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STUDY REPORT

Evaluation of the diagnostic of urogenital mycoplasma with Mycoview test in the laboratory : Laboratoire d'Analyses de Biologie Médicale Pavat-Huguenin – 39105 DOLE

This report contains 4 pages.

Purpose

Evaluate the performance of MycoView for the diagnostic of urogenital mycoplasma compared to the culture methods used routinely in the laboratory and referred as reference method.

Introduction

This study has been carried out in the Laboratory : PAVAT-HUGUENIN, Dole (39) – France in the microbiology department for the period between February '08 and September '08.
This prospective study has included urogenital specimens collected in duplicate by the gynecologists and transmitted to the laboratory for the diagnosis of urogenital mycoplasmas *Ureaplasma urealyticum* (U.u) and *Mycoplasma hominis* (M.h).

Material and Methods

The specimens :
The diagnosis of mycoplasma was done on 210 specimens composed as follow :
Vaginal (VS) n=185 ; Endocervical (ES) n=11 ; Vulva, n=7 ; Urethral (US) n=3 ; Urine, n= 2 and Semen, n=2.
VS and ES represents the great majority (93%) of the specimens.
The samples have been taken in duplicate and transported following the usual laboratory recommendations.

The culture methods used routinely in the laboratory and referred as reference method :
The laboratory uses the conventional culture methods to carry out the mycoplasma diagnosis.
- The diagnosis of U.u. is done by using culture in liquid medium U9 (Biorad) with an estimation of the numeration by dilution serials from 10 to 10 of the original sample and reading after 48 hours of incubation at +37°C.
- The diagnosis of M.h. is carried out using culture A7 agar (Elitech) after 48 hours of incubation under anaerobic conditions at +37°C.

Principle of the test to be evaluated :
The Mycoview test is a liquid culture method in tray. It allows the detection and differentiation of both species U.u and M.h with the diagnostic of levels considered

as significant. This diagnosis is done in 24 h for U.u ; the diagnosis of M.h requires a reading after 48h incubation period.
The tray includes also a resistance test to some antibiotics used in mycoplasmology. The evaluation of this part of the tray was not the purpose of this study.
The laboratory has used the test following strictly the recommendations of the supplier and described in the instruction for use of the kit (Mycoview, ref. 2040, ed. 2008.03).

Evaluation protocol

Reminder : 2 specimens taken by the same person have been collected for each patient.
One sample was used following the 2 techniques of culture used currently in the laboratory. The second sample was tested simultaneously with Mycoview test.
The interpretation of the mycoplasma diagnostic was done after different reading times depending on the method used and the manufacturer's recommendations.
The diagnosis of both species U.u. or M.h. was considered as positive in the case of the most frequent specimens (VS, ES and US) for rates estimated as significant when $\geq 10^4$ CCU/mL in liquid medium, or many colonies of M.h. on the A7 agar medium.
In the case of rarer specimens such as urine or semen, the diagnostic was considered as positive just with the presence of U.u. or M.h..

The performance study compares the results of the mycoplasma diagnosis for both species U.u and M.h :
- A negative or positive diagnostic obtained with the test and the reference method is considered as concordant. Any other discordant result with the test is considered as a false positive or false negative.
- The performance of Mycoview test compared to the reference method is given in terms of sensitivity and specificity. The overall agreement of Mycoview test is expressed as a percentage of the overall concordance.

Results

Among the 210 analysis carried out, only 208 are taken into account for comparison methods. 2 Mycoview tests could not be interpreted and are discussed here under (§ Conclusion and discussion).
The results of the analysis of the mycoplasma diagnosis with Mycoview method compared to the reference method (208 samples) is presented in the following table.

		Reference	
		(+)	**(-)**
Mycoview	**(+)**	29	11 [(b)]
	(-)	3 [(a)]	165

(a) False négative, (b) false positive ;

- The **sensitivity** of Mycoview test is **91%** ; its **specificity** is **94%**.

Details of the results [a,b] observed as discordant :
- 3 false negative : Vaginal specimens were concerned for which U.u rates were considered as significant. For these samples (N° 15279, 0526260 0603135), U.u diagnosis has not been determined with Mycoview test after 24 hours of incubation.

- 11 false positive : Vaginal specimens were concerned for which U.u rates were considered as commensal ($<10^4$ CCU/mL) and not significant. For these samples (N° 27262, 0514122, 0519104, 0527310, 0528039, 0529147, 0604254, 060146, 0709246, 0813248 et 0819042), U.u diagnosis has been determined as significant with Mycoview test after 24 hours of incubation.

Conclusion and Discussion

The performance of Mycoview test kit has been evaluated by comparison of conventional culture methods used in the laboratory and considered as the reference method.
210 urogenital specimens have been included in this study. The prevalence of the mycoplasma diagnosis in this study was quite low (15%).

- The **sensitivity** and **specificity** of Mycoview test obtained from the 208 samples are respectively **91%** and **94%**.
- The **efficiency of Mycoview test** for the diagnosis of urogenital mycoplasma has shown an **overall agreement of 93% with the reference method.**

Regarding the correct identification of both species U.u and M.h (no discrepancy was observed between Mycoview test and the reference method), we can consider that only the well N°2 ($U.u \geq 10^4$) of the test should be a little bit more discriminatory.
Nevertheless, in some analysis serials, it had been reported that the reading times, either for the conventional cultures, or for the Mycoview tray could not be done respecting the incubation times.
Therefore, for any future study done on the Mycoview test, the incubation times should be indicated on the result data sheets, in order to allow accurate discussions on the discrepancies observed.
About both samples excluded from the results analysis (VS N°15046 and 0804165), the Mycoview test could not be interpreted; all the wells of the tray were showing a doubtful (orangey pink) or frank (fuchsia red) color change after 24 hours of incubation. A doubtful color changing should lead to the repetition of the test and a frank color change of all 12 wells of Mycoview tray should lead to the repetition of the test with the sample diluted at 1/10. However, these samples were corresponding to specimens containing a high level of *Escherichia coli*. This flora in excess could not be sufficiently inhibited after inoculation of the selective medium and was leading to a color change of the medium because of its alcalinisation. According to the instructions of use of the Mycoview kit, it would have been preferable to repeat the test, but it could not be done in the laboratory.

General Conclusion :

The analysis of the study has shown that Mycoview test has an overall agreement of 93% with reference methods.
As for any culture method with a threshold level for quantitative determination, the limit of the test is that depending on the mycoplasma strains, the differentiation

between a 10^3 CCU/mL and a 10^4 CCU/mL sample is not always as accurate as it should be. This is the limit of any culture test in liquid medium using a cut-off.
The Mycoview test kit is easy to use and includes all the required materials to perform the test that are not provided by other competitors.
The Mycoview test is suitable for routine diagnostic of urogenital mycoplasma but reading times should be respected as late readings (>24h) could lead to false positive results, especially for *Ureaplasma urealyticum*.

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