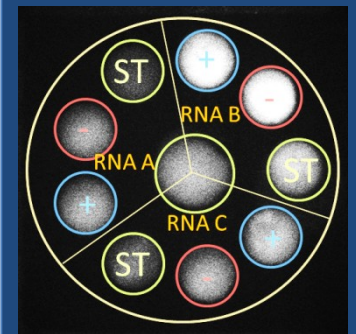
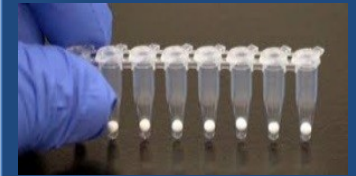
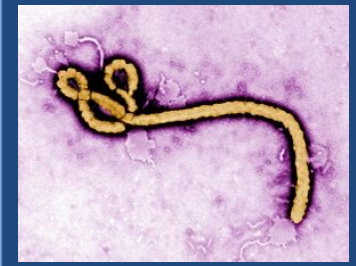


Point-of-care diagnostic tools for Ebola virus and other epidemic-prone viruses

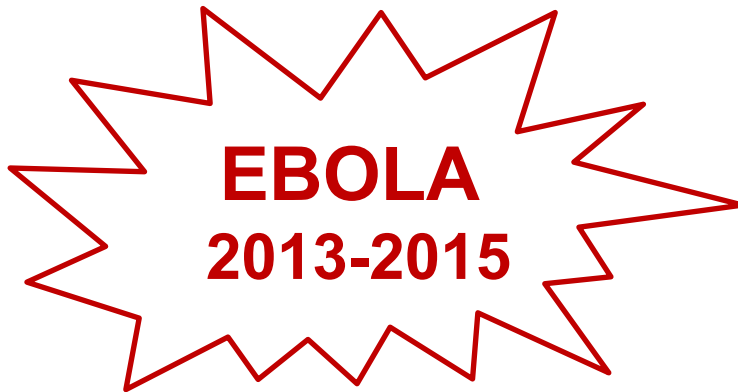
Camille ESCADAFAL, PhD

Laboratory for Urgent Biological Threats (CIBU)

Institut Pasteur, Paris, France



Context



- Inexpensive, portable, and easy-to-use diagnostic devices are **urgently needed** to ensure rapid detection of epidemic-prone viruses at the point-of-care
- Nucleic acid testing can provide access to **rapid and accurate** diagnostic methods

Methodology

Isothermal amplification compared to PCR is :

- faster
- cheaper
- no or small instruments
- more robust
- transportable to the point of care
- user-friendly

Methodology

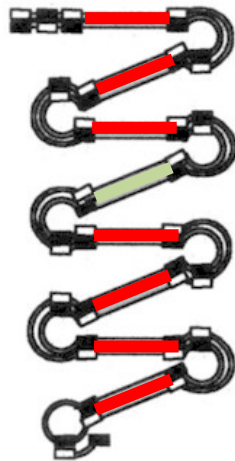
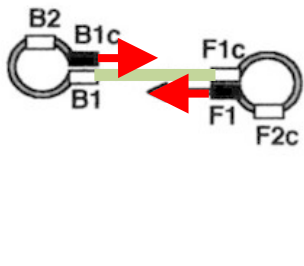
Isothermal amplification

LAMP

Loop-mediated isothermal amplification

Notomi et al., *Nucleic Acids Research* 2000

- LAMP – 65°C – 30 min
- Design of 6 primers
- Dumbbell-like form

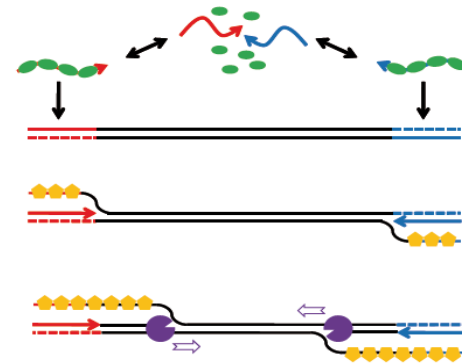


RPA

Recombinase Polymerase Amplification

Piepenburg et al., *Mutation Research* 2007

- RPA - 40°C – 30 min
- Stabilisation of single strands



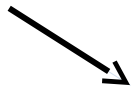
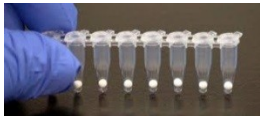
Methodology



Tube-format

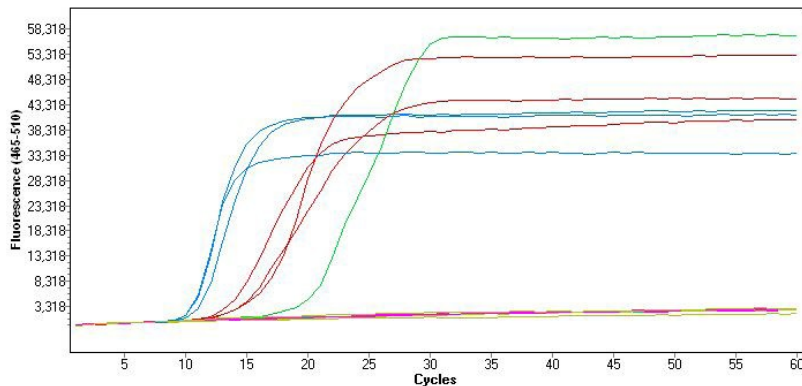


Paper-format



Why paper?

- Capillary pump
- Multiplexing
- Destruction by fire after use
- Cost





Detection of Ebola Zaïre virus RNA

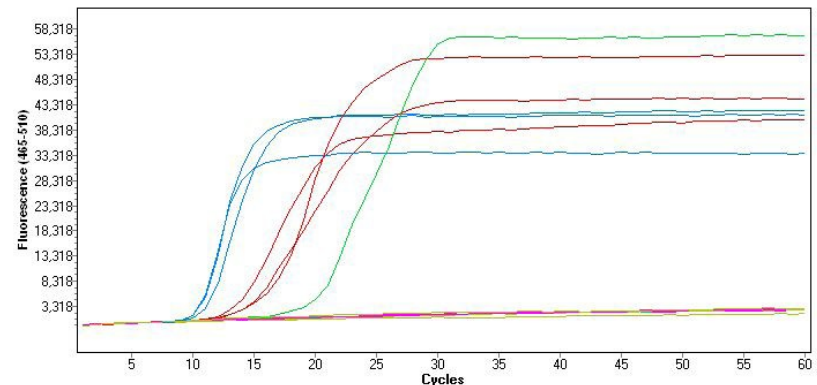


Microtube-format real-time RT-LAMP assay for Ebola Zaïre virus detection

- Microtube format with lyophilised reagents
- Real-time detection of fluorescence
- Amplification time < 30 minutes
- Detection limit < 5 RNA copies per reaction



Instrument Genie III
Optigene



Microtube-format real-time RT-LAMP assay

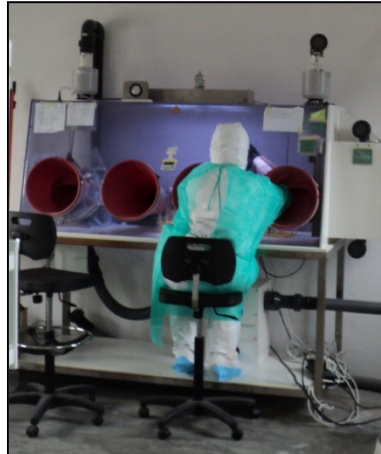
Clinical performances (Field evaluation)

High risk area

Sampling



Sample preparation



Diagnostic
qRT-PCR (Altona kit)

ARN viral extract

Storage (-20°C)

RT-LAMP assay

Use of samples for research purposes:

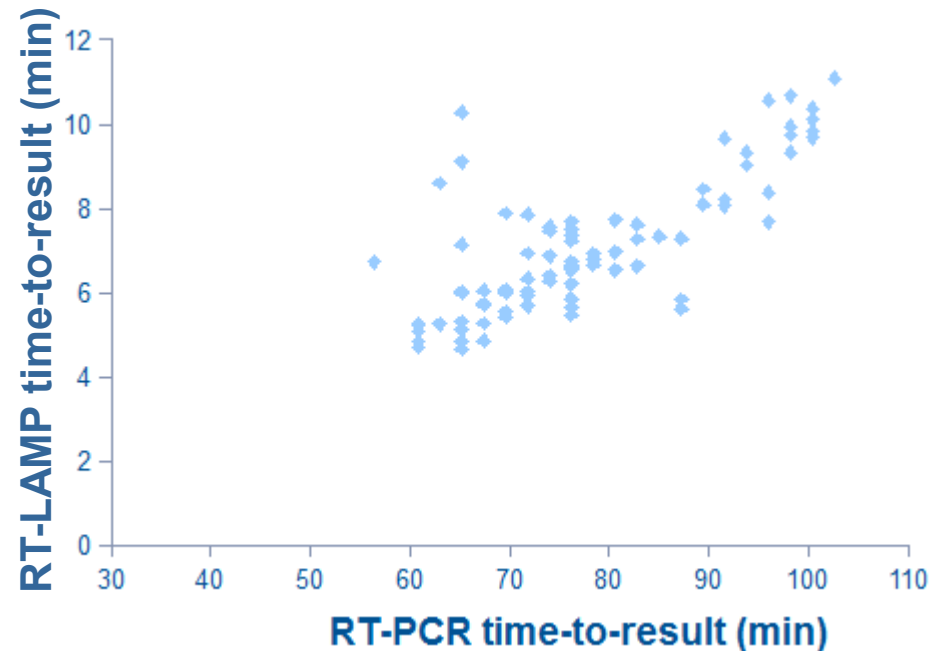
- Patient consent
- Approval from ethical committees

Microtube-format real-time RT-LAMP assay

Clinical performances (Field evaluation)

Real-time RT-LAMP performances compared to real-time RT-PCR

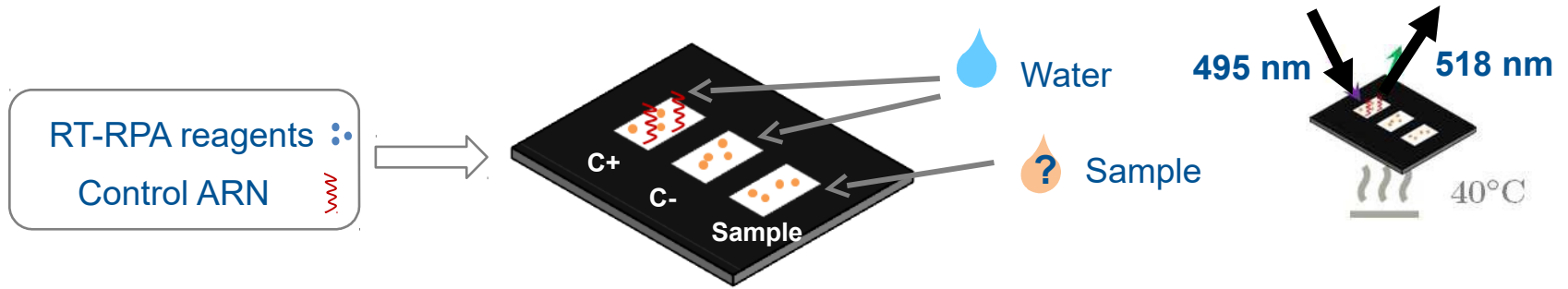
No. of samples	174
True positive	90
False negative	0
True negative	84
False positive	0
Sensitivity	100.0%
(95% CI)	(96.0 to 100.0 %)
Specificity	100.0%
(95% CI)	(95.7 to 100.0 %)



Three samples with false negative results for the reference RT-PCR were classed as true positives after retesting

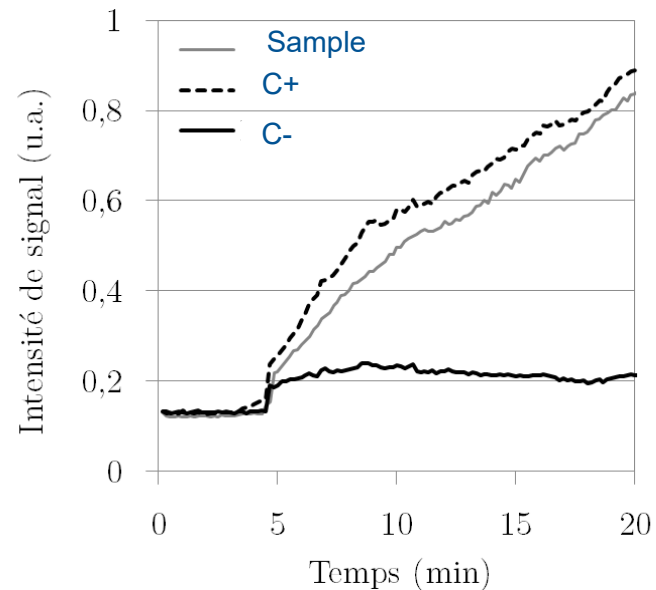
Equivalent clinical performances and 10 x faster time-to-result compared to real-time RT-PCR

Paper-based EBOV RT-RPA assay



Fluorescence detector

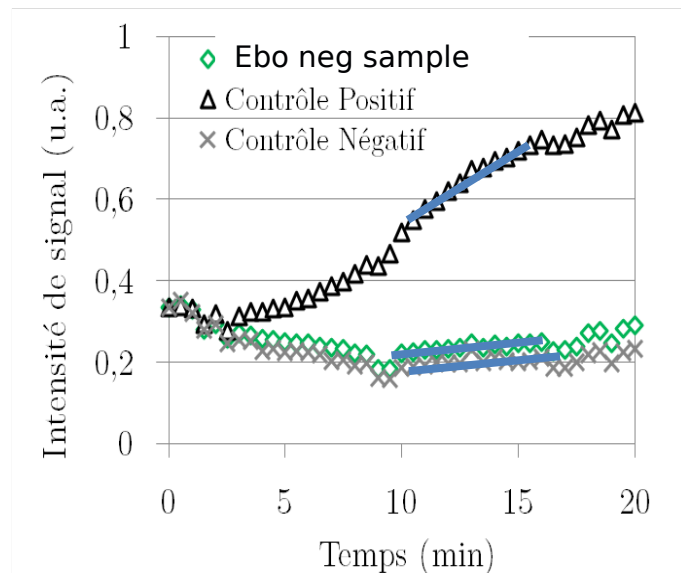
Sample / Negative control / Positive control



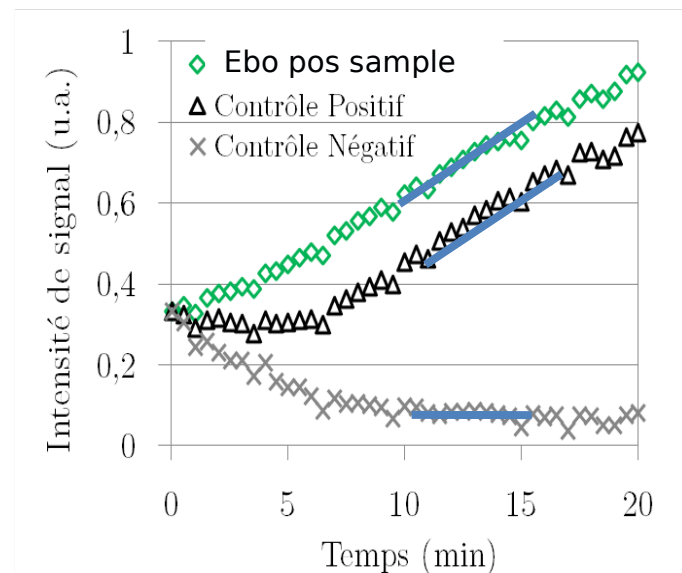
Paper-based EBOV RT-RPA assay

Clinical performances (Field evaluation)

Viral ARN of Ebola negative patient



Viral ARN of Ebola positive patient

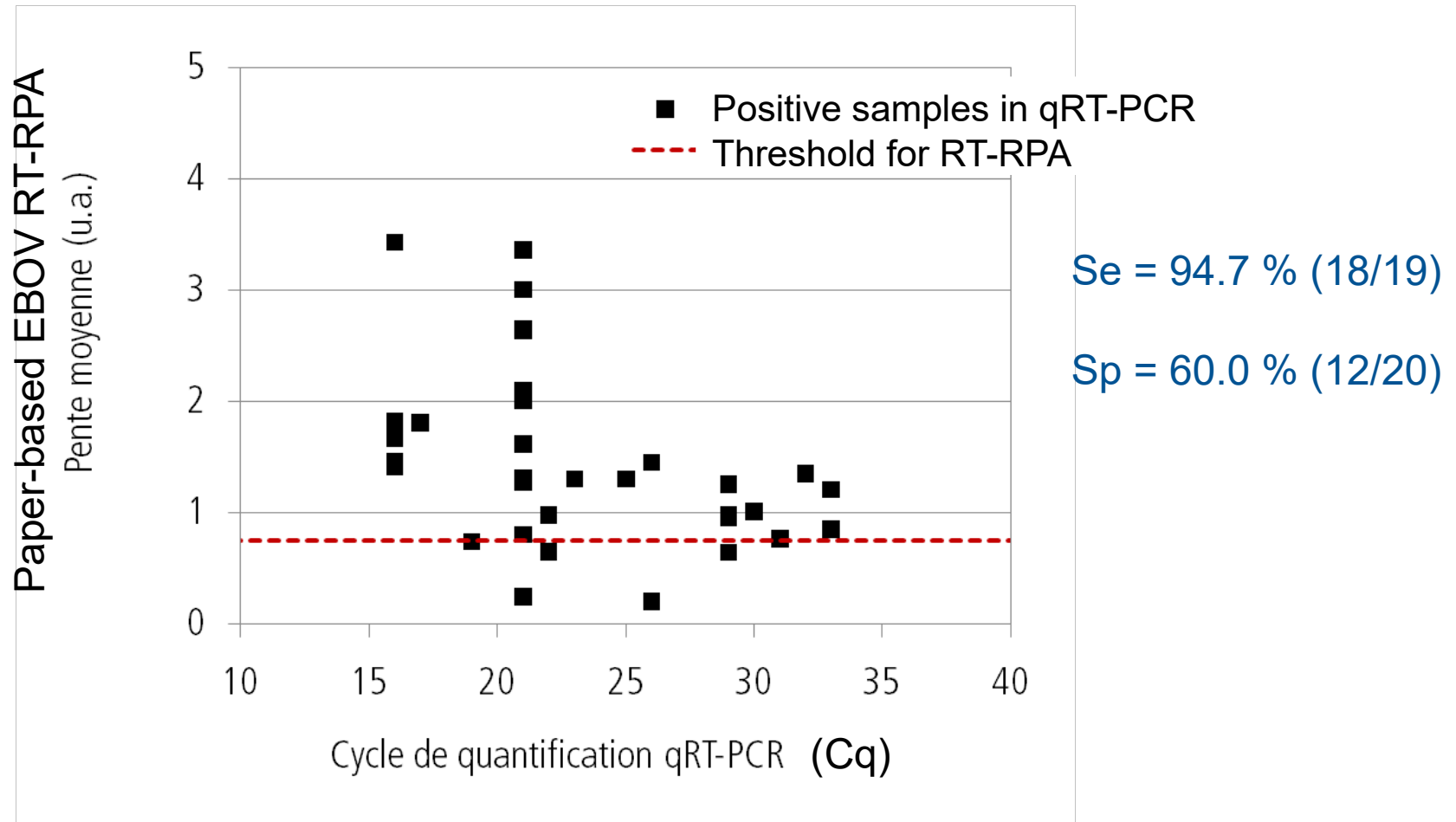


Selection criteria for positivity

→ sliding average slope starting 10 min from start of reaction

Paper-based EBOV RT-RPA assay

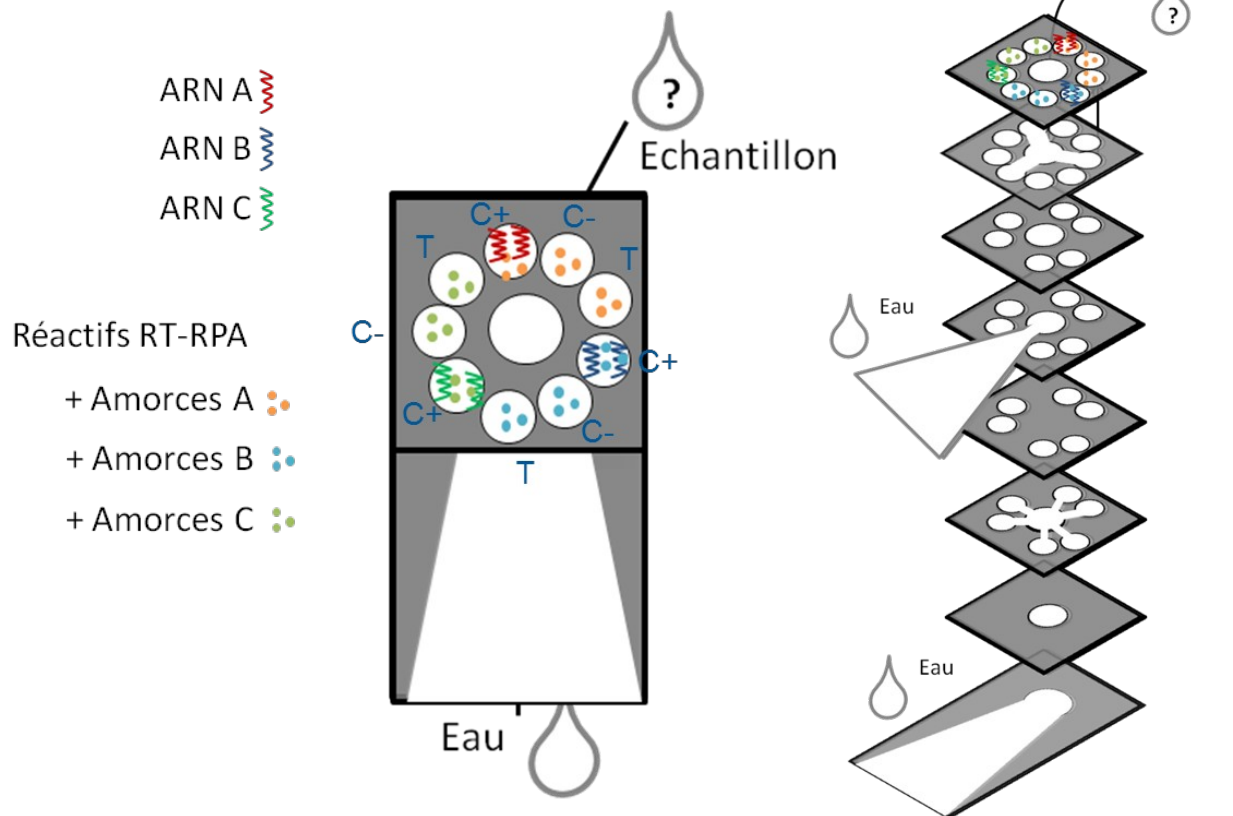
Clinical performances (Field evaluation)



Multiplexing

3 tests in parallel for 3 different Ebola synthetic ARN targets:

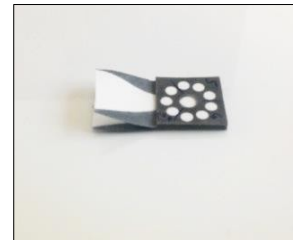
- ARN A: Test sample, Control +, Control -
- ARN B: Test sample, Control +, Control -
- ARN C: Test sample, Control +, Control -



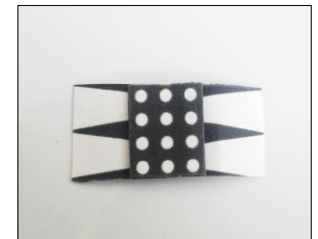
Multiplexing

Multi-layered folded paper for triplex (1) or more tests in a matrix format (2)

1



2



- Vanhomwegen et al., *Patent in 2015*
- Magro et al., *Patent FR1551189 fév. 2015*
- Magro et al., *article under peer review Nature Biotech.* Sept. **2016**
- Review on paper-based microfluidics for molecular diagnostics in preparation for **Lab on Chip**

Assays for other epidemic-prone viruses

Micro-tube format

	Chikungunya		Zika		Dengue	
	RT-LAMP	RT-RPA	RT-LAMP	RT-RPA	RT-LAMP	RT-RPA
Detection limit RNA copies/reaction	< 30	100	< 10	100	Not done	100

Paper-based format

Still in development

Investigating new materials and formats

To improve test sensitivity and reproducibility

Perspectives

- Optimise paper-based tests to improve sensitivity and reproducibility
- Simplify sample preparation and detection method
- Develop multiplex tests for parallel detection of epidemic-prone viruses
- Include expertise in synthetic biology

communication tools

integrated systems

Conclusion

Development and implementation of such diagnostic tools would enable :

- Rapid detection of epidemic-prone viruses
- Timely outbreak response
- Decentralised diagnostic capacities

Acknowledgments



Laura
Magro

Pierre Garneret



Fabrice Monti

Pierre Lafaye



Institut Pasteur



Jean-Claude Manuguerra

Aurélia Kwasiborsi

Jessica Vanhomwegen

Béatrice Jaquelin



Financial support from Carnot Pasteur Maladies Infectieuses (ANR 11-CARN 017-01)



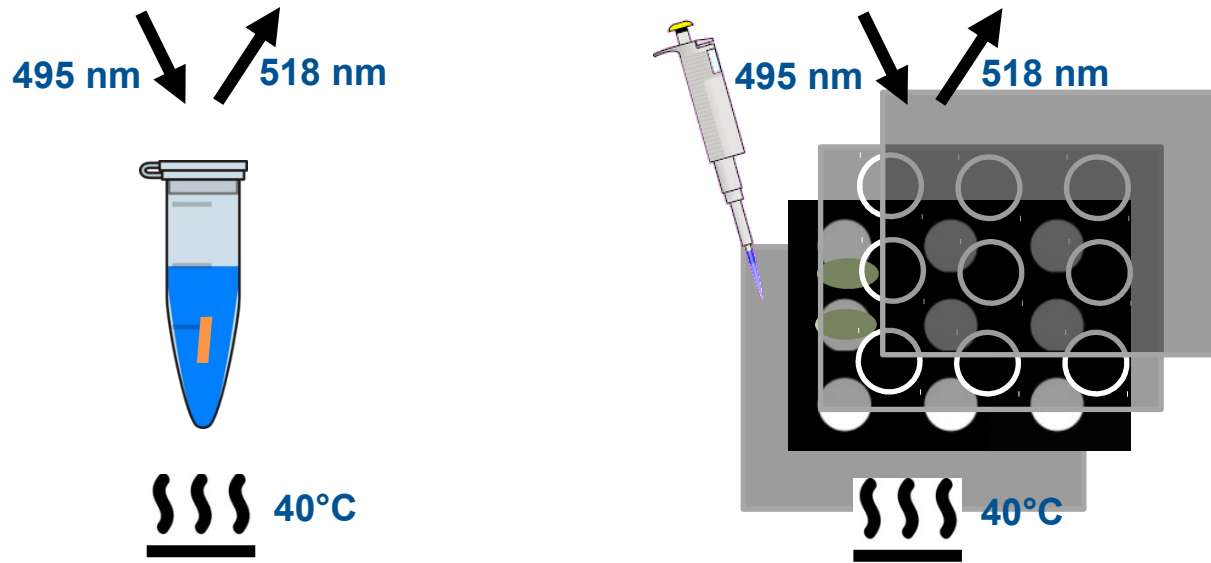
Questions



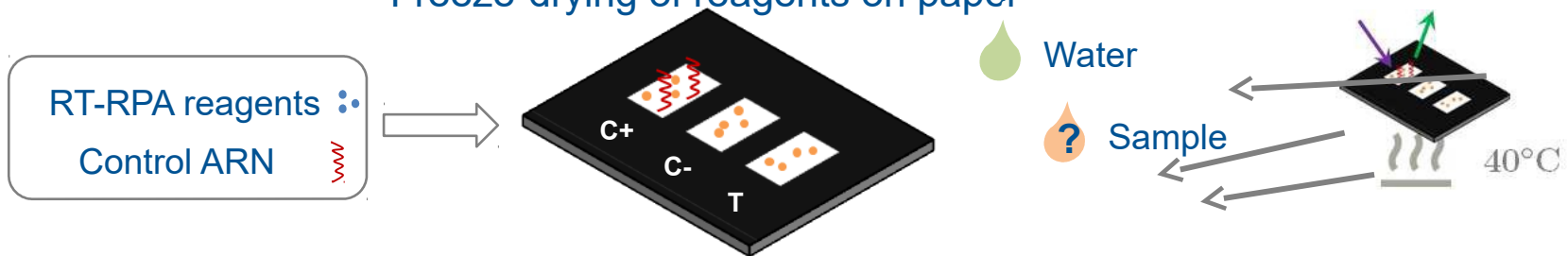
camille.escadafal@pasteur.fr

EBOV RT-RPA on paper

Laboratory development with synthetic ARN

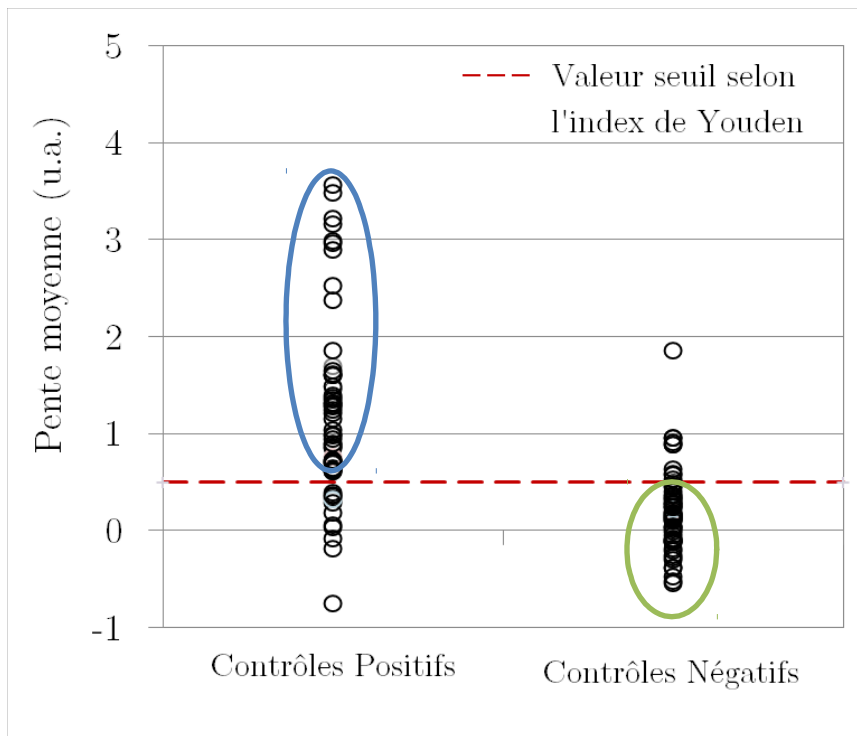


Freeze-drying of reagents on paper



Performances de diagnostic

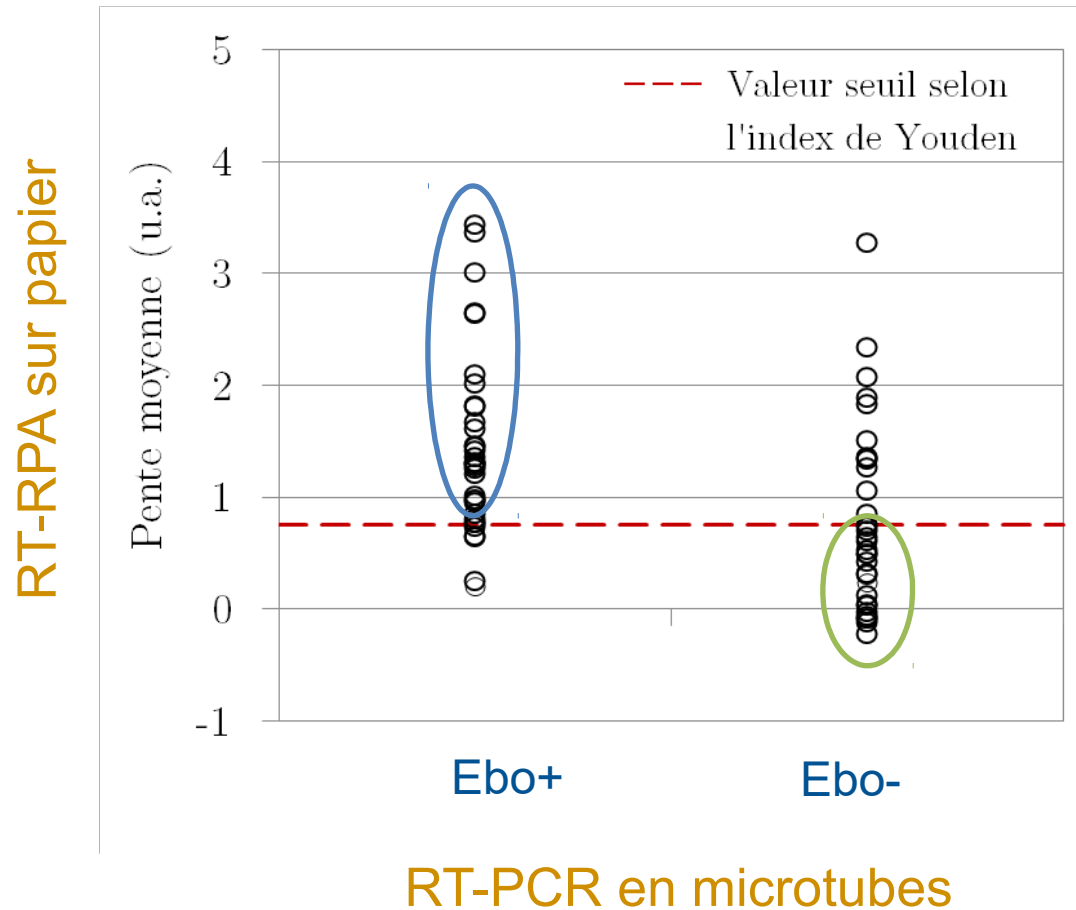
Analyse statistique : CONTROLES = ARN synthétique - c= 10^7 copies/ μ L



Positifs : 82.3 % (56/68)
Négatifs : 83.8 % (57/68)

Comparaison avec la RT-PCR

Analyse statistique : ECHANTILLONS = ARN viral – différentes concentrations



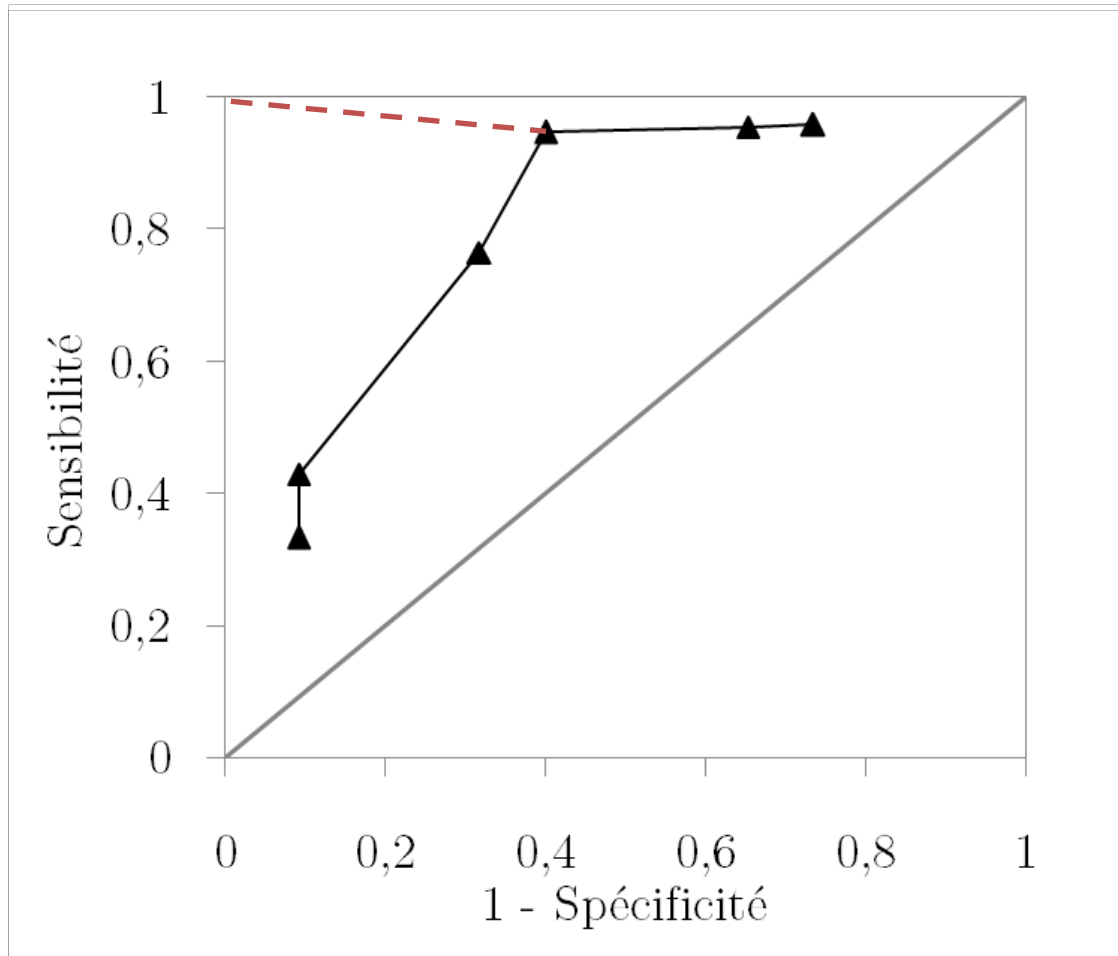
Sensibilité : % de cas Ebo+ bien identifiés

Spécificité : % de cas Ebo- bien identifiés

Paper-based EBOV RT-RPA assay

Clinical performances (Field evaluation)

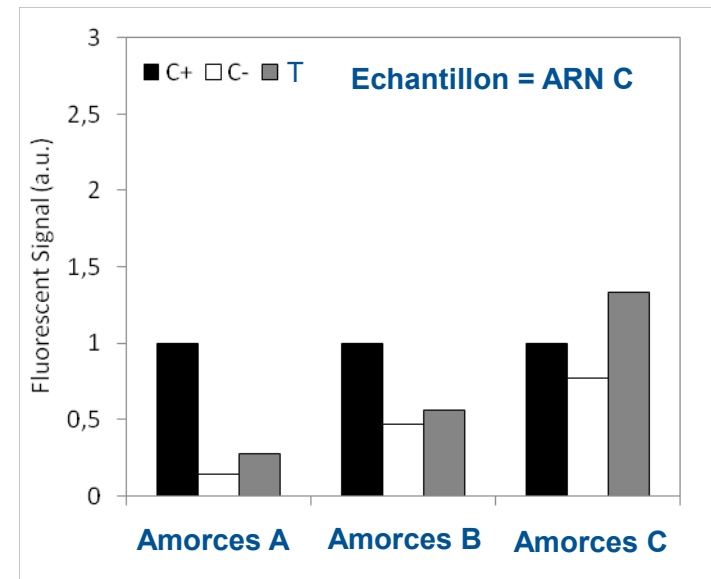
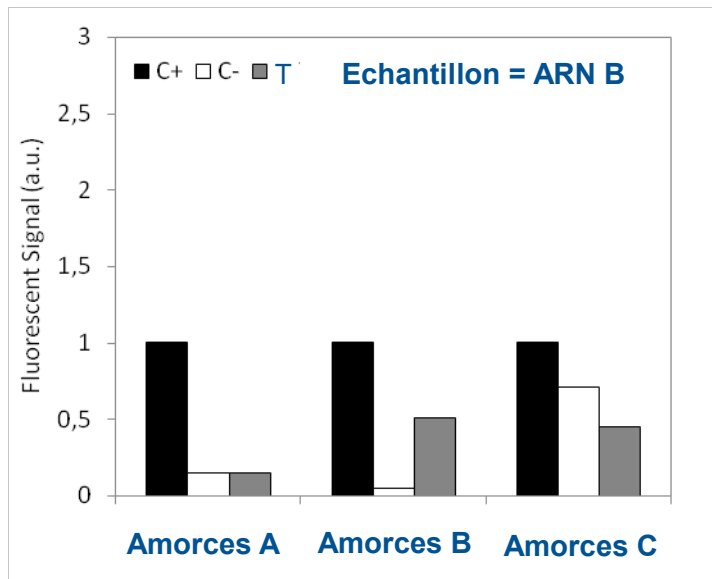
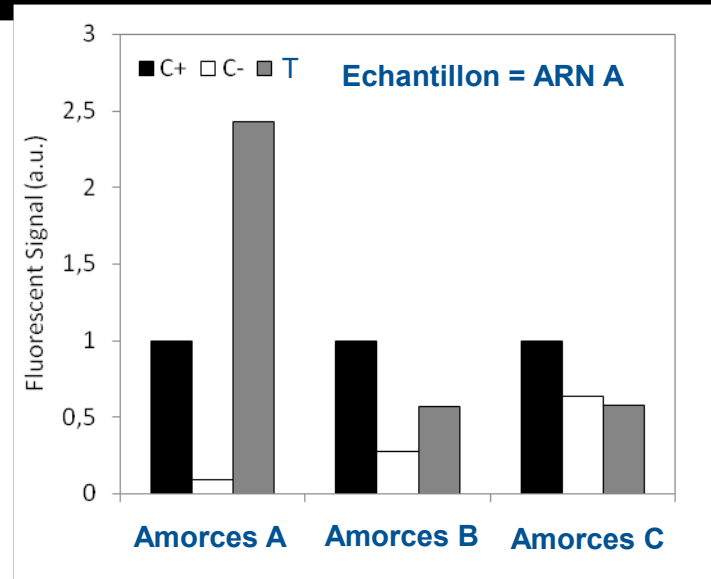
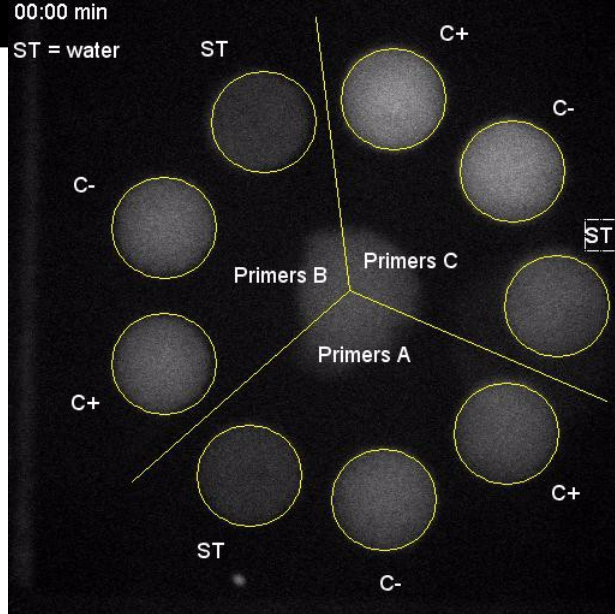
ROC curve



Se = 94.7 % (18/19)

Sp = 60.0 % (12/20)

Réaction RT-RPA multiplexée



Trajectoires d'écoulement

Parallélisation des réactions par l'écoulement

Visualisation colorimétrique des fonctions

Contrôles



t = 0 min



t = 10 min



Echantillon



t = 0 min



t = 10 min



Puits
individuels



t = 0 min

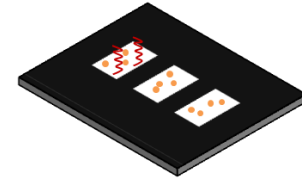


t = 10 min



Conclusions sur l'amplification

Démonstration RT-RPA sur papier prêt à l'emploi
avec contrôle de fonctionnement

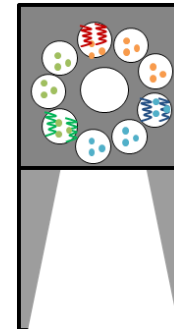


Echantillons synthétiques au laboratoire



Echantillons cliniques en Guinée

Puce papier multiplexée

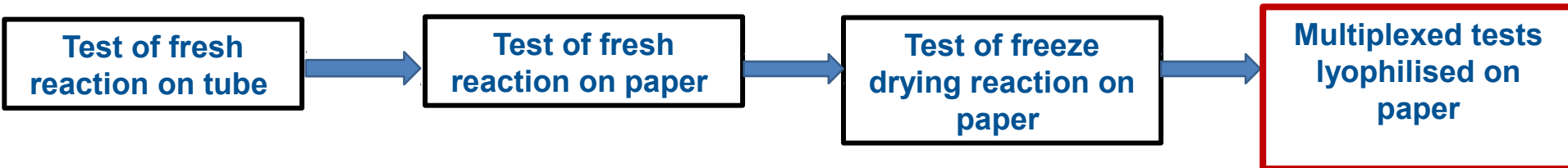


Magro et al., *Patent FR1551189* fév. **2015**

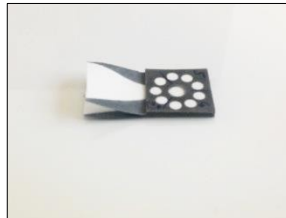
Magro et al., *μ TAS* oct. **2015**

Magro et al., *article under peer review Nature Biotech.* sept. **2016**

Multilayered format for multiplexing



1



2



Assays for other epidemic-prone viruses

- RT-LAMP assay very difficult to design to detect all dengue serotypes
- One primers/probe set for RT-RPA able to detect all dengue virus serotypes
- Limit of detection still needs to be determined with a quantified standard and performances optimised
- Dengue RT-RPA tested on paper as well as the primer sets from El Wahed's publication → Some good results but problems of reproducibility

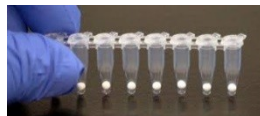
- First primer/probe sets designed to detect all Zika lineages but limit of detection is not low enough (> 100 copies/ μ l)
 - Many parameters were modified for optimisation (T° , concentration of primers, probe, MgAc, sequence of primers and probe, mixing time, etc...)
- initial setting was the best



- Performances of the Zika RT-RPA developed by IP Dakar ?

MATERIAL & METHODS

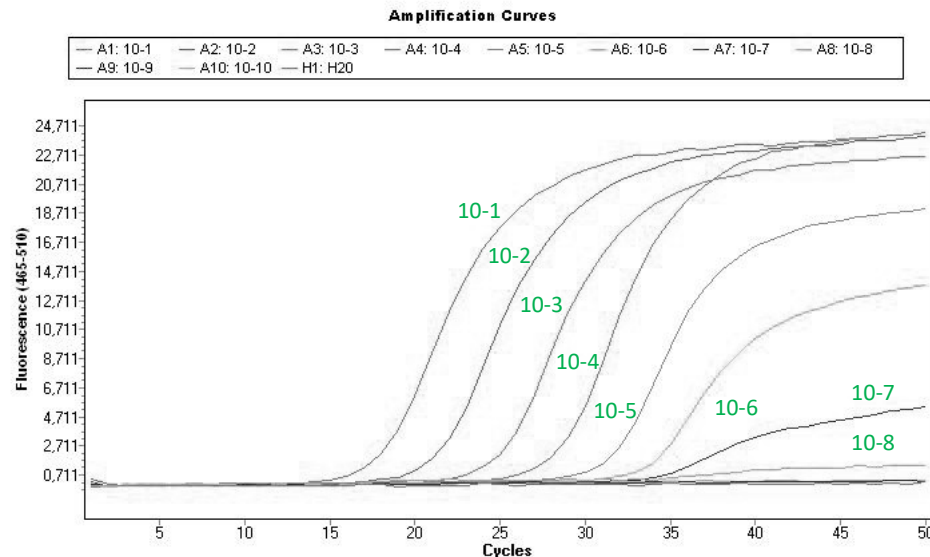
- ISOTHERMAL MASTER MIX: ISO-004 from Optigene
- Primer design: LAMP Designer software
- Set of 6 primers to increase sensitivity
- Design of several primer sets based on Asian and/or African lineages of ZIKV
- RT-PCR from Lanciotti was used as a reference method



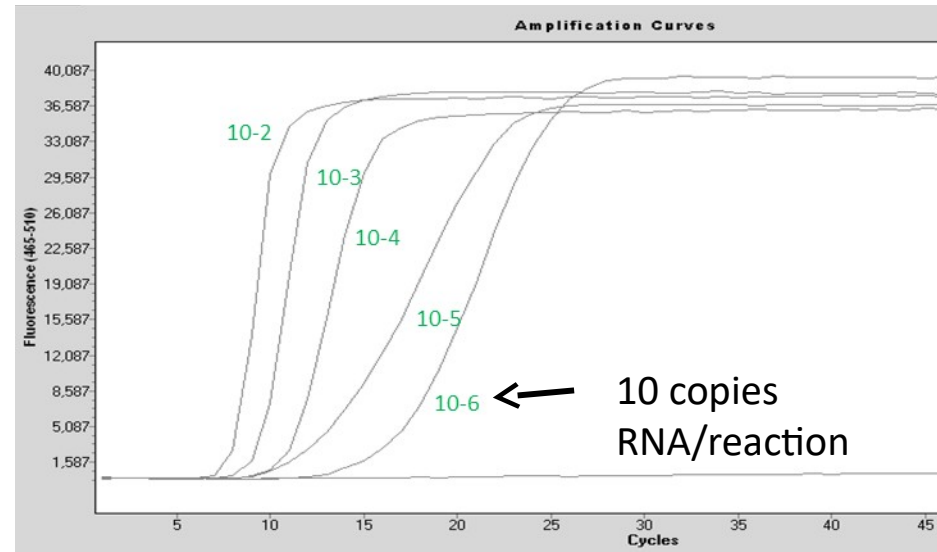
Optigene Genie III

RT-LAMP for Zika virus detection

- Good sensitivity (10 copies of RNA/reaction) for Asian and African strains independently but not simultaneously → Two different primer sets at this stage with sensitivities depending on Zika lineage
- First tests on biological samples (whole blood, serum, saliva and urine) spiked with Zika virus provided good results with a sensitivity comparable to RT-PCR



RT-PCR Lanciotti 2 with PF13 strain



RT-LAMP with PF13 strain

RT-LAMP for Chikungunya virus detection

RESULTS

Detection limit in number of copies detected per reaction				

- Sybr Green protocol demonstrated false positive results
- Pastorino protocol detects up to the 10⁻⁹ dilution
- 7 sets of primers were tested for RT-LAMP
- First RT-LAMP assays demonstrated similar performances for 3 sets of primers detecting at least the 10⁻⁸ dilution
- Comparison with published RT-LAMP assays did not provide better results

Questions/Perspectives

ZIKA

- What is the best compromise between sensitivity (low detection limit) and spectrum of detection (detecting the different Zika lineages)?
- Further testing with other Zika strains (strains from Brazil, Martinique have been received)

CHIK

- Test first RT-RPA designs
- Sensitivity testing for RT-LAMP

CHIK/ZIKA/DENGUE

- Use quantified RNA standards to determine clear limits of detection for developed isothermal assays
- Develop lyophilised tube-format
- Develop multiplex assays
- Validation with clinical samples → IP French Guyana for Zika? Other ?

Assays for other epidemic-prone viruses

- RT-LAMP assay very difficult to design to detect all dengue serotypes
- One primers/probe set for RT-RPA able to detect all dengue virus serotypes
- Limit of detection still needs to be determined with a quantified standard and performances optimised
- Dengue RT-RPA tested on paper as well as the primer sets from El Wahed's publication → Some good results but problems of reproducibility