

**Micro-test system for rapid isolation and identification of *Candida* species in urinary tract infections****DOI:** <http://doi.org/10.26758/7.1.7>

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**Abstract**

**Objectives.** We tried to create a culture medium and a method used to rapidly isolate and identify the yeasts of *Candida* genus.

**Material and Methods.** The isolation and identification of *Candida* genus yeasts have been accomplished using the micro-test system alongside the classical method. The strains of reference were collected from the Bacteriological Laboratory of the National Centre for Public Health.

**Results.** Choosing the culture medium for a prompt isolation of the germs represents a primordial element for determining the correct diagnosis. The elaborated medium increases the sensibility, selectivity and the specificity of the test used to identify *Candida* genus yeasts. The method involves the insertion of the material into the culture medium followed by a further incubation and identification of the *Candida* genus yeasts.

**Conclusions.** We have created a culture medium that allows a fast identification of the *Candida* genus yeasts. The medium is sensitive, economical and easy to use, as well as accessible for the microbiological labs of various levels. Comparing to the usual practices, the elaborated method gives us the possibility to obtain a result in 9 hours after the inoculation of the pathological material.

**Keywords:** *Candida* species; fast identification; culture medium; urinary tract infections.

**Introduction**

The urinary tract infections (UTIs) are among the most often infectious diseases in the medical practice. The positive prediction for UTIs clinical symptoms varies from 0,5 or lower in case of urethral symptoms for women. A significant number of UTIs evolve asymptomatic, and in case of two-year-old children the symptomatology of urinary tract infections is non-specific (Buiuc and Negut, 1999).

The frequent occurrences of mycosis, their slow and often unpredictable evolution are determined by sometimes thoughtless use of a wide range of antibiotics that have modified the biological balance between the bacteria and micromycetes in different ecological niches (Coman and Mares, 2000).

According to specialists „conditions that predispose to candiduria include diabetes mellitus, antibiotic and corticosteroid therapy, as well as factors such as local physiology and disturbance of urine flow. Lower urinary tract candidiasis is usually the result of a retrograde infection, while renal

parenchymal infection most often follows candidemia. In addition to asymptomatic candiduria, recognized clinical forms of candidal urinary tract infections include bladder infection, renal parenchymal infection, and infections associated with fungus ball formation” (Fisher et al., 1982).

The situation could be improved if removal of some predisposition factors is considered. The study of Malani and Kauffman found out that in almost 50% of asymptomatic patients candiduria could be cleared by the elimination of indwelling catheters and antibiotics (Malani and Kauffman, 2007).

The diseases caused by micromycetes are not properly addressed in terms of etiopathogenesis and therapy, as they become the object of study for the practitioner or the laboratory only in critical situations when the clinical evolution of the disease or the classical treatment take a dramatic turn. (Coman and Mares, 2000; Bouza et al., 2001).

Very often, urogenital mycoses have a chronic evolution with frequent recurrences. Among *Candida* spp., *Candida albicans* is the most common infectious agent that colonize the skin, gastrointestinal tract and reproductive tract (Parveen Surain Dabas, 2013).

*C. albicans* is reported as the most common species that causes urinary tract infections, non-*albicans* *Candida* species are better adapted to the environment urinary tract. According to some studies, that more 50% of urinary isolates belong to non-*albicans* species (*C. tropicalis*, *C. glabrata*) (Sobel et al., 2011).

The incidence of candiduria depends on the setting and population that are studied and has to be carefully compared because of the above-outlined discrepancies with opinions on candiduria. The general consensus is that *Candida* spp. is the most frequently isolated pathogen in nosocomial urinary tract infections (Alvarez-Lerma et al., 2003, Richards et al., 2007).

There is evidence that the incidence is linked to antibiotic usage (Weinberger et al., 2003). In general, most incidence rates that based on culture results are likely underestimated, because standard urine culture is not very sensitive. Shay and Miller estimated in 2004 that the incidence of candiduria was ~25,000 cases per year in the United States.

UTIs caused by *C. albicans* are reported more frequently in catheter-associated UTIs than in non-catheter-associated infections (Achkar & Fries, 2010).

The presence of *Candida* spp. on any surface of the body is abnormal, even if it does not cause any health injuries, because *Candida* spp. develops some toxins that provoke the weakening of the immune system (Lipovsky and Hoepelman, 1999).

If UTIs, caused by *Candida* spp., are not diagnosed and treated earlier, can lead to the development of severe complications (recurrent UTIs, blood infection and renal parenchyma damage, renal scarring, hypertension, renal insufficiency or renal failure) (Gelotar, Gandha and Sinha, 2012).

## Material and the Methods

This study was completed using materials and standard materials recorded in the Republic of Moldova by the Ministry of Health. The study included 126 urine samples collected from the hospitalized patients with UTIs.

Sample collection: All samples were collected by midstream urine samples in a sterile container.

Sample testing: All the samples were tested as follows.

Step 1: Direct microscopy by using gram stain to identify yeast cells, budding yeast cells, hyphae and pseudohyphae.

Step 2: Culture was done in 2 Sabouraud Dextrose Agar plates with antibiotics (chloramphenicol and cycloheximide). One plate was incubated at room temperature (25°C-28°C) for mycelia growth and other at 37°C for yeast growth. Both plates were examined every 3rd day for a period of three weeks before declared negative.

Step 3: The growth of bacterial cultures was examined by culture characteristics, using Lactophenol Cotton Blue solution, germ tube test, sugar assimilation test.

The reference strains, *C. albicans* ATCC 10231, *C. albicans* ATCC 38248, *C. albicans* ATCC 36232, *C. tropicalis* ATCC 1369 and *C. krusei* ATCC 24480 were received from the Microbiological Laboratory of the National Centre for Public Health.

Data analysis was performed using statistical and computerized data processing (Epi Info 7 software) through the comparative method with the calculation of the standard errors, confidence intervals with the appreciation of differences between the experimental group and control group according to the criteria "t-Student" and significance threshold "p".

## Results

During the conducted research, a new culture medium has been created in order to rapidly indicate the presence of *Candida* yeasts - DSMCand (Dosed Selective Medium for Candida).

This specially created environment contains the following ingredients: dry peptone broth, glucose, gelatine, the 199 medium, sodium dihydrogen, potassium hydrogen, phenol red and ciprofloxacin. The dry peptone broth, the glucose, gelatine and the 199 medium serve as a nutritional basis including all the necessary components that facilitate the growth and development of the *Candida* genus yeasts.

The Ciprofloxacin is the selectivity factor because it inhibits the growth and development of other microorganisms, thus ensuring the specificity of the growth environment.

The identification of the *Candida* genus yeasts occurs under conditions of pH formed of sodium dihydrogen phosphate, potassium hydrogen and the substances obtained after splitting the glucose with phenol red.

The culture medium is fixed on the bottom of a 10,0 mL vial which serves at the same time as a vessel for multiplication and identification of *Candida* genus yeasts. In order to identify the yeasts, 2.0 mL of sterile physiological serum, in which the media is dissolved and the seeds are inoculated, is added in the vial. The vial is incubated for 9-24 hours at a temperature of 37°C. In case *Candida* is present, the mixture turns from red to yellow.

We created 9 types of DSMCand with different ingredients as seen in Table 1.

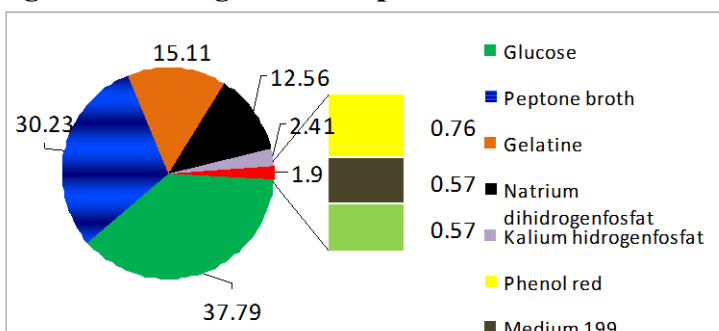
**Table 1. The influence of culture medium ingredients on *Candida* genus yeasts identification**

№	Media Ingredients	The options of media composition								
		1	2	3	4	5	6	7	8	9
1.	Dry peptone broth	+	-	+	+	+	+	+	+	+
2.	Glucose	+	+	-	+	+	+	+	+	+
3.	Gelatin	+	+	+	-	+	+	+	+	+
4.	Sodium Hidrogen	+	+	+	+	-	+	+	+	+
5.	Potassium dihydrogen	+	+	+	+	+	-	+	+	+
6.	199 Media	+	+	+	+	+	+	-	+	+
7.	Phenol red	+	+	+	+	+	+	+	-	+
8.	Ciprofloxacin	+	+	+	+	+	+	+	+	-
Results of identification		◇	-	-	◇	⊠	⊠	x	x	◇

Note: “+” – indicates the presence of the ingredient, “-” – the absence of the ingredient, x - the growth and development of yeasts that allow a 24 hours identification in incubation conditions under 37°C; ✕ - doesn't allow the yeasts identification; ◇ - the growth and development of yeasts that allow on 4-5 hours identification in incubation conditions under 37°C.

Also, the sensibility of the medium according to the quantitative composition of the ingredients has been tested. Therefore, 5 different quantitative compositions of the culture medium have been created and the best option representing an optimal ratio of ingredients for a fast *Candida* genus yeast identification has been detected (fig. 1).

**Figure 1. The ingredients report in the DSMCand**



It is worth mentioning the fact that in order to prepare the right medium to conduct an analysis, the ingredients are used in small proportions, which makes the growth medium economical enough. The identification time for *Candida* genus yeasts depends on the initial concentration of the germs in 1 mL/g of research material (Table 2).

**Table 2. The identification time of *Candida* genus yeasts according to their initial concentration in the research material**

Concentration (CFU/mL, g)	Identification time in hours									
	1	2	3	4	5	6	7	8	9	24
10 <sup>1</sup>	-	-	-	-	-	-	-	+	+++	+++
10 <sup>2</sup>	-	-	-	-	-	-	-	+	+++	+++
10 <sup>3</sup>	-	-	-	-	-	+	++	+++	+++	+++
10 <sup>4</sup>	-	-	-	-	+	++	+++	+++	+++	+++
10 <sup>5</sup>	-	-	-	+	++	++	+++	+++	+++	+++
10 <sup>6</sup>	-	-	+	+	++	++	+++	+++	+++	+++
10 <sup>7</sup>	-	+	+	++	++	+++	+++	+++	+++	+++
10 <sup>8</sup>	-	+	+	++	+++	+++	+++	+++	+++	+++
10 <sup>9</sup>	-	+	++	+++	+++	+++	+++	+++	+++	+++
The control	-	-	-	-	-	-	-	-	-	-

Note: “-” – the initial red color (negative result); “+” – light yellow (weakly positive result); “++” – yellow (positive result); “+++” – dark yellow (strong positive result).

The identification of the unique *Candida* yeasts cells is possible after 9-24 hours of incubation at 37°C, while for the concentration of 10<sup>3</sup>-10<sup>4</sup> CFU/mL within 5-6 hours of incubation at 37°C.

In order to determine DSMCand medium selectivity, several serial experiments were performed using 4 clusters of microorganisms for 30 times. The results of the experiments prove

that the DSMCand medium has a high selectivity towards *C. albicans* depending on the initial concentration of the *Candida* and microorganisms in the mixture. The identification of *C. albicans* in a concentration of  $10^5$  CFU/mL of the following microbial associations: *Candida albicans*  $10^5$  CFU/mL + *Staphylococcus aureus*  $10^6$  CFU/mL; *Candida albicans*  $10^5$  CFU/mL + *Pseudomonas aeruginosa*  $10^6$  CFU/mL; *Candida albicans*  $10^5$  CFU/mL + *Escherichia coli*  $10^6$  CFU/mL is possible after 6 hours of incubation at a temperature of  $37^\circ\text{C}$ . The identification of *C. albicans* in a concentration of  $10^2$ ,  $10^3$ ,  $10^4$  in 1 mL in the mixture of *S. aureus*  $10^6$  CFU/mL; *P. aeruginosa*  $10^6$  CFU/mL; *E. coli*  $10^6$  CFU/mL is succeeded after 9 hours (Table 3).

We have established the statistical accuracy of the indices assessing DSMCand selectivity in the following clusters: *C. albicans*  $10^5$  CFU/mL + *S. aureus*  $10^6$  CFU/mL; *C. albicans*  $10^5$  CFU/mL + *P. aeruginosa*  $10^6$  CFU/mL; *C. albicans*  $10^5$  CFU/mL + *E. coli*  $10^6$  CFU/mL comparing to the combinations in which the concentration of *C. albicans* is of  $10^2$ ,  $10^3$ ,  $10^4$  CFU/mL ( $p < 0.001$ ).

**Table 3. The DSMCand medium selectivity**

Evaluated associations among microorganisms	Nr. exp	Identification time			p	
		6	9	24	6 - 9 hours	9 - 24 hours
		% $\pm$ ES <sub>p</sub>	% $\pm$ ES <sub>p</sub>	% $\pm$ ES <sub>p</sub>		
<i>Candida albicans</i> ( $10^2$ ) <i>Staphylococcus aureus</i> ( $10^6$ )	40	0	100	100	-	-
<i>Candida albicans</i> ( $10^2$ ) <i>Pseudomonas aeruginosa</i> ( $10^6$ )	40	0	97.5 $\pm$ 1.42	97.5 $\pm$ 1.42	-	<0.05
<i>Candida albicans</i> ( $10^2$ ) <i>E. coli</i> ( $10^6$ )	40	0	100	100	-	-
<i>Candida albicans</i> ( $10^3$ ) <i>Staphylococcus aureus</i> ( $10^6$ )	40	10.0 $\pm$ 0.71	97.5 $\pm$ 1.42	100	<0.001	<0.05
<i>Candida albicans</i> ( $10^3$ ) <i>Pseudomonas aeruginosa</i> ( $10^6$ )	40	12.5 $\pm$ 0.86	100	100	<0.001	-
<i>Candida albicans</i> ( $10^3$ ) <i>Escherichia coli</i> ( $10^6$ )	40	17.5 $\pm$ 0.94	95.0 $\pm$ 1.53	100	<0.001	<0.05
<i>Candida albicans</i> ( $10^4$ ) <i>Staphylococcus aureus</i> ( $10^6$ )	40	60,0 $\pm$ 1.22	97,5 $\pm$ 1.42	100	<0.001	<0.05
<i>Candida albicans</i> ( $10^4$ ) <i>Pseudomonas aeruginosa</i> ( $10^6$ )	40	70,0 $\pm$ 1.26	100	100	<0.05	-
<i>Candida albicans</i> ( $10^4$ ) <i>Escherichia coli</i> ( $10^6$ )	40	65.0 $\pm$ 1.25	100	100	<0.001	-
<i>Candida albicans</i> ( $10^5$ ) <i>Staphylococcus aureus</i> ( $10^6$ )	40	90.0 $\pm$ 1.41	100	100	<0.001	-
<i>Candida albicans</i> ( $10^5$ ) <i>Pseudomonas aeruginosa</i> ( $10^6$ )	40	95.0 $\pm$ 1.53	100	100	<0.05	-
<i>Candida albicans</i> ( $10^5$ ) <i>Escherichia coli</i> ( $10^6$ )	40	95.0 $\pm$ 1.53	100	100	<0.05	-

We proved the statistical veracity of selected indices for identification of *C. albicans* in bacterial combinations during 6 - 9 hours ( $p_{6-9}$ ) and 9-24 hours ( $p_{9-24}$ ), where  $(0.05 > p < 0.001)$ . It's worth mentioning that the DSMCand medium has a selectivity towards *C. albicans*, preventing the growth and development of other microorganisms of the mixture.

In order to decide the sensibility of the DSMCand medium, a series of experiments have been conducted using 7 *Candida* genus yeasts strains with concentrations of  $10^4$  and  $10^5$  CFU in 1 mL, in 12-19 repetitions. By comparison with it, two other media were used for the growth of yeasts: Agar Sabouraud with glucose and Sabouraud broth with glucose (Table 4).

**Table 4. The sensibility of *Candida* genus yeasts identification test using DSMCand medium**

№	The microbial species	Nr. of repetitions	The concentration of microorganisms CFU/mL suspension, and the indication (by color) during 6 hours of incubation at 37 ° C (%)					
			DSMCand		Agar Sabouraud		Sabouraud Broth	
			$10^4$	$10^5$	$10^4$	$10^5$	$10^4$	$10^5$
1.	<i>C. albicans</i> ATCC 10231	15	93.3± 1.61	100	0	0	0	0
2.	<i>C. albicans</i> ATCC 38248	12	100	100	0	0	0	0
3.	<i>C. albicans</i> *	16	93.7 ±1.53	100	0	0	0	0
4.	<i>C. albicans</i> *	13	84.6±1.42	100	0	0	0	0
5.	<i>C. albicans</i> ATCC 36232	18	100	100	0	0	0	0
6.	<i>C. tropicalis</i> ATCC 1369	12	88.9±1.63	100	0	0	0	0
7.	<i>C. krusei</i> ATCC 24480	19	94.7±1.25	100	0	0	0	0
Total		102	93.6±0.41	100	0	0	0	0

Note: \* - clinical strain

We established that the DSMCand medium is much more sensible than the other two (Agar Sabouraud with glucose and Sabouraud Broth with glucose): in 93,6% of the cases, at a concentration of  $10^4$  CFU/mL, the identification of the *Candida* genus yeast was possible within 6 hours of incubation at a temperature of 37°C; the rate of success reached 100% for a concentration of  $10^5$  CFU/mL.

The resulting culture medium has been kept at the bottom of the vial at a temperature of 4-7°C for 2 years (observational study), without changing its initial properties.

126 urine samples from the hospitalized patients with UTIs were examined. *Candida* genus yeasts were detected in 5 patients accounting for 3,9% of the total sample. The results have been confirmed in 100% of the cases using the classical methods of investigation.

## Conclusions

Considering the fact that the UTIs are frequent in medical practice, the improvement and development of rapid isolation and identification methods of pathogenic and conditioned pathogenic microorganisms is most welcome allowing early treatment and preventing complications.

The DSMCand medium has a high specificity and sensitivity. The method allows an identification of *Candida* genus yeasts within a short period of time: from 4-5 hours up to 9-24 hours, depending on the initial concentration of the germs in 1 mL or gram of product.

DSMCand is cheap, easy to use and accessible to any microbiological labs. The validity of DSMCand expires after 24 months.

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