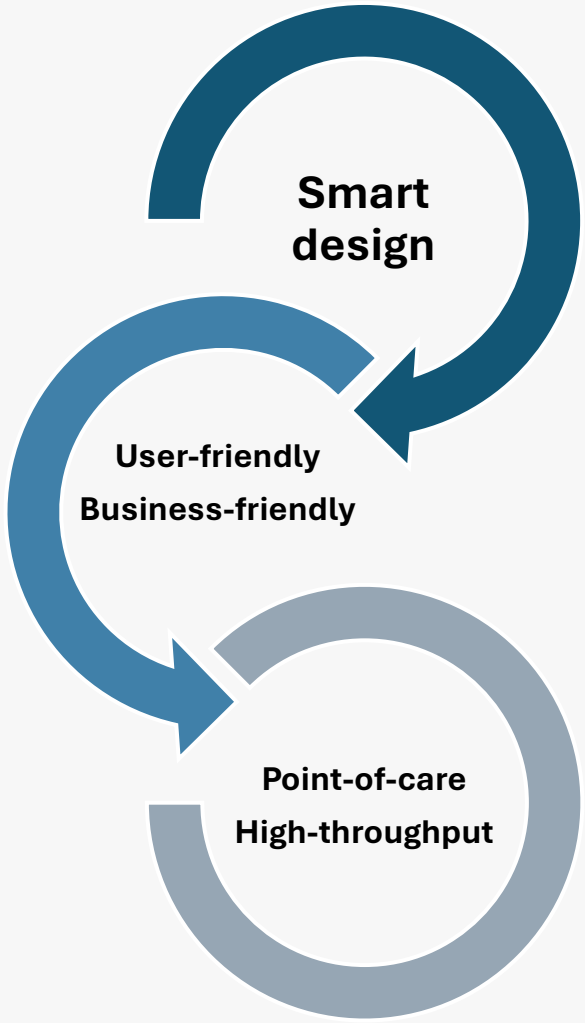
3. Test Execution
10-20 minutes

Conclusions

ISTITUTO ITALIANO DI TECNOLOGIA

Lyo-LAMP

High-performing LAMP in Lyophilized format



- Fast
- Sensitive
- Specific
- No DNA-purification
- RT stable
- Scalable

Customizable

SAMPLE matrix and volume

Technology FLEXIBILITY

- Challenging targets (SNP)
- Ultrasensitivity detection (e.g., microorganisms)

Application VERSATILITY

- Infectious diseases
- Genetic predisposition and susceptibility
- Pharmacogenetics and nutrigenetics
- Food safety and traceability
- Environmental monitoring (water, biosolids, waste-water)
- Bio-terrorism

What the Future Holds

ISTITUTO ITALIANO DI TECNOLOGIA

If you are interested in collaborating, we would be excited to hear from you!

Amplify Diagnostics With Lyo-LAMP, your partner in Point-of-Care Excellence!

Dr. Paola Cecere
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Istituto Italiano Di Tecnologia

Dr. Pier Paolo Pompa
Research Director –
Istituto Italiano Di Tecnologia

Dr. Giuseppina Sannino
Global Business Development Manager –
Medix Biochemica

Thank You – Any Questions?


Thank you
Medix Biochemica

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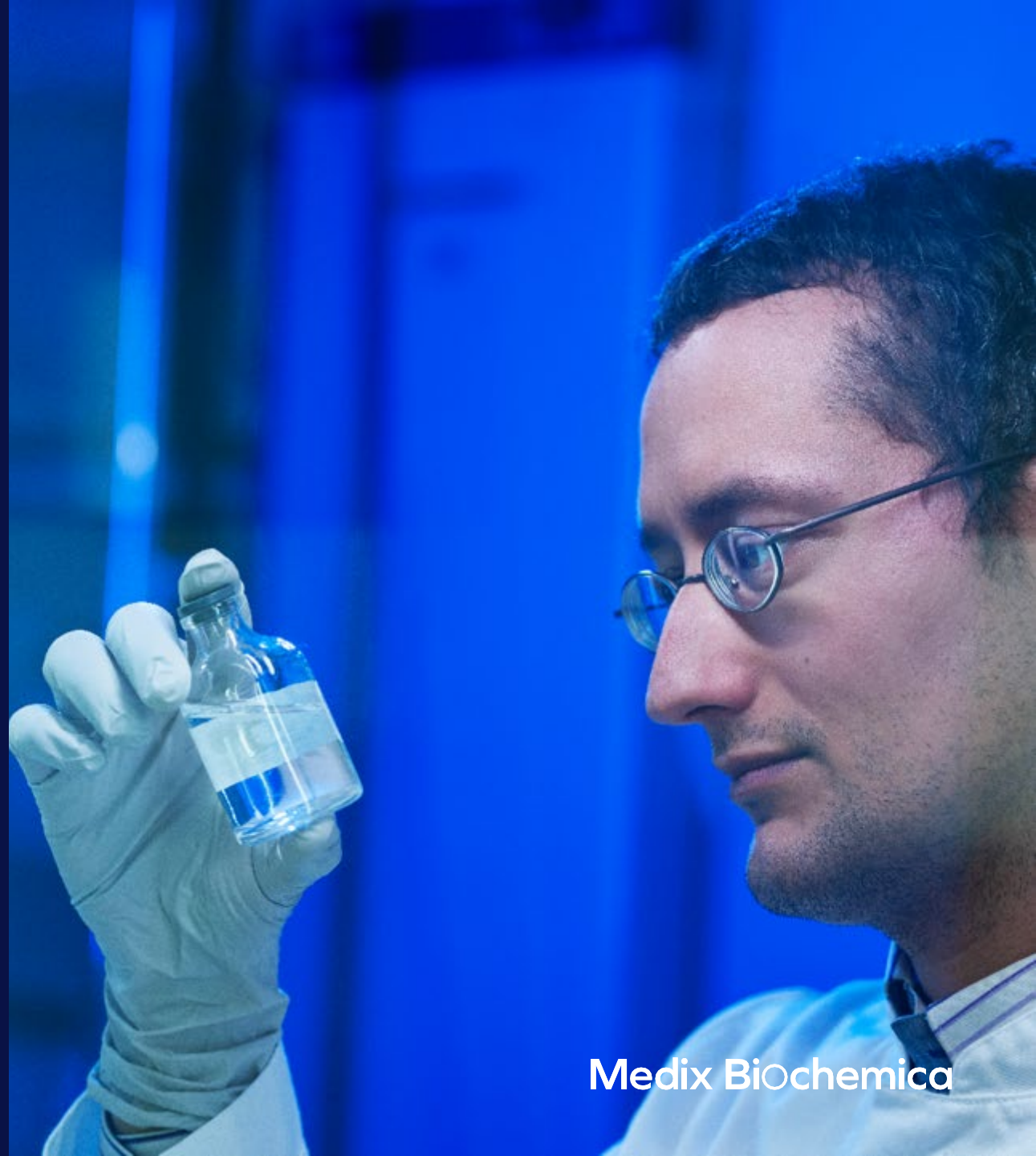

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