

Fiocruz Paraná Pesquisa, Desenvolvimento e Produção





Gelified real-time PCR assay for Malária Diagnosis

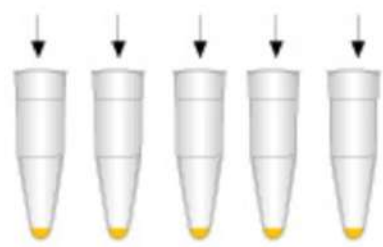
December/2011

Responsável: Cheysa Biondo

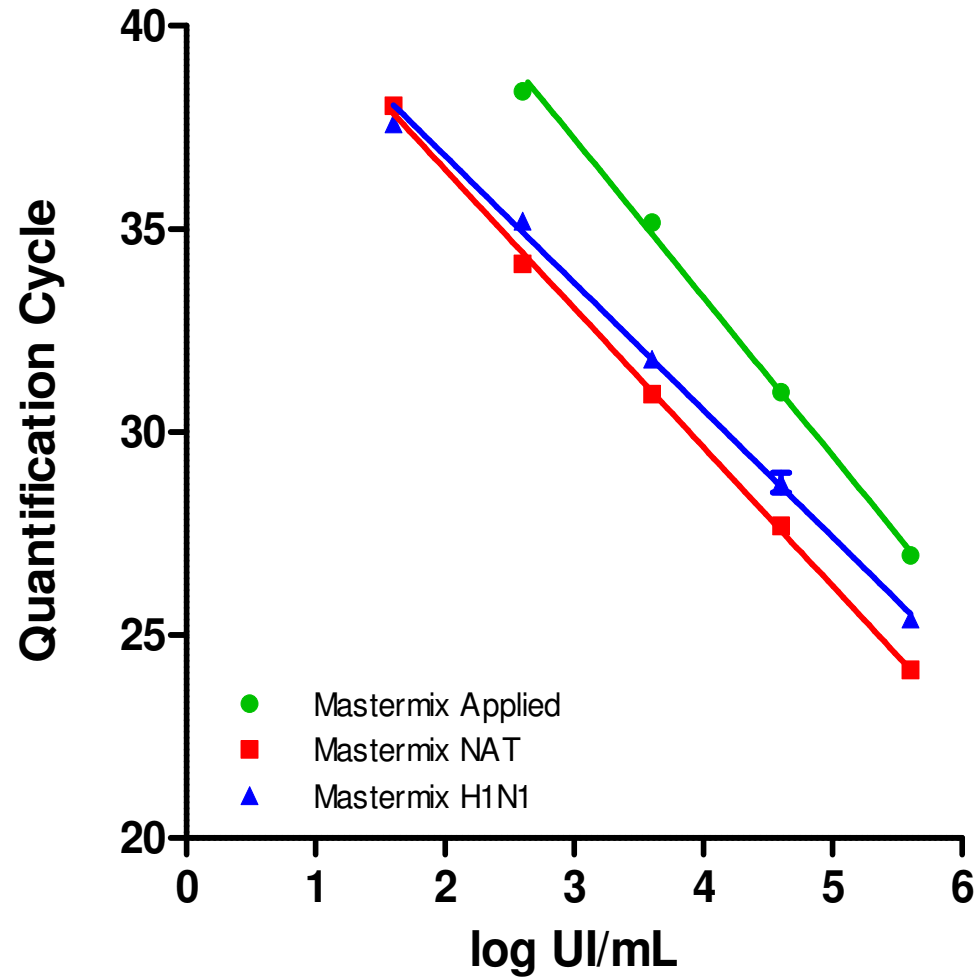
Data: 08/12/2011

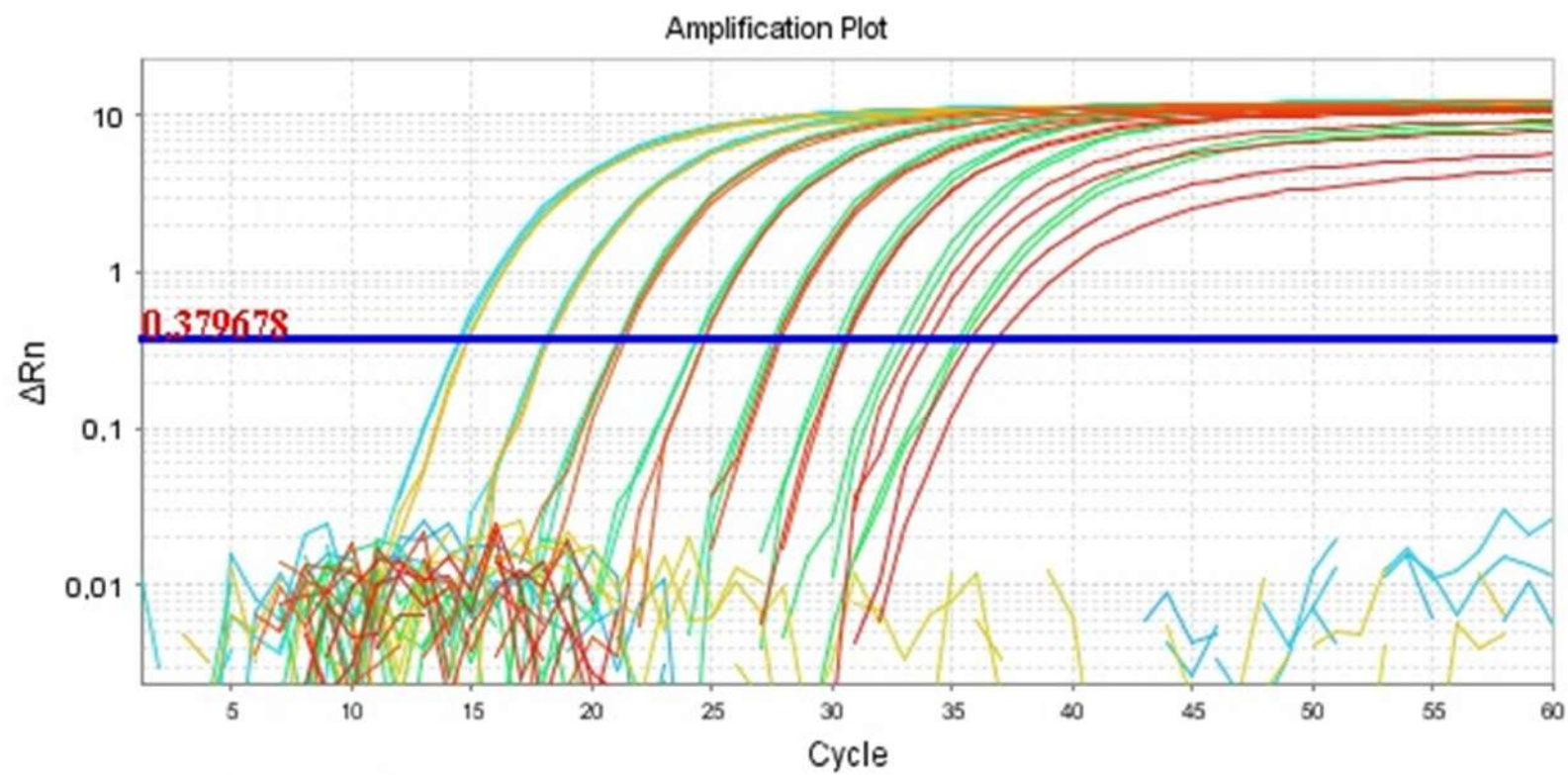


H₂O
+
Purified Nucleic Acid
from clinical sample



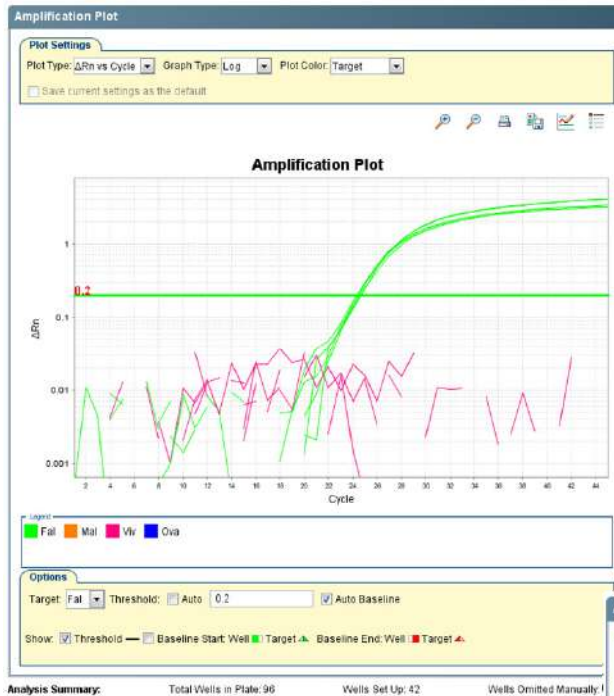
Comparação de desempenho das Mastermix:





HBV gelificada e líquida sobrepostas

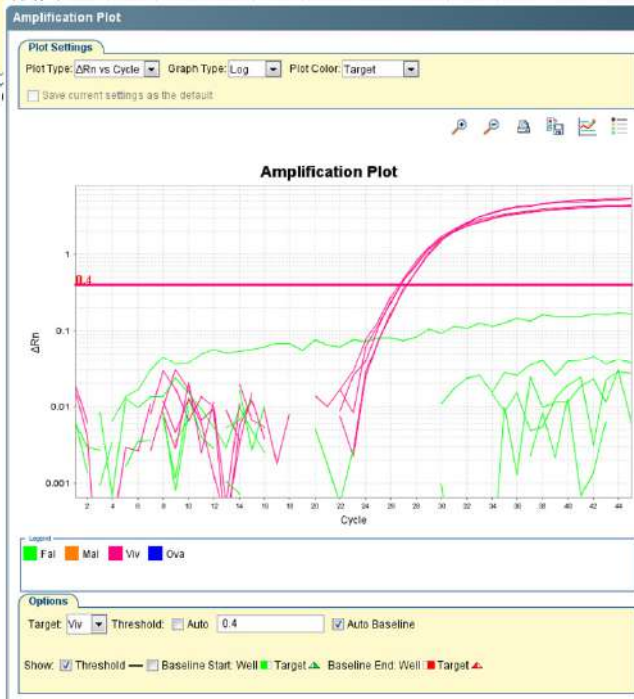
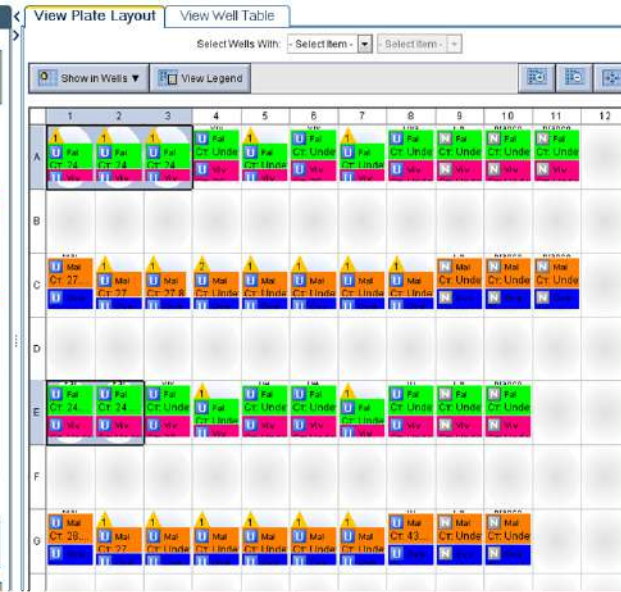
After 24 hours 4°C



Analysis Summary: Total Wells in Plate: 96 Wells Set Up: 42 Wells Omitted Manually: 0

P. vivax
Gelified + Liquid

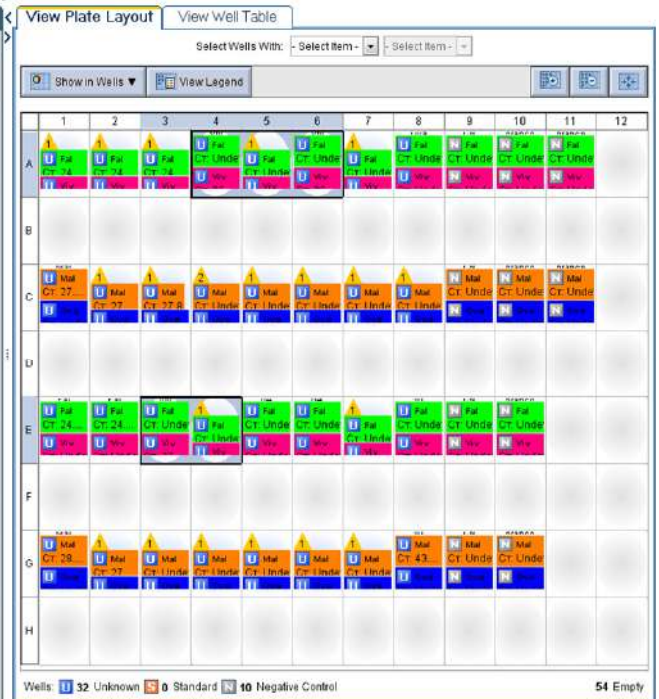
Gel mean Ct → 26,8
Liq mean Ct → 27,3



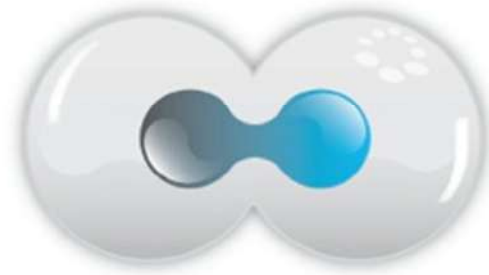
Analysis Summary: Total Wells in Plate: 96 Wells Set Up: 42 Wells Omitted Manually: 0

P. falciparum
Gelified + Liquid

Gel mean Ct → 24,6
Liq mean Ct → 24,7



Wells: 32 Unknown 0 Standard 10 Negative Control 54 Empty
Wells Flashed: 70 Wells Omitted by Analysis: 0 Samples Used: 8 Targets Used: 4



Gen Cell

b i o s y s t e m s

Company and Technology Introduction to:

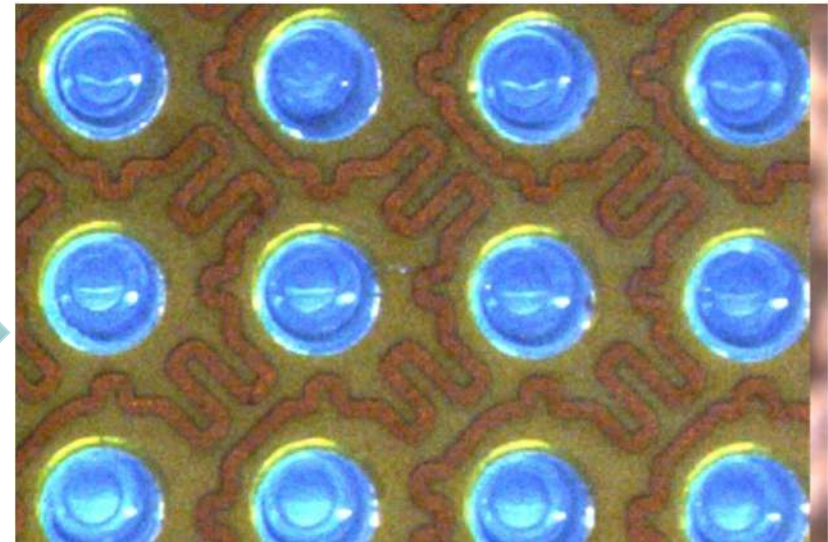
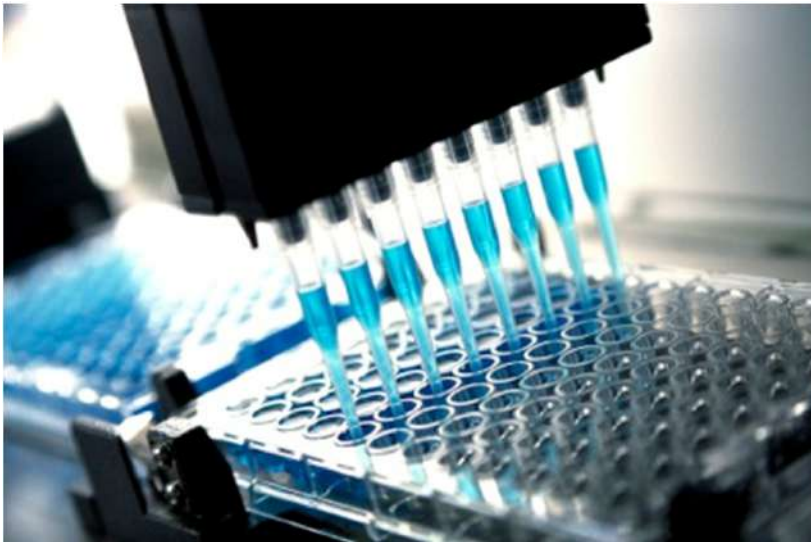


24th February 2013

Kieran Curran Ph.D
CEO

Mission:

To develop and commercialize *better* automated solutions for life sciences.

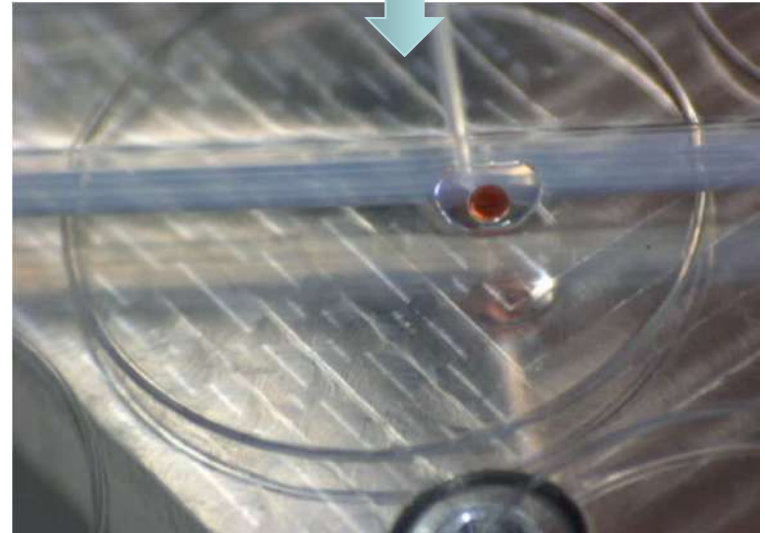
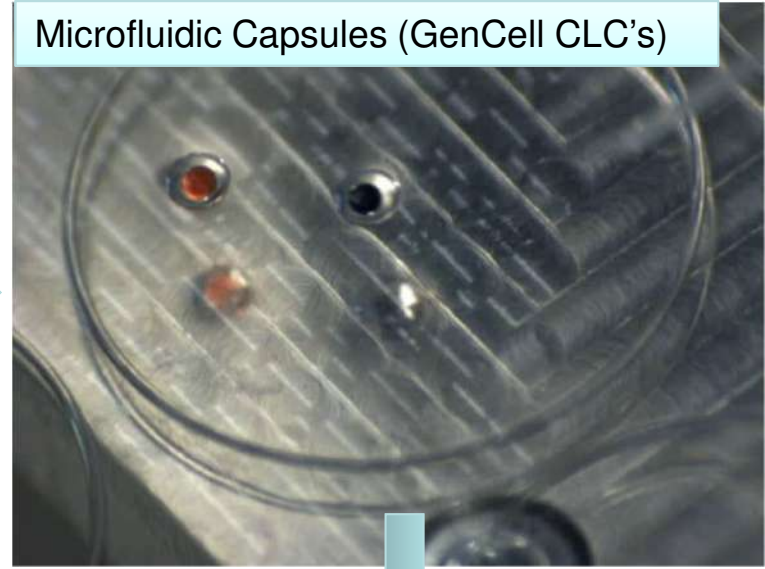
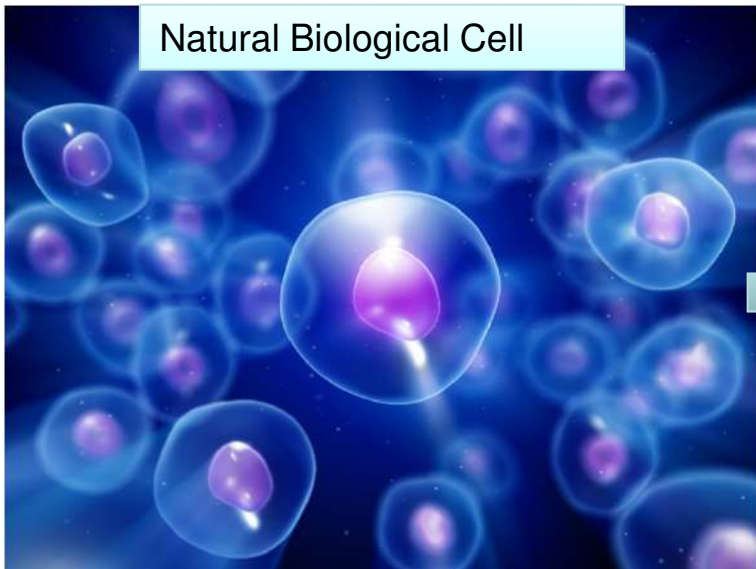


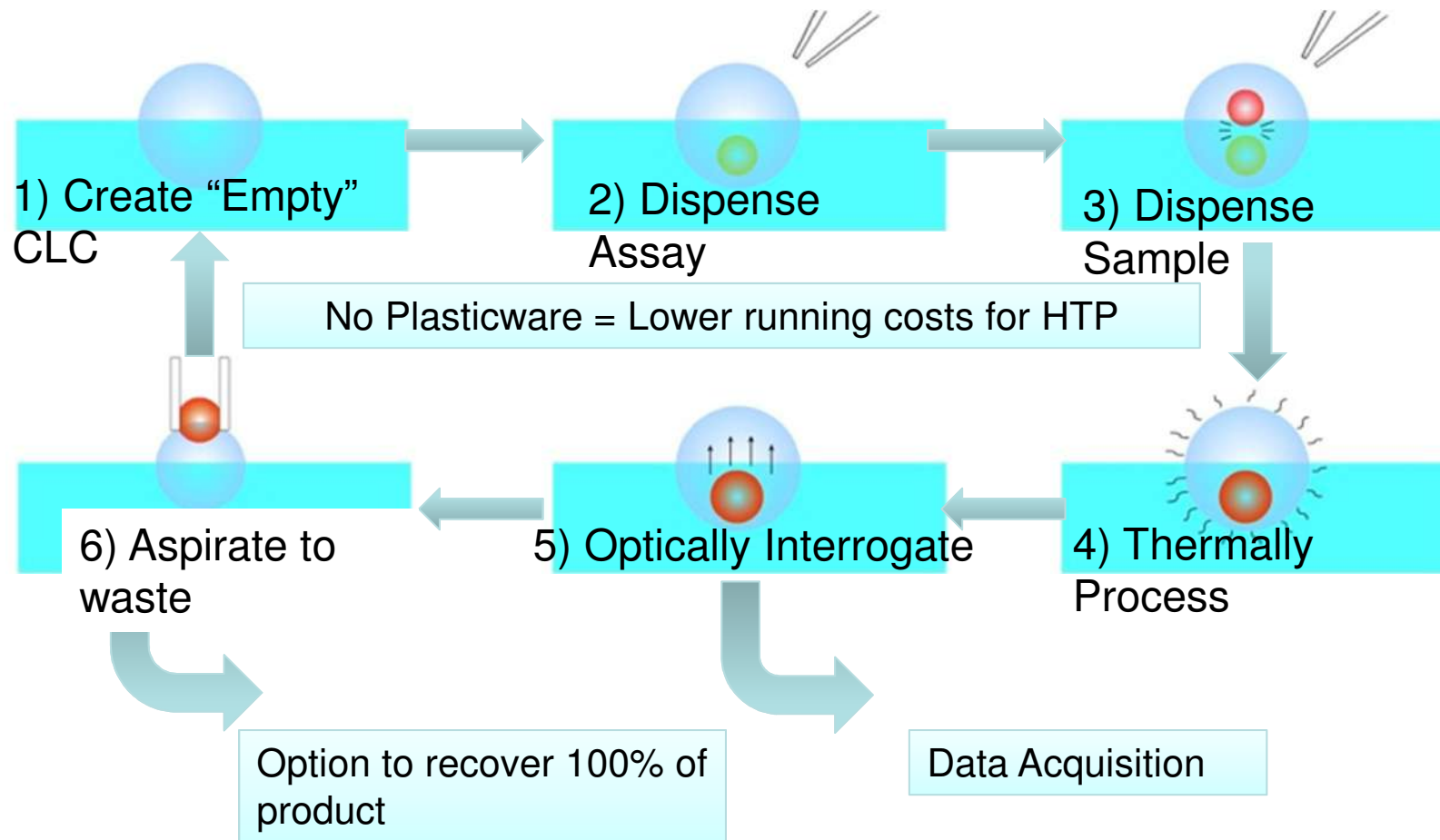
Conventional Automation Tech

- ✓ Widely applicable
- ✗ Miniaturization a challenge
- ✗ Consumable plasticware

GenCell's *Composite Liquid Cell* Tech

- ✓ Widely applicable
- ✓ Automation "friendly" microfluidics
- ✓ No plastic consumables





High Throughput: 960 libraries per day

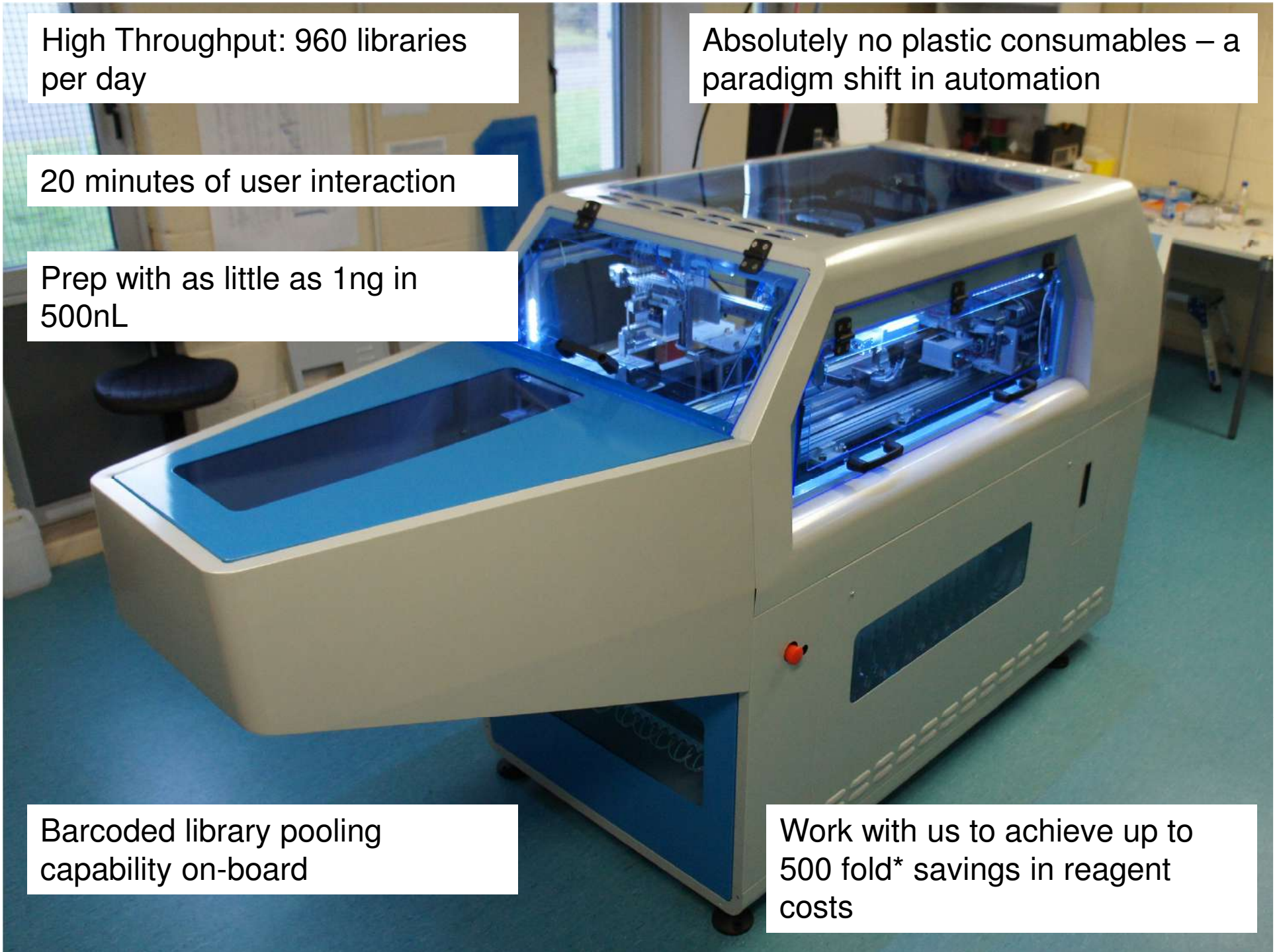
Absolutely no plastic consumables – a paradigm shift in automation

20 minutes of user interaction

Prep with as little as 1ng in 500nL

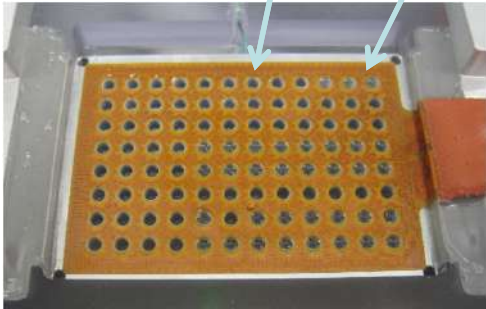
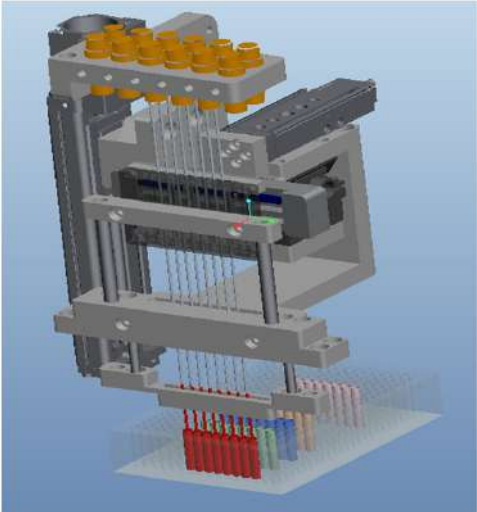
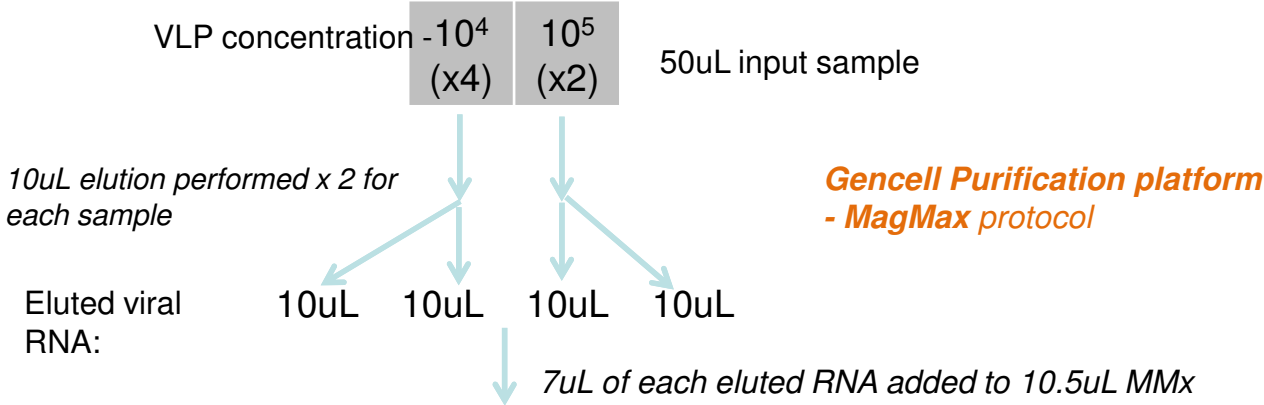
Barcoded library pooling capability on-board

Work with us to achieve up to 500 fold* savings in reagent costs





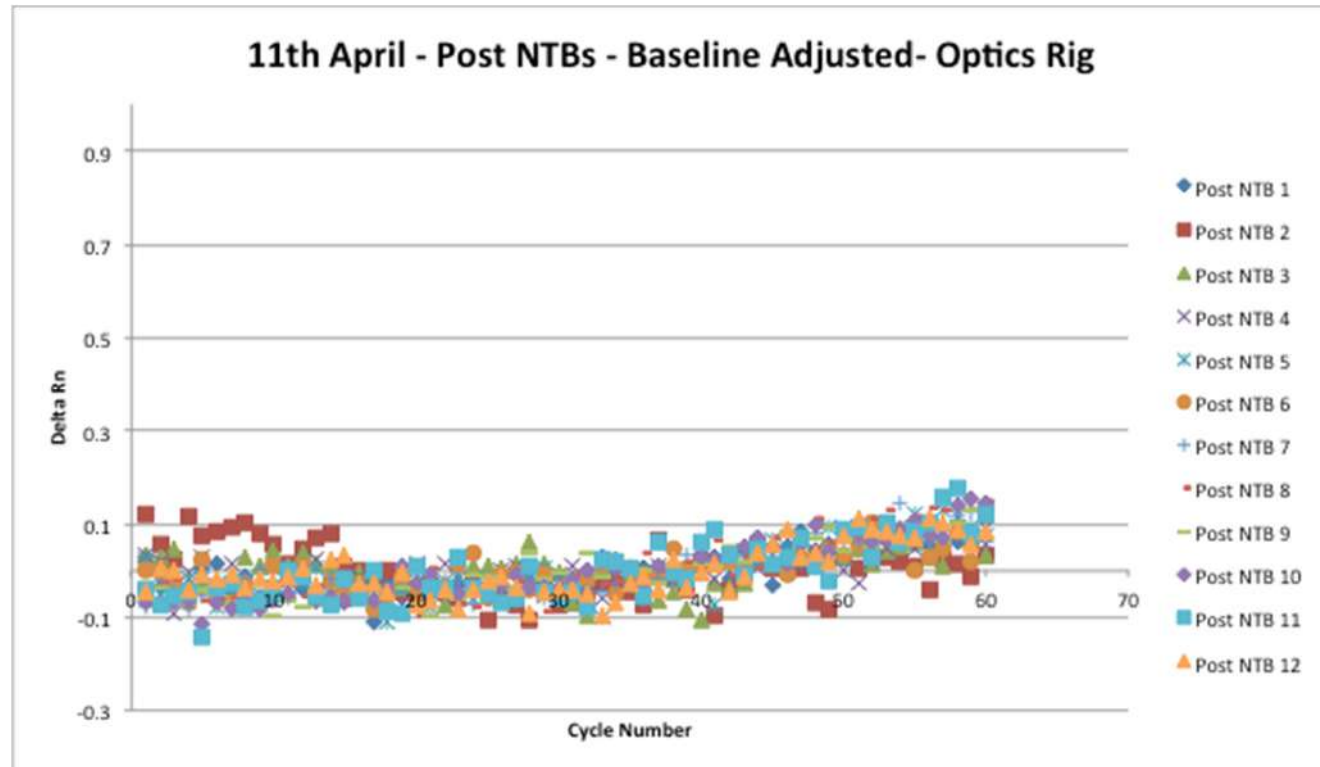
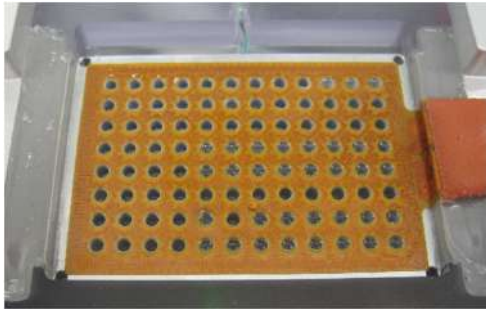
Gencell Purification Instrument followed by amplification in CLC on Gencell qPCR platform and on ABI StepOne. Magmax kit.



Viral RNA Purification and amplification on commercial instrument:

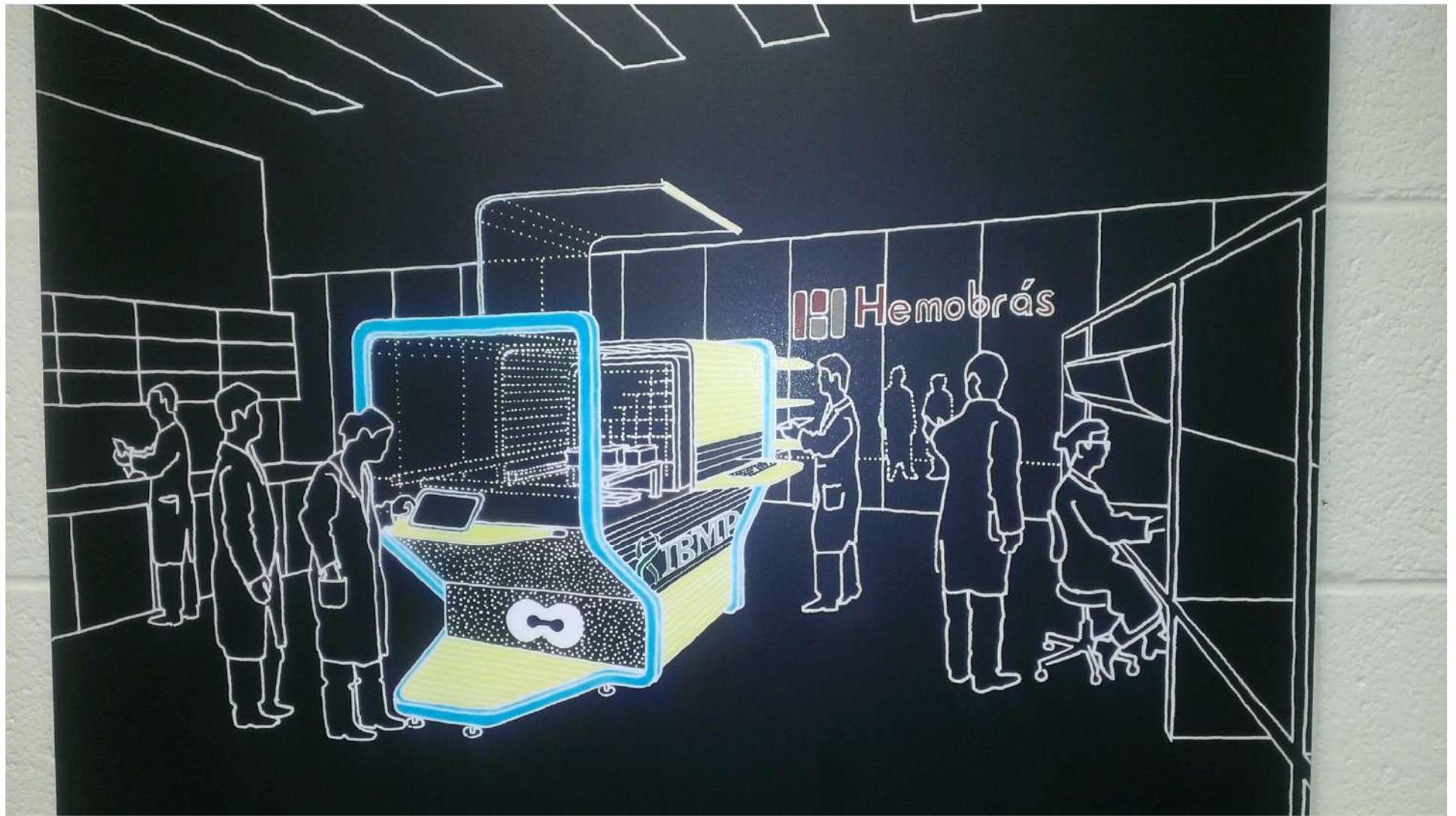
Viral Copies	Spin Column IBMP	Standard Magnetic Plate	Gencell
20,000	✓	✓	✓
2,000	✓	✓	✓
200	✓	✗	✓
20	✓	✗	✓
7	✓	✗	✓

12 x 5uL CLCs containing No Template Blanks (NTB's) performed on Gencell Purification Platform post run on Wednesday 10/4/13



Conclusion: No detectable carry over contamination

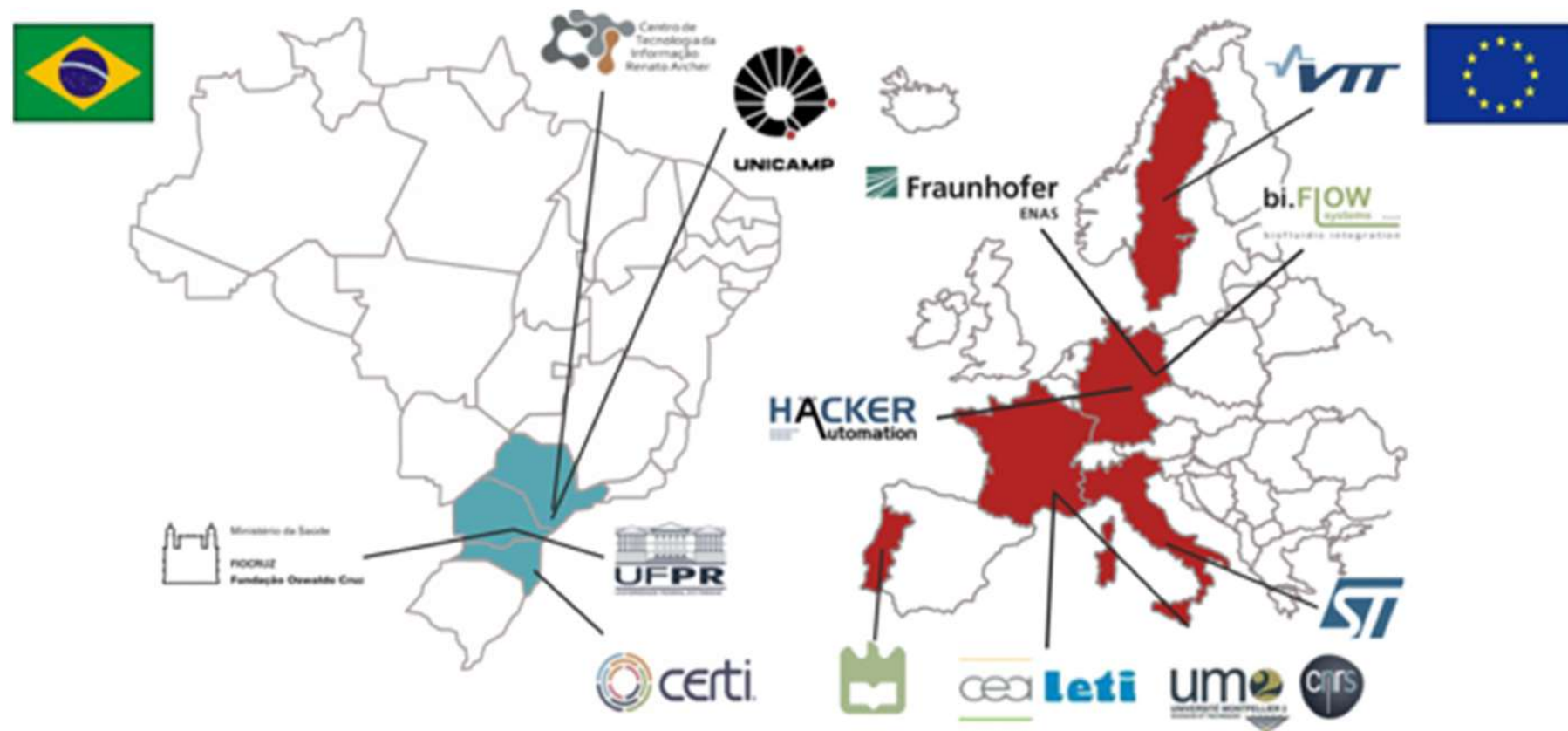
Confidential





PodiTrodi

Point-of-care diagnostics for tropical diseases

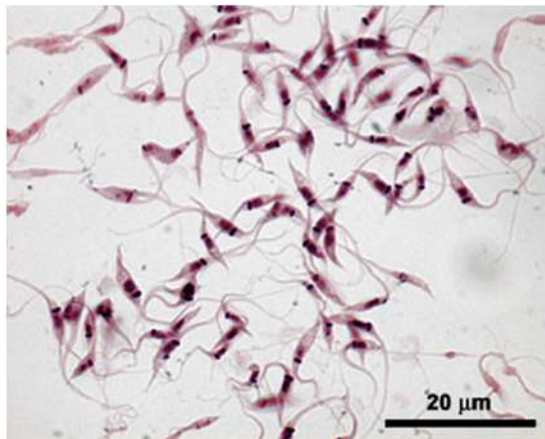


POC Real Time qPCR (detecção com quantificação 1 amostra /hora)



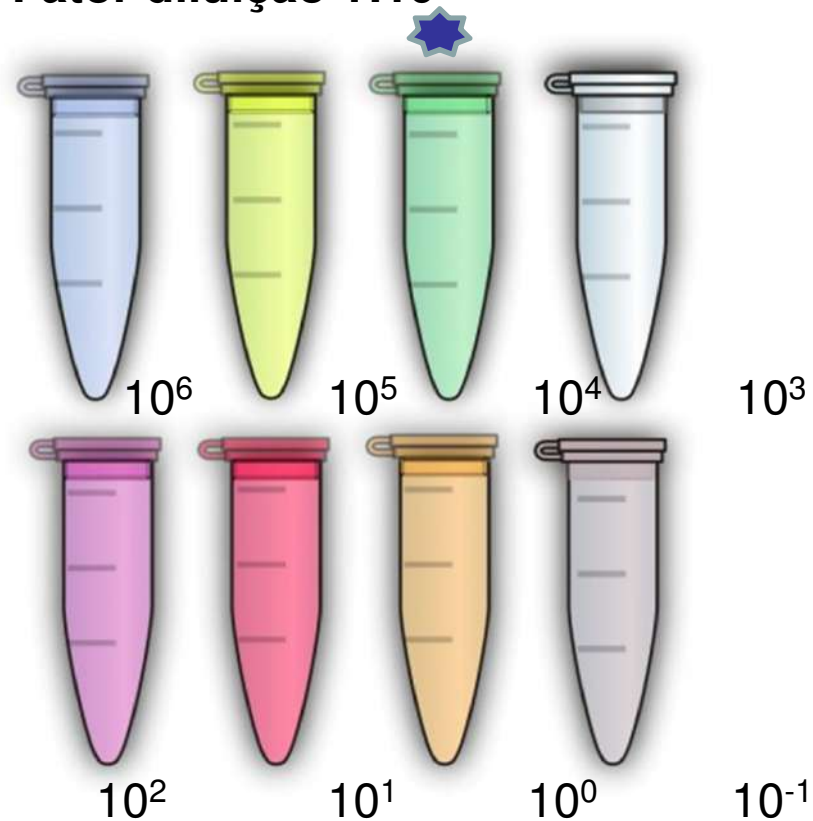
Procedimento Experimental

Extração de DNA
 10^7 epimastigotas



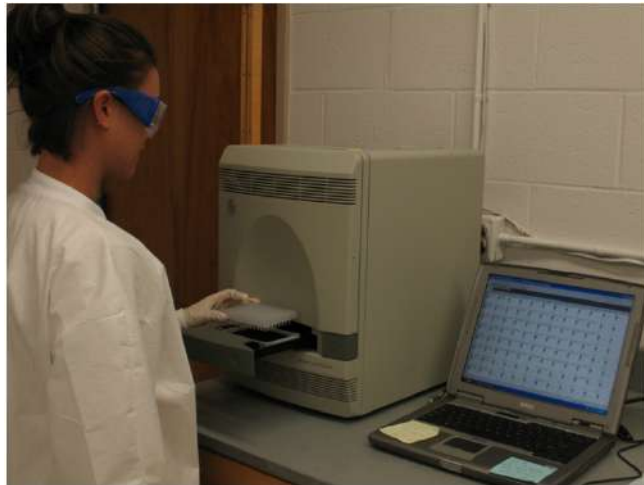
FONTE Portal Fiocruz

Diluições em PBS: $10^7 - 10^{-1}$
Fator diluição 1:10



Procedimento Experimental

Comparação de 2 tipos de equipamentos de PCR em Tempo Real



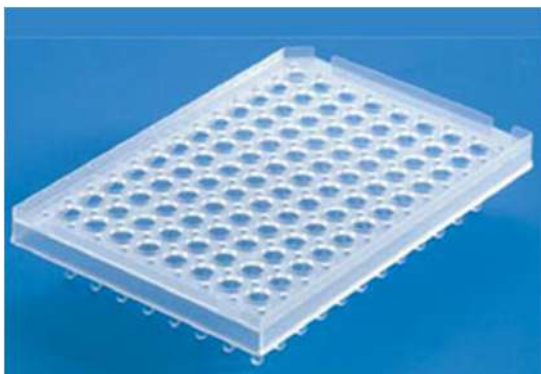
**DENATURAÇÃO
ANELAMENTO
EXTENÇÃO**



40.000 DÓLARES

1.000 DÓLARES

VOLUME DOS REAGENTES E AMOSTRA



1 hora e 30 minutos

Plástico



30 minutos

Silício

Resultados e Discussão

3 experimentos distintos

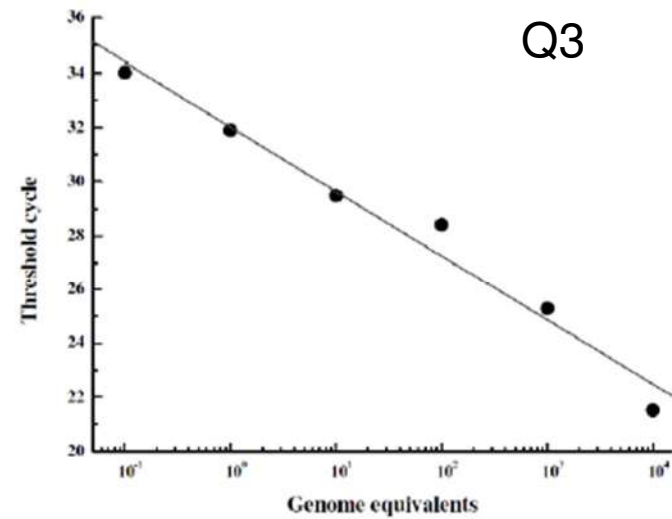
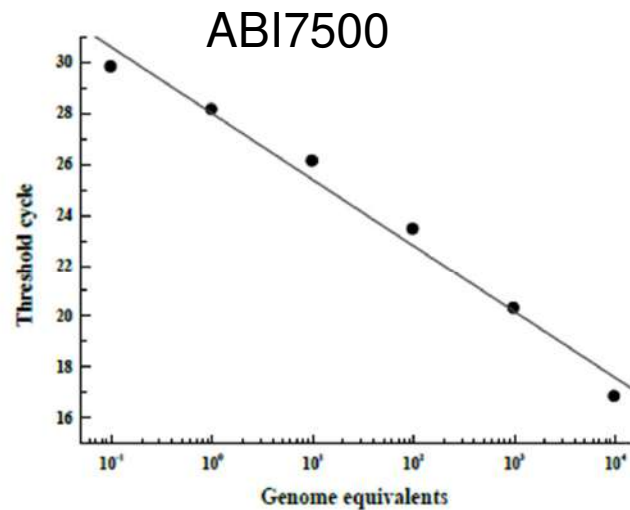
Equivalentes de genoma	ABI7500	Q3
10,000	16.8±0.4	21.5±0.3
1,000	20.3±0.6	25.3±0.6
100	23.4±0.7	28.4±0.7
10	26.1±0.1	29.5±1.9
1	28.1±2.2	31.9±3.6
0.1	29.8±1.8	34.0±1.9

ABI7500

Eficiência: 98,2%

Q3

Eficiência: 97,4%



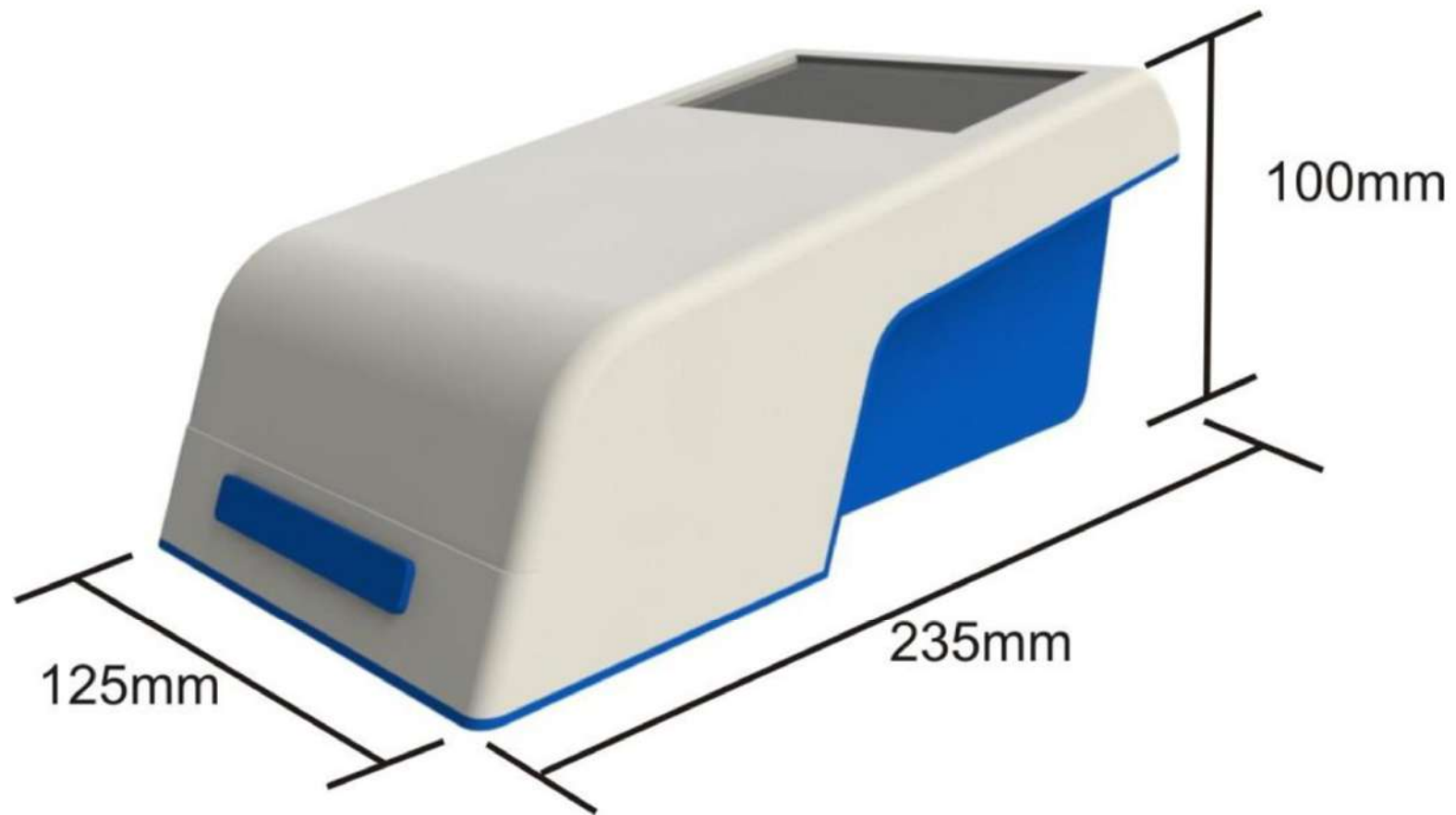


Figure 6 – Perspective view of the revised design concept.

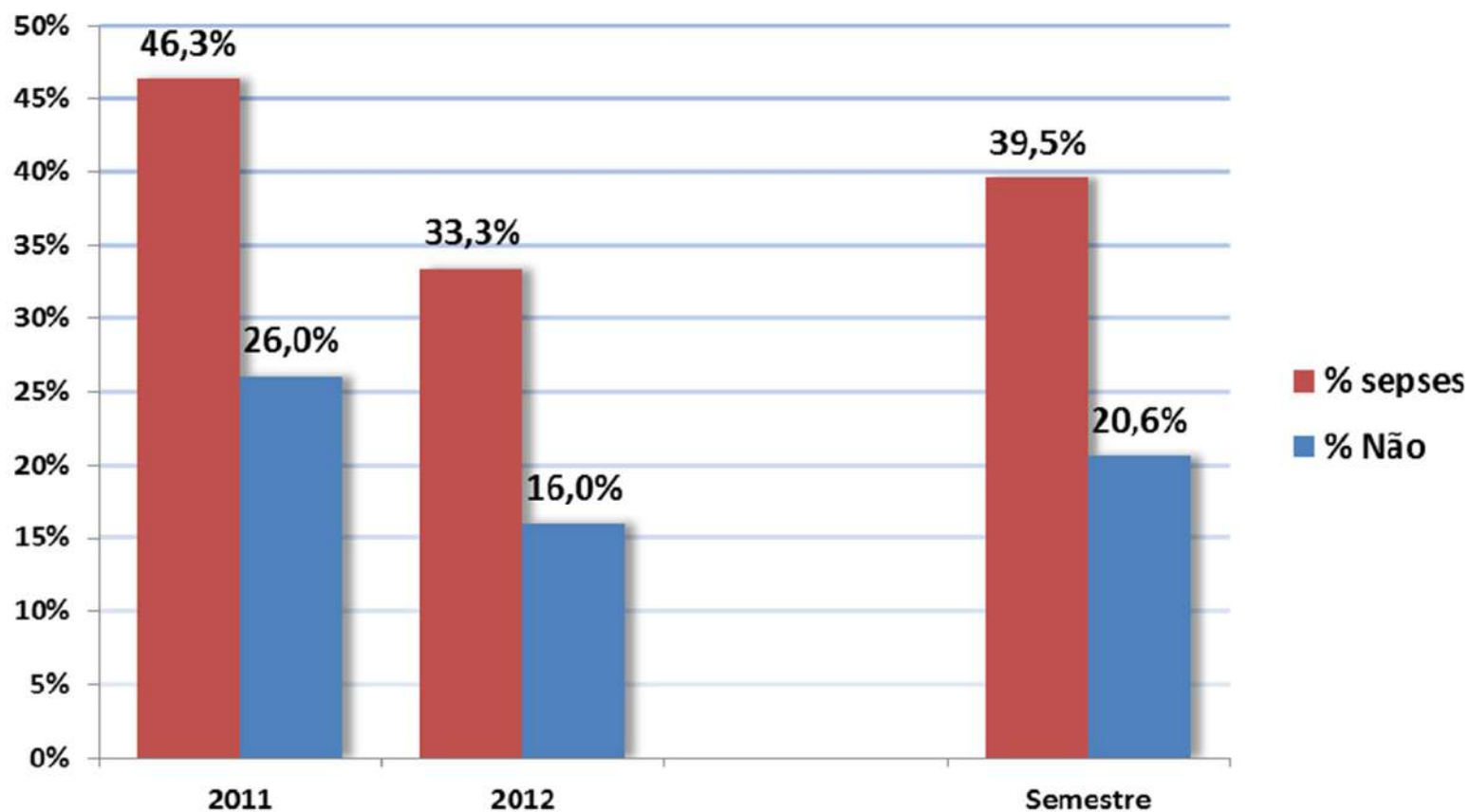




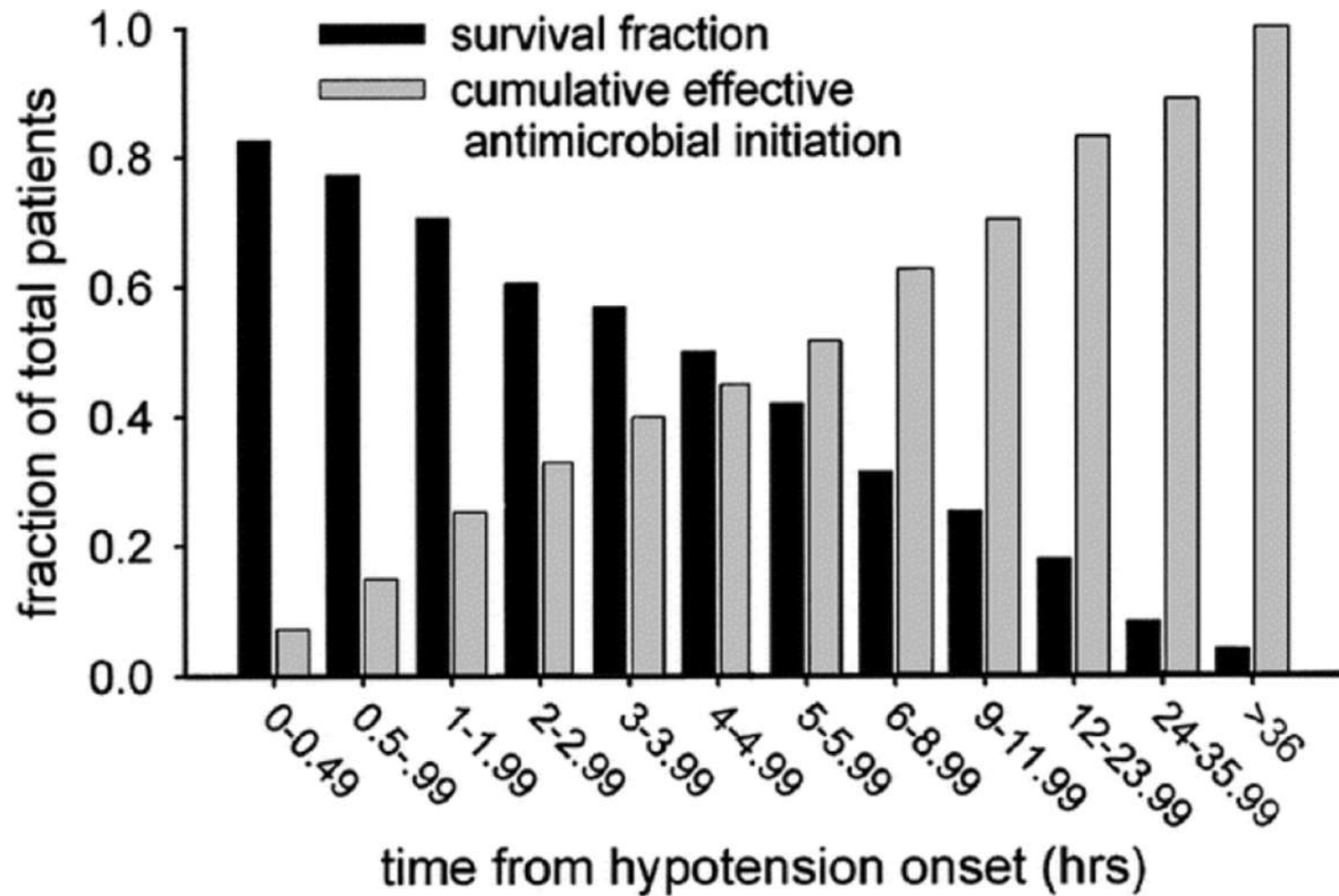
Desenvolvimento de Testes para Diagnóstico Molecular de patógenos Associados a Sepses.

Mortalidade observada por grupo

Últimos 6 meses – 114 admissões na UTI



Iniciar logo o antibiótico



Principais microorganismos causadores de sepse:

Enterobactérias (bacilos Gram-negativos)

Gram Negative

Escherichia coli

*Klebsiella (pneumoniae /
oxytoca)*

Serratia marcescens

*Enterobacter (cloacae /
aerogenes)*

Proteus mirabilis

Pseudomonas aeruginosa

Acinetobacter baumannii

Stenotrophomas maltophilia

Cocos Gram- positivos

Gram Positive

Staphylococcus aureus

*CoNS (Coagulase negative
Staphylococci)*

Streptococcus pneumoniae

Streptococcus spp.

Enterococcus faecium

Enterococcus faecalis

Fungos

Fungi

Candida albicans

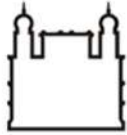
Candida tropicalis

Candida parapsilosis

Candida krusei

Candida glabrata

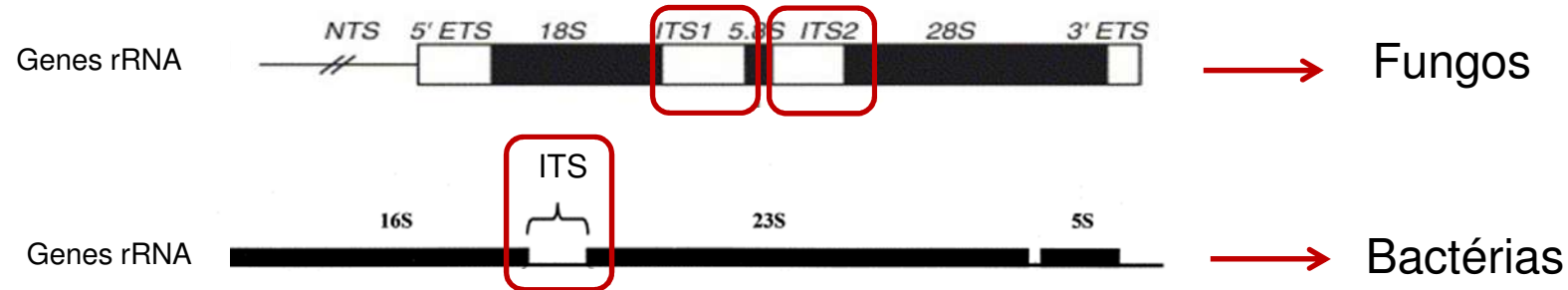
Aspergillus fumigatus

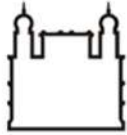


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Fundação Oswaldo Cruz

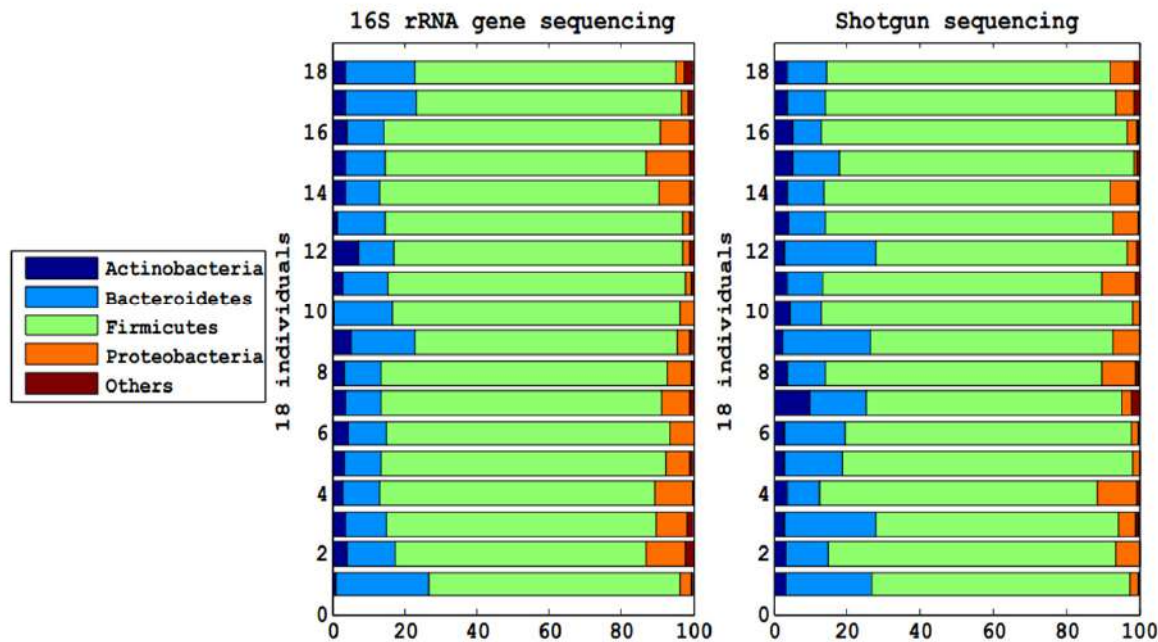


- Seleção das sequências para identificação dos microorganismos:





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16S rRNA gene sequencing can profile the bacterial communities in a greater detail than can metagenomics. In addition low abundance species are best identified through 16S rRNA gene sequencing



Contamination and Sensitivity Issues with a Real-Time Universal 16S rRNA PCR

C. E. CORLESS,^{1*} M. GUIVER,¹ R. BORROW,¹ V. EDWARDS-JONES,²
E. B. KACZMARSKI,¹ AND A. J. FOX¹

Meningococcal Reference Unit, Manchester Public Health Laboratory, Withington Hospital, Manchester M20 2LR,¹ and Department of Biological Sciences, Manchester Metropolitan University, Manchester M1 5GD,² United Kingdom

Received 8 September 1999/Returned for modification 3 November 1999/Accepted 14 January 2000

A set of universal oligonucleotide primers specific for the conserved regions of the eubacterial 16S rRNA gene was designed for use with the real-time PCR Applied Biosystems 7700 (TaqMan) system. During the development of this PCR, problems were noted with the use of this gene as an amplification target. Contamination of reagents with bacterial DNA was a major problem exacerbated by the highly sensitive nature of the real-time PCR chemistry. This was compounded by the use of a small amplicon of approximately 100 bases, as is necessary with TaqMan chemistry. In an attempt to overcome this problem, several methodologies were applied. Certain treatments were more effective than others in eliminating the contaminating DNA; however, to achieve this there was a decrease in sensitivity. With UV irradiation there was a 4-log reduction in PCR sensitivity, with 8-methoxypsoralen activity facilitated by UV there was between a 5- and a 7-log reduction, and with DNase alone and in combination with restriction digestion there was a 1.66-log reduction. Restriction endonuclease treatment singly and together did not reduce the level of contaminating DNA. Without the development of ultrapure Taq DNA polymerase, ultrapure reagents, and plasticware guaranteed to be free of DNA, the implementation of a PCR for detection of eubacterial 16S rRNA with the TaqMan system will continue to be problematical.

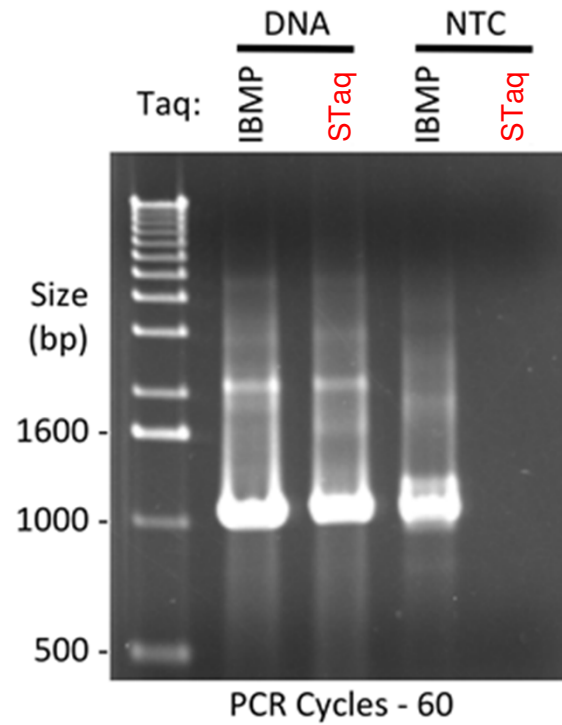
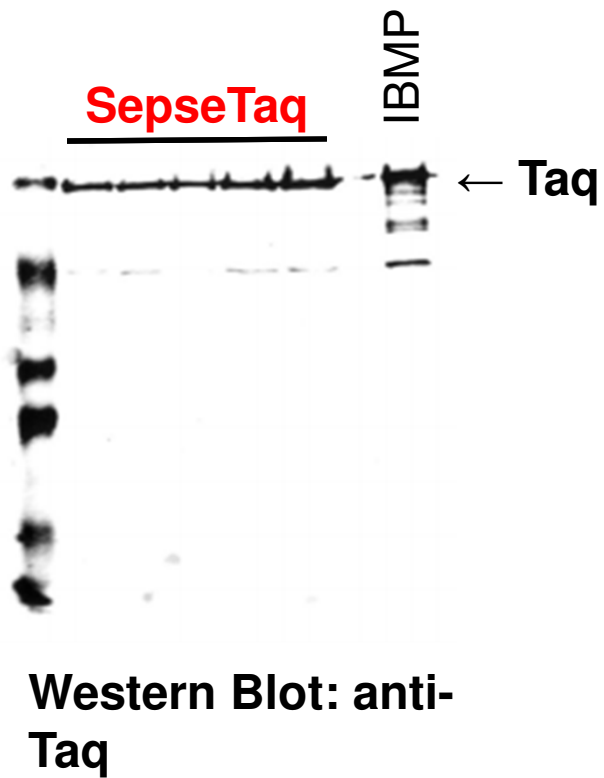
EXPERT
REVIEWS

New technology for rapid molecular diagnosis of bloodstream infections

Expert Rev. Mol. Diagn. 10(4), 399–415 (2010)

nation. Virtually all commercially obtained materials contain small amounts of bacterial genomic material [74]. This material in our automated clean-up procedure. An important source of adventitious DNA is the enzymes used in the PCR reactions.

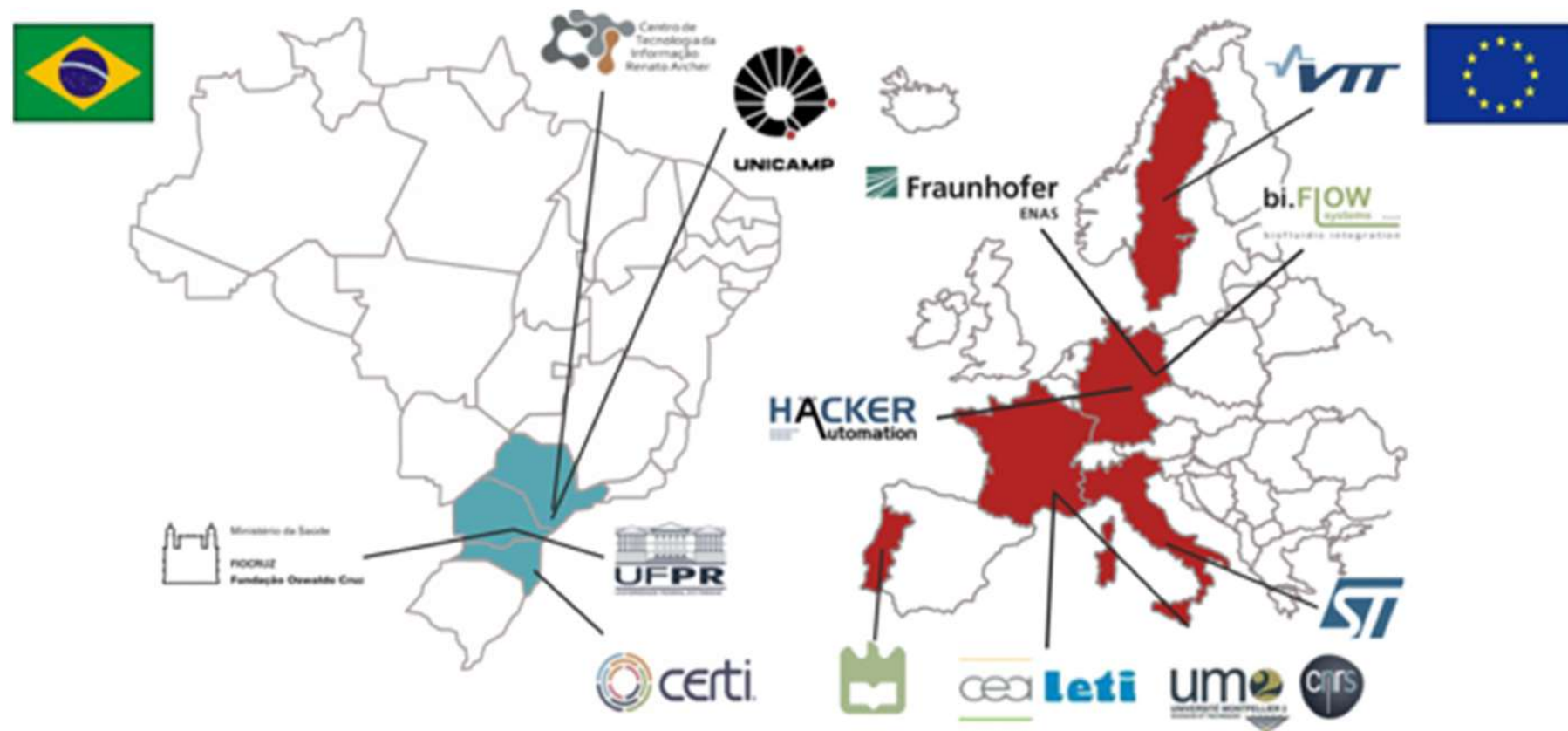
Pathogen DNA-free Taq Polymerase (**SepseTaq**)





PodiTrodi

Point-of-care diagnostics for tropical diseases



Biological Content
(developed by partners)

Pathogen A



Pathogen B



Pathogen C



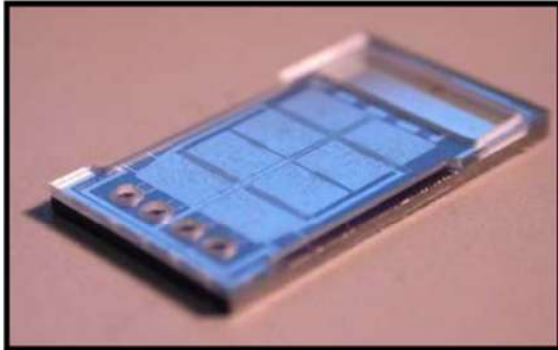
Marker A



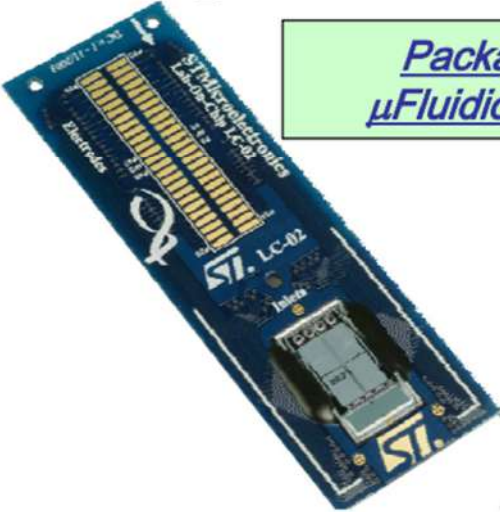
Marker B



Lab-on-Chip core
MEMS based Si-chip



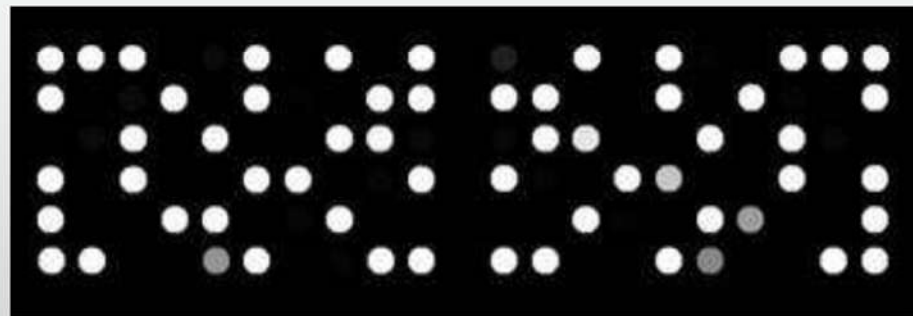
Packaged Biochip
μFluidics - Chemistry





All-in-one device for fast PCR
amplification, hybridization and detection
on...

...a low density, customizable
microarray with increased
performances



Software E@syCheck



Lavador e Secador do Biochip

Extrator e purificador de DNA

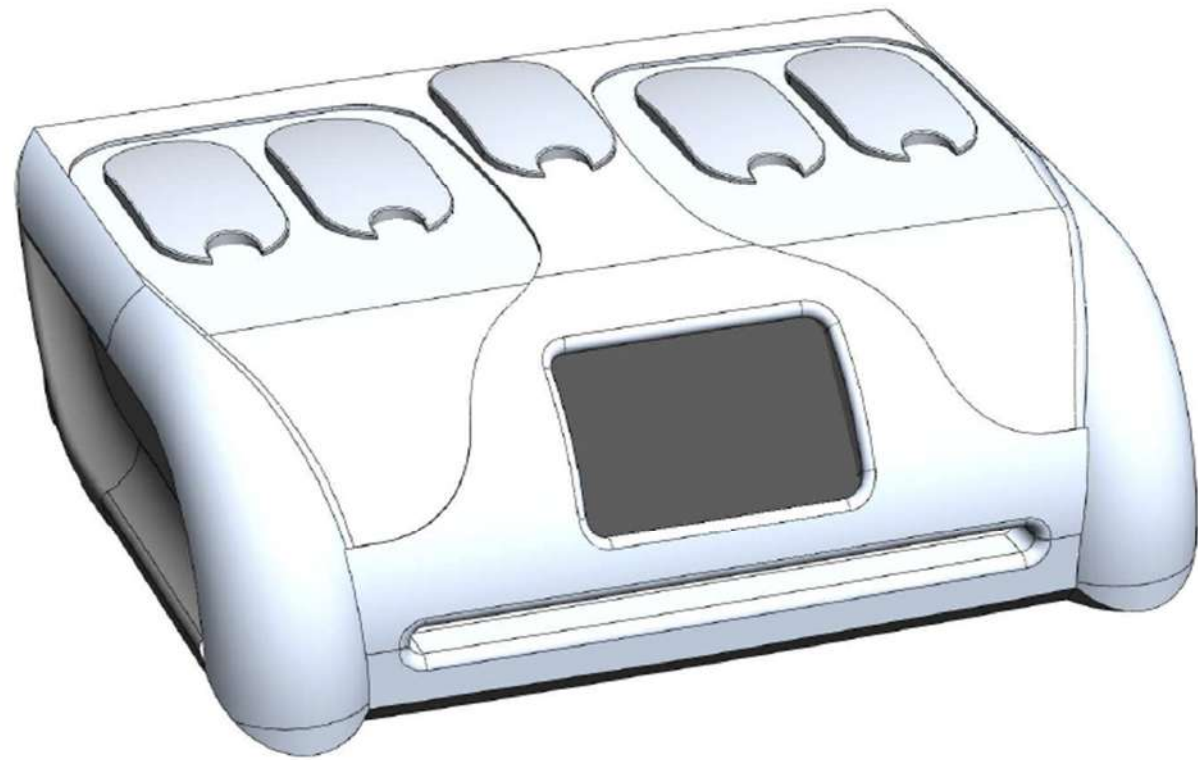
Leitor óptico (OCR)

Sistema de controle de Temperatura do Chip (TCS)



Biochip LC03







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