Evaluation of ESwab (COPAN) for the detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* from genital specimens with Mycoplasma Duo kit (BIO-RAD)

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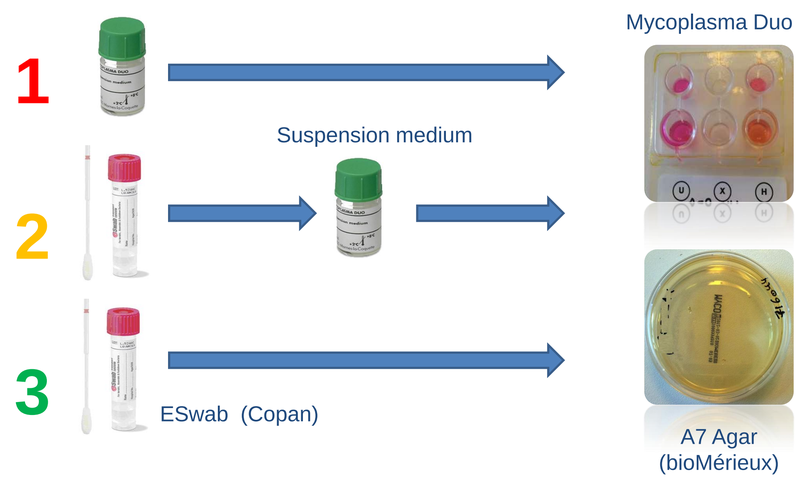
OBJECTIVES :
To validate ESwab as an alternative collection and transport medium for identification and titration of genital mycoplasma by using the Mycoplasma Duo kit

METHODS :

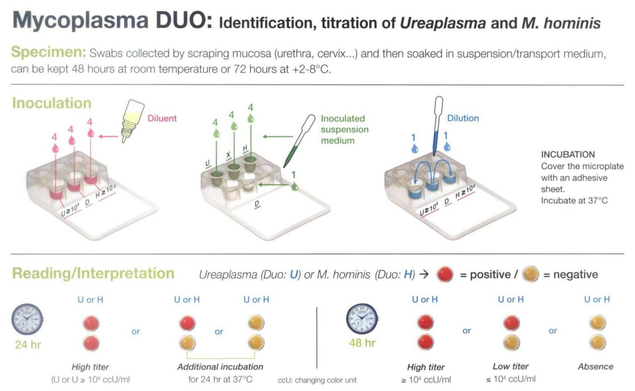
1) Comparison between 3 different methods for Mycoplasma culture, identification and titration of 25 clinical specimens and various dilutions of *U. parvum* ATCC 27815 (*Up*) and *M. hominis* ATCC 27618 (*Mh*) strains :

- **Method 1 :** collection by a Rayon swab, transfer into the suspension medium of the Mycoplasma Duo kit (Bio-Rad France) and microplate seeding according to manufacturer's instructions
- **Method 2 :** collection and transport by Eswab (Copan, Italy) and transfer of 200 µl of ESwab medium into the suspension medium of the Mycoplasma Duo kit before microplate seeding
- **Method 3 :** collection by ESwab and direct seeding of the microplate by 100µl of Eswab medium



- Transport < 48 hours at room temperature
- Inoculation of an A7 agar (BioMérieux, France) as gold standard, in each case
- Incubation, reading and interpretation according to manufacturer's instructions

2) Statistical analysis of patient's results during one year before and after introducing Eswab for mycoplasma diagnosis in our lab (comparing percentages)



Bio-Rad instructions for using Mycoplasma Duo kit

RESULTS :

ATCC strains

Results	Method 1		Method 2		Method 3	
***U. parvum* ATCC 27815**	Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar
Dilution 1/10	$\geq 10^4$	10^4	$\geq 10^4$	10^4	$\geq 10^4$	10^5
Dilution 1/20	$\geq 10^4$	10^3	$\geq 10^4$	10^3	$\geq 10^4$	10^5
Dilution 1/100	$\geq 10^4$	10^4	$\geq 10^4$	10^3	$\geq 10^4$	10^4
Dilution 1/1000	$\leq 10^3$	10^3	$\leq 10^3$	10^3	Ø	Ø

Results	Method 1		Method 2		Method 3	
***M. hominis* ATCC 27618**	Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar
No dilution	$\geq 10^4$	10^5	$\geq 10^4$	10^5	Ø	10^3
Dilution 1/10	$\leq 10^3$	10^3	$\geq 10^4$	10^3	Ø	Ø
Dilution 1/100	$\leq 10^3$	10^3	$\leq 10^3$	10^3	Ø	Ø

With ATCC strains, there was no difference between the method 1 and 2. The method 3 didn't allow the detection of *Mh* at every concentration and *Up* at the lowest.

Clinical specimens

About the 25 clinical specimens :

- 17 negative cultures with every method
- 2 samples contaminated by yeast
- 3 positive cultures with *U. urealyticum* (*Uu*) $\geq 10^4$ colour changing unit/ml for the 3 methods
- 1 culture positive with *Uu* $\leq 10^3$ CCU for methods 1 and 2 and negative for method 3
- 1 culture positive with *Uu* $\geq 10^4$ CCU for the 3 methods and *Mh* $\geq 10^4$ CCU for methods 1 and 3 and *Mh* $\leq 10^3$ CCU with method 2
- 1 culture positive with *Uu* $\geq 10^4$ CCU for methods 1 and 3 and *Mh* $\leq 10^3$ CCU with method 2

Results	Specimen	Method 1		Method 2		Method 3	
		Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar	Mycoplasma Duo kit	A7 Agar
Patient 1	Urethral	Uu $\geq 10^4$	Uu $< 10^3$	Uu $\geq 10^4$	Uu 10^5	Uu $\geq 10^4$	Uu 10^5
		Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø
Patient 2	Vaginal	Uu $\geq 10^4$	Uu 10^5	Uu $\geq 10^4$	Uu 10^5	Uu $\geq 10^4$	Uu 10^5
		Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø
Patient 3	Vaginal	Uu $\geq 10^4$	Uu 10^6	Uu $\leq 10^3$	Uu 10^5	Uu $\geq 10^4$	Uu 10^6
		Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø
Patient 4	Vaginal	Uu $\geq 10^4$	Uu 10^5	Uu $\geq 10^4$	Uu 10^4	Uu $\geq 10^4$	Uu 10^5
		Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø
Patient 5	Vaginal	Uu $\leq 10^3$	Uu $\leq 10^3$	Uu $\leq 10^3$	Uu $\leq 10^3$	Uu Ø	Uu $\leq 10^3$
		Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø	Mh Ø
Patient 6	Vaginal	Uu $\geq 10^4$	Uu $\geq 10^4$	Uu $\geq 10^4$	Uu $\geq 10^4$	Uu $\geq 10^4$	Uu $\geq 10^4$
		Mh $\geq 10^4$	Mh $\leq 10^3$	Mh $\leq 10^3$	Mh $\leq 10^3$	Mh $\geq 10^4$	Mh $\leq 10^3$

Results of the six positive clinical specimens

Statistical analysis of patient's results during one year before and after introducting Eswab as collection medium

No statistically significant difference between the two study periods was found : 269 specimens (27%) positive in 2009 versus 320 (26%) in 2010 with about a thousand clinical specimens in each cohort

Year	2009	2010
Uu negative	729 (73%)	918 (74%)
Uu $\leq 10^3$	61 (6%)	62 (5%)
Uu $\geq 10^4$	208 (21%)	258 (21%)
TOTAL	998	1238

Year	2009	2010
Mh negative	960 (96%)	1200 (98%)
Mh $\leq 10^3$	16 (1,6%)	62 (0,9%)
Mh $\geq 10^4$	22 (2,2%)	27 (2,2%)
TOTAL	998	1238

Comparison of *U. ureaplasma* and *M. hominis* results between 2009 and 2010

CONCLUSION :

- **Transfer of 200 µl ESwab into the Bio-Rad suspension medium** provides equivalent results in comparison with the method recommended by Bio-Rad.
- **Use of ESwab** for collection and transport of mycoplasma with Mycoplasma Duo kit **simplifies the detection of genital mycoplasma** by allowing multiple testing from the same original specimen.