



# Lack of sensitivity of four multiplex PCR kits detecting sexually transmitted pathogens

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41<sup>e</sup>

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pas de lien d'intérêt potentiel à déclarer

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# Background

## WHO 2016 : 376 million new cases for curable non viral STIs

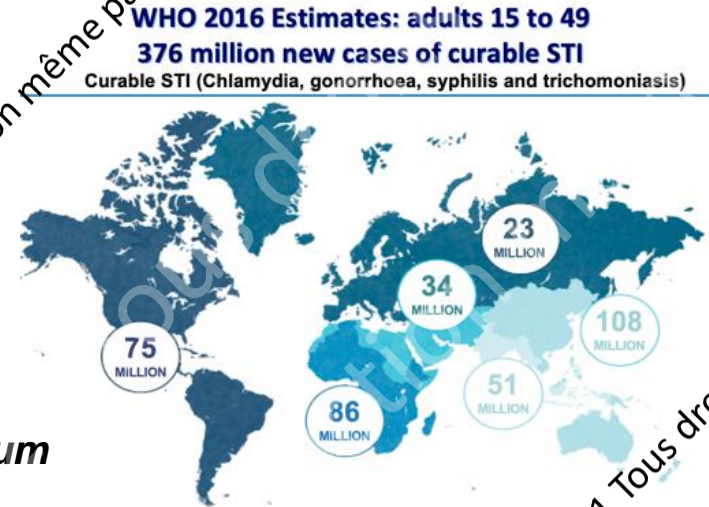
(+5% increase in 4 years)

- **Chlamydia** 127 million cases
- **Gonorrhoea** 87 million
- **Syphilis** 6 million
- **Trichomonas vaginalis** 156 million
- More than 1 million of acquired STIs everyday
- Emerging STI pathogen: **Mycoplasma genitalium**

👉 Improve diagnosis: **new molecular tools**

👉 **Antimicrobial resistance**: *N. gonorrhoeae* and *M. genitalium*

→ antimicrobial treatment and control



Newman et al PLoS One 2015  
Rowley J et al Bull World Health Organ 2019

# Antibiotic resistance threat report, CDC 2019



U.S. Department of  
Health and Human Services  
Centers for Disease  
Control and Prevention

👉 4 categories of 21 AMR bacteria and fungi based on level of concern on human health

- Urgent threats: 5 microbes including drug-resistant *N. gonorrhoeae*
- Serious threats
- Concerning threats
- Watch list: 3 microbes including drug-resistant *M. genitalium*

➔ High-quality diagnostic tests are required



# Aim of the study

- Evaluate the clinical performance of four multiplex real-time PCR commercial kits for the detection of non-viral STIs

## STI PLUS ELITE MGB kit



*N.gonorrhoeae/C. trachomatis*  
*/M. genitalium/T. vaginalis*

Real-TM kit



## Allplex STI Essential kit




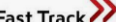


## FTD Urethritis plus kit



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# Main characteristics of the commercial kits

Kits	STI PLUS ELITE MGB 	Allplex STI Essential 	NG/CT/MG/TV Real-TM 	FTD Urethritis plus* 
Detected targets	CT: <i>dnaB</i> gene and <i>ompA</i> gene; NG: <i>pivNG</i> gene; MG: 23S rRNA gene; TV: L23861 repeated sequence	CT, NG, MG, TV, UU, UP and MH Undisclosed	CT, NG, MG and TV Undisclosed	CT, NG, MG, TV, UU, UP and MH Undisclosed
Validated specimen types	Cervico-vaginal swabs, urine	Genital swabs, urine, liquid-based cytology	Cervical, urethral, conjunctival swabs, urine, prostatic liquid, semen	Genital and rectal swabs, urine
Input sample volume	200 µL of primary specimen or 20 µL DNA	5 µL DNA	10 µL DNA	10 µL DNA
IC (extraction and amplification)	Endogenous IC and additional exogenous IC for urine	Endogenous IC and additional exogenous IC for urine	Exogenous IC	Exogenous IC
Automation	DNA extraction, PCR set up, amplification and analysis	Amplification and analysis	Amplification	Amplification
Number of test / kit	12	50	100	32 or 64
Number of reaction / run	Batches of 12 tests **	Batches; up to 94 samples per PCR run	Batches; up to 72 samples per PCR run	Batches; up to 34 samples per PCR run
Hands-on time (min)***	30	30 – 60 ****	80 – 240 ****	30 – 60 ****
Test turnaround time	3h including DNA extraction	1.75 h, excluding DNA extraction	2 h excluding DNA extraction	1.5 h excluding DNA extraction
Data analysis software	ELITE InGenius software	Seegene Viewer	Rotor-Gene Q software	Applied Biosystems 7500 software

\*commercialization recently discontinued

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# Methods

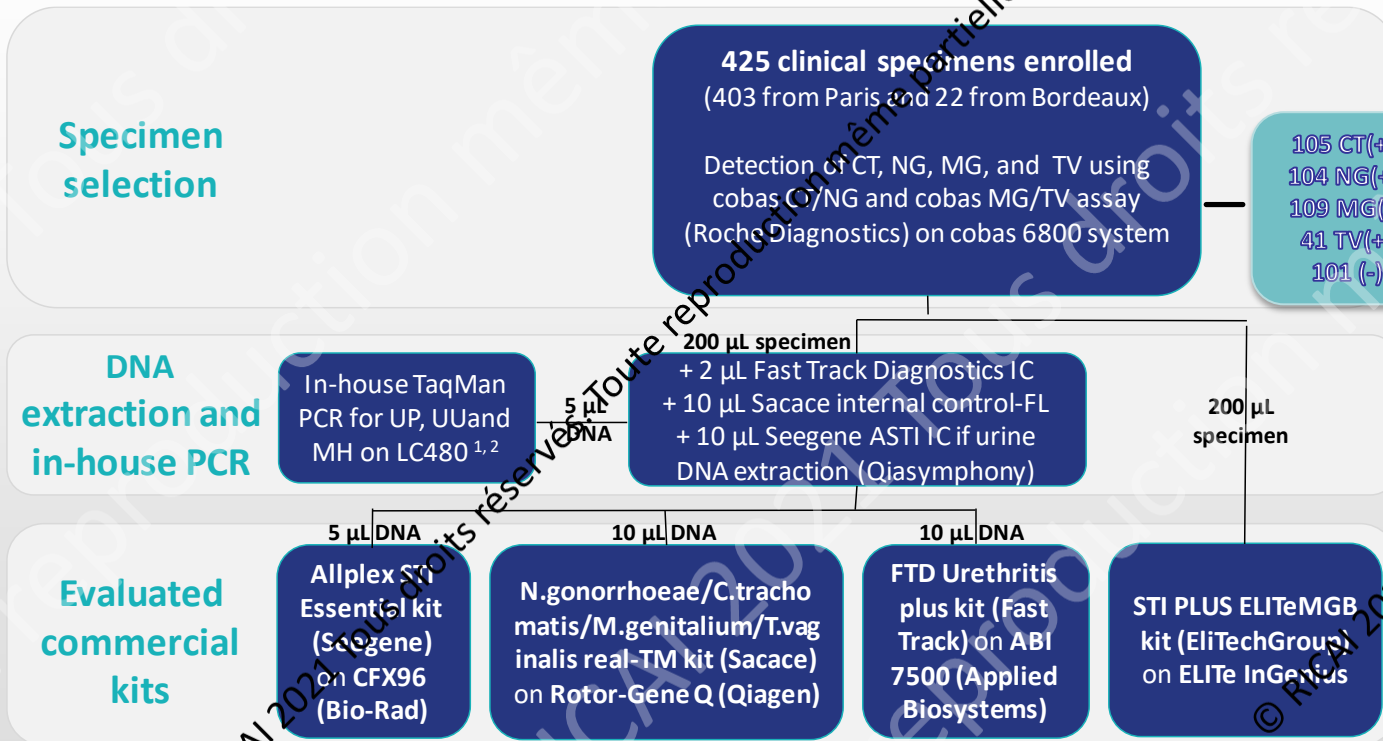
- In comparison to cobas CT/NG and TV/MG kits (Roche diagnostics) on cobas 6800, used as reference



- In comparison to published in-house Taqman PCRs for kits detecting *U. urealyticum*, *U. parvum*, and *M. hominis*<sup>1</sup>

<sup>1</sup> Yi Mol Cell Probes 2005, Férandon Clin Microbiol Infect 2011

# Methods



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# Results: *C. trachomatis* and *N. gonorrhoeae*

Micro-organisms	Evaluated kit	Overall % agreement [CI 95%]	Positive % agreement [CI 95%]	Negative % agreement [CI 95%]	$\kappa$
CT	Elitech kit	96.9 [94.8-98.2]	89.5 [82.2-94.1]	99.4 [97.7-99.8]	0.92
	Seegene kit	94.3 [91.7-96.1]	81.6 [73.0-87.9]	98.4 [96.4-99.3]	0.84
	Sacace kit	94.3 [91.7-96.2]	84.8 [76.7-90.4]	97.5 [95.1-98.7]	0.84
	Fast Track kit	95.9 [93.3-97.5]	88.3 [80.3-93.3]	98.5 [96.3-99.4]	0.89
NG	Elitech kit	91.4 [88.4-93.8]	88.1 [80.4-93.1]	<b>92.5 [89.1-94.9]</b>	0.77
	Seegene kit	92.6 [89.7-94.8]	<b>71.2 [61.8-78.9]</b>	99.7 [98.2-99.9]	0.78
	Sacace kit	89.3 [86.0-91.9]	<b>63.1 [53.5-71.8]</b>	97.8 [95.5-98.9]	0.68
	Fast Track kit	95.6 [93.0-97.3]	90.5 [83.1-95.0]	97.4 [94.7-98.7]	0.89

***C. trachomatis* detection : Good detection performances** were observed for the four kits, with **no statistically significant difference**.

***N. gonorrhoeae* detection :** The Seegene and the Sacace kits showed a significantly lower positive agreement. The ELITEch kit presented a significantly lower negative agreement.

# Results: *M. genitalium* and *T. vaginalis*

Micro-organisms	Evaluated kit	Overall % agreement [CI 95%]	Positive % agreement [CI 95%]	Negative % agreement [CI 95%]	$\kappa$
MG	Elitech kit	93.1 [90.3-95.2]	74.1 [65.1-81.4]	99.7 [98.4-99.9]	0.80
	Seegene kit	89.8 [86.5-92.3]	63.3 [54.0-71.8]	99.8 [97.2-99.7]	0.70
	Sacace kit	90.5 [87.3-92.9]	68.5 [59.3-76.5]	99.1 [95.9-99.1]	0.73
	Fast Track kit	87.7 [83.9-90.7]	72.7 [63.2-80.5]	<b>93.2 [89.6-95.7]</b>	0.68
TV	Elitech kit	96.2 [93.9-97.7]	61.0 [45.7-74.3]	100 [99.0-100]	0.74
	Seegene kit	95.2 [92.8-96.9]	51.2 [36.5-65.8]	100 [99.0-100]	0.65
	Sacace kit	95.2 [92.8-96.9]	63.4 [46.9-76.4]	98.7 [97.0-99.4]	0.70
	Fast Track kit	94.2 [91.4-96.2]	68.1 [46.0-84.6]	95.7 [93.0-97.4]	0.52

***M. genitalium* detection** : The four kits presented a **low positive agreement** (PPA range : 63.3-74.1).

A significantly lower negative agreement was observed for the Fast-Track kit.

***T. vaginalis* detection** : The four kits presented a **very low positive agreement** (PPA range: 51.2%-68.4%). The Seegene kit missed half (20/41) of the *T. vaginalis* positive samples.

The Fast-Track kit had a significantly lower negative agreement (95.7%) than the ELITech and Seegene kits.

# Analysis per sample type

- **First-void urines and cervicovaginal swabs (all kits validated)**

- Good performance of the four kits for *C. trachomatis* and *N. gonorrhoeae* detection

- **No kits validated on throat swabs or rectal swabs**

- ✓ Except Fast-track kit on rectal swabs

- Lower positive % agreement for *C. trachomatis* on rectal swabs (Seegene, Sacace)
- Lower positive % agreement for *N. gonorrhoeae* on throat swabs (Seegene, Sacace)

→ **Caution for the use of the kits in these types of samples**

# Results: *Ureaplasma* spp. and *M. hominis*

Micro-organisms	Evaluated kit	Overall % agreement [CI 95%]	Positive % agreement [CI 95%]	Negative % agreement [CI 95%]	$\kappa$ <sup>1</sup>
UU	Seegene	91.2 [88.1-93.6]	100 [93.2-100]	89.9 [86.5-92.6]	0.69
	Fast Track	87.9 [84.2-90.9]	98.0 [89.7-99.7]	86.3 [82.1-89.7]	0.63
UP	Seegene	98.3 [96.6-99.2]	96.9 [89.3-99.1]	98.6 [96.8-99.4]	0.94
	Fast Track	<b>93.4 [90.4-95.5]</b>	<b>59.6 [46.1-71.8]</b>	99.0 [97.2-99.7]	0.69
MH	Seegene	98.6 [96.9-99.4]	93.3 [85.3-97.1]	95.7 [98.4-100]	0.95
	Fast Track	95.9 [93.3-97.5]	93.3 [84.1-97.4]	<b>96.4 [93.7-98.0]</b>	0.86

Allplex STI Essential kit



FTD Urethritis plus kit



• **Asymptomatic carriage is frequent. Colonized individuals do not develop any disease.**

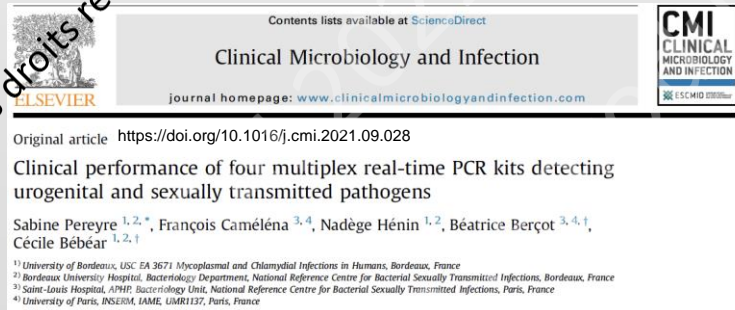
→ Only high load of UU might be associated with few cases of NGU

• **Routine screening and subsequent antibiotic treatment is not recommended by IUSTI<sup>1</sup>**

<sup>1</sup>Horner et al. JEADV 2018

# Conclusion

- Multiplex real-time PCR kits are **convenient methods** for the detection of several STI-associated pathogens at the same time.
- Users should be aware of the **lack of sensitivity for the detection of *M. genitalium* and *T. vaginalis*.**
- The commensal *U. parvum*, *U. urealyticum* and *M. hominis* species **should not be included in these kits for a screening in the lower urogenital tract**





<https://www.cnr-ist.fr/>