

Original papers

Characteristics of growth of yeasts and yeast-like fungi on chromogenic medium CHROMagar® Candida (GRASO)

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ABSTRACT. Early detection and correct diagnosis of fungal infection is important for further therapy and prophylaxis. Currently, there are a number of proposals related to rapid diagnostic tests. To those we can include chromogenic media, such as CHROMagar® Candida (GRASO). This medium has been developed to differentiate four species of genus *Candida*. Its use in laboratory has opened up possibilities for growing not only the genus *Candida*, but also other fungi. The study suggests that medium CHROMagar® Candida can provide an excellent pre-test, or be used as complementary to biochemical tests. Establishing a full template of colours would enable more efficient and fuller use of the medium properties, especially in the discrimination of the teleomorphic form from anamorphic.

Key words: CHROMagar® Candida, chromogenic medium, yeasts, yeast-like fungi, colour of growth

Introduction

Over the past 20 years mycological literature has shown rapid increase in the frequency of fungal invasion and growing number of etiological agents of systemic fungal infections and organ damage [1–3]. Yeasts and yeast-like fungi show significant expansiveness, particularly to the organisms of patients with higher risk of fungal infection. However, owing to the increase in the number of factors predisposing to infections, fungi have become more pathogenic [4,5]. Early detection and correct diagnosis of mycosis can reduce duration of disease and increase the effectiveness of treatment.

Standard identification of yeasts and yeast-like fungi involves several steps: direct preparations, cultures and biochemical series [6]. Microscopic slides afford possibilities for the assessment of population size, stage of fungi development and mutual proportions between the different components of microbiota [7]. Preparation and microscopic observation enable appropriate culturing of fungi. Classical diagnostic methods use morphological characteristics of fungi (size, colour and shape of the colony, the shape and size of blastospores, creation and arrangement of

pseudohyphae and hyphae, production of chlamydospores, artrospores, etc.) as well as biochemical (fermentation of selected carbohydrates, assimilation of carbon or nitrogen from selected organic compounds, acid production, etc.). These methods, however, require long time waiting for the final score, in case of microculture lasting up to 1–2 weeks. Therefore, laboratories increasingly choose the so-called rapid diagnostic tests, such as API Candida, API 20C AUX, ID32C. They shorten the time of identification to 24–48 hours. Commercial tests are based on evaluation of selected biochemical properties with assigned values, which in turn are given a numerical code designating the species [6,8].

Among rapid tests used recently in mycological diagnostics there are chromogenic media such as CHROMagar® Candida (GRASO, bioMérieux, MastDiagnostica) and CandiSelect® 4 (BioRad). Chromogenic media differentiate individual species of fungi on the basis of the colour of growth. CHROMagar® Candida and CandiSelect® 4 contain similar ingredients: peptone, glucose, agar, chloramphenicol or gentamicin, and chromogenic mixture, the composition of which is not released by the producers. Owing to predominance of the

Candida genus isolated from human organ ontocenoses [9–11], the company focused mainly on its identification.

The aim of the study was evaluation of growth of yeasts and yeast-like fungi on chromogenic medium CHROMagar® *Candida* and the analysis of the medium appropriateness in mycological diagnostics.

Materials and methods

Isolates of fungi used for testing originate from the collection of the Department of Mycology, University of Warmia and Mazury in Olsztyn, Poland. They were obtained in scientific cooperation from patients of Independent Public Centre of Pneumonia and Pulmonary Diseases, Endoscopic Laboratory of the Municipal Hospital and the Clinical Ward of Oncological Surgery at the Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn.

354 mono-species, 46 two-species and 4 three-species isolates of yeasts and yeast-like fungi were used in the study. The mono-species isolates belonged to 38 species, the dominant was the classical form of *Candida albicans* – 75 isolates. A significant share among the examined strains had *Saccharomyces cerevisiae* – 44 isolates, *Candida dubliniensis* – 33, *Issatchenkia orientalis/Candida krusei* – 27, *Pichia guilliermondii/Candida guilliermondii* – 22, *Candida albicans var. stellatoidea* – 19, and *Dipodascus capitatus/Trichosporon capitatum* – 16. Other species showed a lower number of isolates ranging from 1 (e.g. *C. lactis-condensi* or *Rhodospodium diobovatum*) to 12 (*Debaryomyces hansenii/Candida famata*). The most common component of mixed isolates was the classic form of *C. albicans* – 24 cases. *C. guilliermondii* was also frequently noted – 14, *S. cerevisiae* – 11 and *C. dubliniensis* – 8.

Fungi were identified on the basis of macroscopic (size, shape, colour, texture of the colony) and microscopic characteristics (shape, size and location of budding cells, chlamydo-spores and blastospores, size and shape of pseudohyphae and hyphae) as well as biochemical properties (the ability of fermentation and assimilation of sugars) using the following keys: Kreger-van Rij [12], De Hoog et al. [13], Kurtzman and Fell [14], and elaboration characterizing taxonomy of the tested group [1,2,15].

CHROMagar® *Candida* by GRASO was employed

for the analysis of colours of growth on chromogenic medium. Assessment of the usefulness of this medium for differentiation and identification of yeasts and yeast-like fungi belonging to other than *Candida* genus was attempted. For this purpose the colours of colonies of all tested isolates were analyzed. Colours characteristic for each species were determined by RAL CLASSIC template of the German Institute for Quality and Designations RAL *Deutsches Institut für Gütersicherung und Kennzeichnung e. V.* [16].

Results

The analyzed species were characterized by good growth on chromogenic medium. Only one species, *Oosporidium margaritiferum*, did not grow.

As expected by the producer, *C. albicans* grew in green colonies (Table 1). A similar growth was found among others, also for *C. albicans var. stellatoidea*, *C. dubliniensis*, *C. guilliermondii* and *S. cerevisiae*. The use of RAL CLASSIC template by German Institute for Quality and Designations allowed capturing subtle differences in the colour of individual isolates. The classic form of *C. albicans* grew in three shades of green: RAL 6002, RAL 6005 and RAL 6033, with the dominant colour of leaf green RAL 6002 – 57.3% of isolates. *C. albicans var. stellatoidea* for 89.5% of isolates had a shade of blue-green RAL 6004, *C. dubliniensis* – pearl opal green RAL 6036 for 75.8%, *C. guilliermondii* – turquoise green RAL 6034 for 70%, and *S. cerevisiae* in 65.9% of cases was characterized by blue-green colour RAL 6004 (Table 1). Different shades of green were also observed for other species, namely *C. catenulata* (RAL 6009), *C. datila* (RAL 6024), *D. occidentalis* (RAL 6020), *P. brasiliensis* (RAL 6001) and *S. fermentans* (RAL 6036).

The characteristic colour of growth for *C. tropicalis* was the azure blue RAL 5009, for *C. pelliculosa* – water blue RAL 5021, *T. capitatum* – pigeon blue RAL 5014 and for *T. cutaneum* – heather violet RAL 4003 (Table 1). For *C. krusei* three different shades of expressly matt colonies were noticed: telemagenta RAL 4010, salmon pink RAL 3022 and ivory RAL 1014. The increase in the colour purple violet RAL 4007 was noted in case of *C. glabrata* (10 isolates), *D. hansenii* (4), *M. reukafii* (1), *K. marxianus* (2) and *S. cerevisiae* (8).

In case of several species the difference in colour of growth between anamorphic and teleomorphic

Table 1. Diversity of growth colour of mono-species isolates of fungi on chromogenic medium

No.	Species	Colour acc. RAL CLASSIC	Number of isolates	% of isolates
1	<i>Candida albicans</i> (Robin) Berkhout (1923)	leaf green RAL 6002	43	57.3
		moss green RAL 6005	25	33.3
		mint turquoise RAL 6033	7	9.3
2	<i>Candida albicans</i> (Robin) Berkhout var. <i>stellatoidea</i> (Jones & Martin) Diddens & Lodder (1942)	blue green RAL 6004	17	89.5
		mint green RAL 6029	2	10.5
3	<i>Candida catenulata</i> Diddens & Lodder (1942)	fir green RAL 6009	3	100.0
4	<i>Candida datila</i> (Kluyver) S.A. Meyer & Yarrow (Yarrow & Meyer 1978)	traffic green RAL 6024	4	100.0
5	<i>Candida dubliniensis</i> Sullivan et. al. (1995)	pearl opal green RAL 6036	25	75.8
		mint turquoise RAL 6033	8	24.2
6	<i>Candida glabrata</i> (Anderson) S.A. Meyer & Yarrow (Yarrow & Meyer 1978)	purple violet RAL 4007	10	90.9
		antique pink RAL 3014	1	9.1
7	<i>Candida lactis-condensi</i> (B.W. Hammer) S.A. Meyer & Yarrow (Yarrow & Meyer 1978)	moss green RAL 6005	1	100.0
8	<i>Candida pelliculosa</i> Radaelli	water blue RAL 5021	5	100.0
9	<i>Candida tropicalis</i> (Castellani) Berkhout (1923)	azure blue RAL 5009	9	100.0
10	<i>Debaryomyces hansenii</i> (Zopf) Lodder & Kreger-van Rij (1952)/ <i>Candida famata</i> (Harrison) S.A. Meyer & Yarrow	purple violet RAL 4007 – anamorphic form	1	100.0
		purple violet RAL 4007 – teleomorphic form	3	27.3
		leaf green RAL 6002 – teleomorphic form	8	72.7
11	<i>Debaryomyces occidentalis</i> (Klocker) Kurtzman & Robnett (1991)	chrome green RAL 6020	5	100.0
12	<i>Debaryomyces polymorphus</i> (Klocker) Price & Phaff (1979)	ivory RAL 1014	1	100.0
13	<i>Dipodascus albidus</i> de Lagerheim (1892)	fern green RAL 6025	1	100.0
14	<i>Dipodascus capitatus</i> de Hoog, M.Th. Smith & Gueho (1986)/ <i>Trichosporon capitatum</i> Diddens & Lodder (1942)	red violet RAL 4002 – teleomorphic form	1	100.0
		pigeon blue RAL 5014 – anamorphic form	15	100.0
15	<i>Dipodascus tetrasperma</i> (Macy & M.W. Miller) von Arx (1977a)	beige RAL 1001	1	100.0
16	<i>Geotrichum fermentans</i> (Diddens & Lodder) von Arx (1977)= <i>Trichosporon fermentans</i> Diddens & Lodder (1942)	red lilac RAL 4001 with curry RAL 1027	3	42.9
		turquoise blue RAL 5018 with pearl blackberry RAL 4012	1	13.3
		ivory RAL 1014	3	42.9
17	<i>Issatchenkia orientalis</i> Kudryavtsev (1960)/ <i>Candida krusei</i> (Castellani) Berkhout	telemagenta RAL 4010 mat	10	37.0
		salmon pink RAL 3022 mat	12	44.4
		ivory RAL 1014 mat	5	18.5
18	<i>Kluyveromyces marxianus</i> (E.C. Hansen) van der Walt (1971)/ <i>Candida kefyr</i> (Beijerinck) van Uden & H.R. Buckley	light pink RAL 3015	2	50.0
		purple violet RAL 4007	2	50.0
19	<i>Kluyveromyces yarrowi</i> van der Walt, E. Johannsen, Opperman & Halland (1986b)	Reseda green RAL 6011 with moss green RAL 6005	2	100.0
20	<i>Metschnikowia pulcherrima</i> Pitt & M.W. Miller (1968)/ <i>Candida pulcherrima</i> (Lindner) Windisch	mint green RAL 6029 – teleomorphic form	3	100.0
		pastel violet RAL 4009 – anamorphic form	1	100.0
21	<i>Metschnikowia reukafii</i> Pitt & M.W. Miller (1968)	purple violet RAL 4007	1	100.0

No.	Species	Colour acc. RAL CLASSIC	Number of isolates	% of isolates
22	<i>Oosporidium margaritifera</i> Stautz (1931)	no growth	2	100.0
23	<i>Paracoccidioides brasiliensis</i> (Splendore) de Almeida (1930)	emerald green RAL 6001	1	100.0
24	<i>Pichia bispora</i> (Wickerham) Kurtzman (1984a)	brown red RAL 3011	3	100.0
25	<i>Pichia guilliermondii</i> Wickerham (1966)/ <i>Candida guilliermondii</i> (Castellani) Langeron & Guerra	antique pink RAL 3014 – teleomorphic form	2	100.0
		pastel turquoise RAL 6034 – anamorphic form	6	30.0
		turquoise green RAL 6016 – anamorphic form	14	70.0
26	<i>Pichia jadinii</i> (A. & R. Sartory, Weill & J. Meyer) Kurtzman (1984a)/ <i>Candida utilis</i> (Henneberg) Lodder & Kreger-van Rij	patina green RAL 6000 – teleomorphic green	2	100.0
		patina green RAL 6000 – anamorphic form	2	40.0
		beige red RAL 3012 – anamorphic form	3	60.0
27	<i>Rhodospiridium diobovatum</i> Newell & I.L. Hunter (1970)	pine green RAL 6028 with red lilac RAL 4001 center	1	100.0
28	<i>Rhodospiridium kratochvilovae</i> Hamamoto, Sugiyama & Komagata (1988a)	patina green RAL 6000	1	100.0
29	<i>Saccharomyces bayanus</i> Saccardo (1895)	purple violet RAL 4007 with cream RAL 9001	3	42.9
		yellow green RAL 6018	4	57.1
30	<i>Saccharomyces carlsbergensis</i> E.C. Hansen (1908)	grey beige RAL 1019	1	100.0
31	<i>Saccharomyces cerevisiae</i> Meyen ex E.C. Hansen (1883)	purple violet RAL 4007	8	18.2
		red violet RAL 4002	7	15.9
		blue green RAL 6004	29	65.9
32	<i>Saccharomyces pastorianus</i> E.C. Hansen (1904)	red violet RAL 4002 with beige red RAL 3012	1	100.0
33	<i>Saccharomycopsis capsularis</i> Schionning (1903)	ocean blue RAL 5020	4	44.4
		patina green RAL 6000	5	55.6
34	<i>Saccharomycopsis fermentans</i> (C.-F. Lee, F.-L. Lee, Hsu & Phaff) Kurtzman & Robnett (1995)	pearl opal green RAL 6036	1	100.0
35	<i>Schizosaccharomyces pombe</i> Lindner (1893)	grass green RAL 6010	2	66.7
		cobalt blue RAL 5013	1	33.3
36	<i>Torulaspora delbrueckii</i> (Lindner) Lindner (1904)	pearl opal green RAL 6036	1	33.3
		moss green RAL 6005	2	66.7
37	<i>Trichosporon cutaneum</i> (de Beurmann, Gougerot & Vaucher) Ota (1926) = <i>Trichosporon beigelii</i> Vuillemin (1902)	heather violet RAL 4003	6	100.0
38	<i>Zygosaccharomyces rouxii</i> (Boutroux) Yarrow (von Arx et al. 1977)	green brown RAL 8000	1	100.0

form was found. *P. guilliermondii* was characterized by colony of colour antique pink RAL 3014, while *C. guilliermondii* – pastel turquoise RAL 6034 and turquoise green RAL 6016. The growth of *M. pulcherrima* was in the colour of mint green RAL 6029, and *C. pulcherrima* – pastel purple RAL 4009. A similar correlation was also obtained for *D. capitatus* and *T. capitatum*, *D. hansenii* and *C. famata*, *P. jadinii* and *C. utilis* (Table 1).

Growth in the form of colourful colonies was observed for 30% of multispecies isolates (Table 2), with each colour corresponding to the colour typical for the component species of the isolate. 70% of

two- and three-species isolates were noticed in the colour typical for the dominant species in the culture.

Discussion

RAL German Institute for Quality and Designations *RAL Deutsches Institut für Gütersicherung und Kennzeichnung e. V.* was founded in 1925 as Reichsausschuss für Lieferbedingungen and since 1927 has dealt with systematization of colour description for the needs of industry and trade [16]. The basic template RAL

Table 2. Diversity of growth colour of multi-species isolates of fungi on chromogenic medium

No.	Species	Colour acc. RAL CLASSIC	Number of isolates
1	<i>C. albicans</i> + <i>C. albicans</i> var. <i>stellatoidea</i>	leaf green RAL 6002	1
2	<i>C. albicans</i> + <i>C. glabrata</i>	purple violet RAL 4007 + mint turquoise RAL 6033	1
3	<i>C. albicans</i> + <i>C. guilliermondii</i>	leaf green RAL 6002 + antique pink RAL 3014	1
		moss green RAL 6005	3
		pastel turquoise RAL 6034	1
		turquoise green RAL 6016	1
4	<i>C. albicans</i> + <i>C. krusei</i>	salmon pink RAL 3022	1
		moss green RAL 6005 + telemagenta RAL 4010	1
5	<i>C. albicans</i> + <i>C. tropicalis</i>	moss green RAL 6005	1
		leaf green RAL 6002	1
		azure blue RAL 5009	1
6	<i>C. albicans</i> + <i>C. utilis</i>	leaf green RAL 6002	1
7	<i>C. albicans</i> + NN	moss green RAL 6005 + red lilac RAL 40011	
		leaf green RAL 6002	1
8	<i>C. albicans</i> + <i>P. bispora</i>	brown red RAL 3011	1
9	<i>C. albicans</i> + <i>S. cerevisiae</i>	blue green RAL 6004 + leaf green RAL 6002	1
		blue green RAL 6004	2
		leaf green RAL 6002	1
		purple violet RAL 4007	1
10	<i>C. albicans</i> var. <i>stellatoidea</i> + <i>C. dubliniensis</i>	ivory RAL 1014	1
11	<i>C. albicans</i> var. <i>stellatoidea</i> + <i>C. guilliermondii</i>	blue green RAL 6004 + leaf green RAL 6002	1
		turquoise green RAL 6016	1
12	<i>C. albicans</i> var. <i>stellatoidea</i> + NN	blue green RAL 6004	1
13	<i>C. dubliniensis</i> + <i>C. guilliermondii</i>	pearl opal green RAL 6036	1
14	<i>C. dubliniensis</i> + <i>C. krusei</i>	salmon pink RAL 3022	1
15	<i>C. dubliniensis</i> + <i>G. fermentans</i>	pearl opal green RAL 6036	1
16	<i>C. dubliniensis</i> + <i>K. marxianus</i>	pearl opal green RAL 6036	1
17	<i>C. dubliniensis</i> + NN	mint turquoise RAL 6033	1
		pearl opal green RAL 6036	1
18	<i>C. dubliniensis</i> + <i>T. beigeli</i>	pearl opal green RAL 6036 + heather violet RAL 4003	1
19	<i>C. guilliermondii</i> + <i>K. marxianus</i>	light pink RAL 3015	1
20	<i>C. krusei</i> + <i>G. fermentans</i>	salmon pink RAL 3022 + turquoise blue RAL 5018 + pearl blackberry RAL 4012	1
21	<i>C. krusei</i> + <i>S. cerevisiae</i>	blue green RAL 6004 + telemagenta RAL 4010	1
22	<i>C. tropicalis</i> + NN	azure blue RAL 5009	2
23	<i>D. polymorphus</i> + <i>S. pombe</i>	ivory RAL 1014 + grass green RAL 6010	1
24	<i>I. orientalis</i> + <i>Pichia bispora</i>	pine green RAL 6028 + telemagenta RAL 4010	2
25	<i>M. reukafii</i> + <i>D. albidus</i>	purple violet RAL 4007 + fern green RAL 6025	1
26	<i>P. bispora</i> + <i>K. marxianus</i>	purple violet RAL 4007	1
27	<i>S. cerevisiae</i> + <i>D. tetrasperma</i>	red violet RAL 4002	1
28	<i>S. cerevisiae</i> + <i>G. fermentans</i>	red lilac RAL 4001 + curry RAL 1027 + blue green RAL 6004	1
29	<i>S. cerevisiae</i> + NN	blue green RAL 6004	1
30	<i>C. albicans</i> + <i>C. guilliermondii</i> + <i>C. krusei</i>	moss green RAL 6005 + turquoise green RAL 6016	1
31	<i>C. albicans</i> + <i>C. guilliermondii</i> + <i>S. cerevisiae</i>	leaf green RAL 6002 + red violet RAL 4002	1
32	<i>C. guilliermondii</i> + <i>C. kefyri</i> + <i>S. cerevisiae</i>	light pink RAL 3015	1
33	<i>C. guilliermondii</i> + <i>C. tropicalis</i> + NN	pastel turquoise RAL 6034 + turquoise green RAL 6016	1

NN – not known

CLASSIC includes 213 shades categorized into 9 groups of shades: RAL 10xx – yellow; RAL 20xx – orange; RAL 30xx – red and pink; RAL 40xx – violet; RAL 50xx – blue; RAL 60xx – green; RAL 70xx – gray; RAL 80xx – brown; RAL 90xx – white and black.

So far the system has been used in paint industry, construction, furniture, advertising and computer graphics. It allows producers of compatible components to unify „the language of colours” and affords possibilities for converting the RGB system into a digital description. Our research suggests implementation of RAL template in microbiology. In the studies requiring evaluation of colour, researchers frequently have problems with subjective and precise perception of shades. It particularly concerns chromogenic media and tests. Nawrot et al. [17] used the RAL template in the evaluation of suitability of chromogenic medium CandiSelect® 4 (BioRad) for preliminary identification of the *Candida* genus. The results obtained by these authors indicate usefulness of the RAL template. The use of RAL symbols allows researchers to evaluate their own isolates easily while comparing them on the same medium.

CHROMagar® *Candida* was developed to differentiate four species of genus *Candida* basing on the colour of colonies: *C. albicans* – green, *C. tropicalis* – blue, *C. krusei* – pink, mat and *C. glabrata* – dark pink, shiny [18]. Employment of this medium in laboratory enables also growth of other fungi, not only genus *Candida*. On this medium Odds and Bernaerts [18] grew colonies of *C. famata*, *C. utilis*, *C. pelliculosa*, *C. laurentii* and *C. kefyr*. Willinger and Manafi [19] obtained *C. lusitanae*, *C. parapsilosis*, *C. guilliermondii*, *S. cerevisiae* and *R. mucilaginosa*. Because some of the abovementioned fungi colonies grew on CHROMagar® *Candida* in colours corresponding to the colours characteristic for species defined by the producer, many authors performed a review of identification on this medium using other methods. Kirkpatrick et al. [20] analyzed 48 isolates of fungi, initially identified on CHROMagar® *Candida* as *C. albicans*, using the API-type tests, germ-tubes and pattern of DNA. It appeared that 22 (46%) isolates tested were in fact *C. dubliniensis*, the remaining 26 were *C. albicans*. Włodarczyk et al. [21] verified 111 isolates identified as *C. glabrata*. Basing on biochemical tests ID32C and analysis of polymorphism 5.8 S rRNA with RFLP method, the preliminary identification was confirmed only in

case of 79 isolates (71%). Others were classified as *S. cerevisiae*, *K. apiculata*, *C. guilliermondii*, *C. parapsilosis* and *C. rugosa*. Simultaneously, several authors noticed that some strains of the four species differentiated on CHROMagar® *Candida* may form colonies of unusual colour on this medium. Bishop et al. [22] obtained white-coloured colonies of *C. glabrata*, while Murray et al. [23] found colonies of lavender, beige, pink and blue in case of several isolates of *C. albicans*, pink and green for *C. glabrata* and lavender for *C. tropicalis*. This data corresponds closely with observations conducted in the course of our studies. Precise analysis of colours of colonies on CHROMagar® medium, using a RAL CLASSIC template by German Institute for Quality and Designations *RAL Deutsches Institut für Gütersicherung und Kennzeichnung e. V.*, allowed us to capture frequently subtle differences between the species and to designate colours characteristic for each species. We also observed appearance of different colours of colonies among species, depending on anamorphic or teleomorphic form. Therefore, it can be suggested that CHROMagar® *Candida* medium may not serve as the only identification method, but be used as preliminary or complementary to biochemical tests. Establishing complete template of colours would enable more efficient and fuller use of the medium properties, especially for discrimination of sexual form from asexual. This may be important during treatment owing to physiological characteristics of both the forms. For many years it was believed that only the budding forms exist in the body [1], then the presence of fungi in mycelial phase was revealed [7]. Recent observations suggest that sexual forms of fungi may also be found in the organism [24].

Laboratory practice of the Department of Mycology UWM shows that fungi often co-exist, two or three species simultaneously. Analyzing bronchoscopic material Biedunkiewicz [25] obtained 15 two-species and 4 – three-species isolates. Dynowska et al. [10] noticed the presence of nine combinations of two-species and one of three-species isolates. Diagnostic methods based solely on rapid biochemical tests are ineffective in case of multispecies isolates. Biochemical properties of one component of a mixed culture may overshadow features of the second one, or the properties of two fungi may overlap to give a combination characteristic for completely different species not present in the isolate. Although the use

of microculture in identification lengthens the time of diagnosis, it allows visualization of mixed isolates. Studies show that microculture is necessary for correct designation of species. Rapid chromogenic tests commonly used in diagnostic laboratories pose a risk of subjective error in distinguishing colour by the examiner, especially in case of mixed isolates. These tests should be used as complementary or differentiating anamorphic and teleomorphic forms within already marked species.

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