# Comparative Evaluation of CHROMagar and Conventional Carbohydrate Fermentation Test for Candida Species Identification in HIV Patients: Assessing Sensitivity and Specificity.

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#### **ABSTRACT**

Context: Candida species are opportunistic fungal pathogens commonly associated with infections in immunocompromised individuals, particularly those with HIV. Reliable identification of Candida species is crucial for appropriately treating and managing these infections. The Conventional Carbohydrate fermentation test, traditionally used for species identification, has limitations, so alternative methods like CHROMagar, a chromogenic culture medium, are being explored. CHROMagar offers faster results and easier interpretation, making it a promising alternative.

Aims: The present study aims to isolate and speciate different candida species among HIV-positive patients by the CHROMagar method and Carbohydrate fermentation test and to determine the sensitivity and specificity of both methods.

Methods and Material: Oral rinse was collected from each subject in sterile sample containers and speciation was done by CHROMagar method and carbohydrate fermentation test.

**Results:** The sensitivity and specificity of the CHROMagar method in the present study were 100% and 45% respectively, whereas the sensitivity and specificity of the carbohydrate fermentation test were 63% and 25% respectively showing that the CHROMagar method is more accurate with 80% accuracy when compared to carbohydrate fermentation test with 53% accuracy.

Conclusions: CHROMagar provides fast and accurate identification of Candida species compared to conventional carbohydrate fermentation tests. This method's potential application in HIV patients to identify specific species and assess their immune status is important for treating infections and understanding the incidence and role of Candida species in invasive and systemic diseases.

**Keywords:** Oral Candida, CHROMagar, HIV, Sensitivity, Specificity

#### 1. INTRODUCTION

Oral candidiasis is an opportunistic infection of the oral cavity caused by Candida species<sup>1</sup>. Candida, a genus containing around 200 species, is a normal commensal flora in the oral microbiota of many healthy individuals. However, nearly 20 of these species are pathogenic to humans, capable of causing a range of infections from mucocutaneous to severe, invasive infections affecting various organs<sup>2</sup>. The most common species in infected and healthy mouths is Candida albicans, estimated to be found in over 80% of oral fungal isolates. Other types of *Candida*, the so-called non-*albicans Candida* species present in the mouth are *C. glabrata*, *C.* dubliniensis, C. parapsilosis, C. krusei, and C. tropicalis<sup>3,4</sup>.

Oral candidiasis is an immunological state marker of HIV-positive patients and therefore represents a clinical predictor of HIV infection progression<sup>5</sup>. Moreover, oral candidiasis may lead to secondary infections, further weakening the already compromised immune system of HIV patients<sup>6</sup>. Therefore, timely diagnosis and management of oral candidiasis are crucial for improving the overall health and well-being of individuals living with HIV.

Sabouraud glucose agar is the primary medium for isolating Candida and other yeast species, but its lack of differential indicators may hinder the detection of mixed cultures, risking underdiagnosis or misdiagnosis of infections. Alternative selective or differential media may be needed for accurate identification. Enhanced detection of specific yeast species can improve clinical outcomes. Awareness of the limitations of Sabouraud agar is crucial for effective diagnosis<sup>7</sup>.

Generally, Yeast identification begins with a rapid germ tube test to distinguish C. albicans from other species, but it may result in false positives or negatives. If unidentifiable, further tests like culturing on cornmeal agar, carbohydrate fermentation, and carbohydrate assimilation are done (24-72 hours to two weeks)<sup>8</sup>. Rapid identification is facilitated by chromogenic substrate culture media, which yield different coloured colonies based on microbial enzymes.

CHROMagar Candida employs this methodology to differentiate several Candida yeasts by colour and morphology. It identifies C. albicans by the growth of green colonies, C. tropicalis as steel blue colonies, C. glabrata as off-white to cream-coloured colonies and C. krusei as pink-coloured colonies<sup>9</sup>.

Carbohydrate fermentation tests assess microbial ability to ferment carbohydrates for energy production. They provide insights into metabolic capabilities and offer valuable information for Candida identification, such as C. albicans' typical glucose fermentation but lack lactose or sucrose fermentation. Combining CHROMagar and carbohydrate fermentation tests enhances Candida species identification and differentiation, facilitating prompt diagnosis and targeted treatment of oral candidiasis in HIV patients.

This study aims to identify and specify different types of candida species in HIV-positive patients using the CHROMagar and carbohydrate fermentation tests and to determine the sensitivity and specificity of both methods.

### 2. SUBJECTS AND METHODS

In this study, there are 50 participants, including 40 HIV-positive patients who are not receiving antifungal treatment. in the Study Group. The other 10 participants are healthy individuals and make up the Control Group. We excluded patients who are on antifungal therapy and those who are immunosuppressed for reasons besides HIV from the study group.

Oral rinses were collected from HIV-positive patients (n=40) residing at HIV rehabilitation centres and healthy individuals (n=10) from RIMS Institute of Dental & Medical Sciences and Hospital, Kadapa. The patients were asked to rinse their mouths with a sterile solution, and the rinse was then collected in a sterile manner to avoid contamination.

### **Preparation of Phosphate Buffered Saline:**

10.7gms of phosphate is added to 1000ml of distilled water and heated to dissolve the medium completely. Sterilised by autoclaving at 10lbs pressure (115°C) for 10 minutes.

## Preparation of phenol red indicator:

1.6 gms of phenol red broth base is suspended in 100 ml of distilled water and heated to ensure the complete solution. It is sterilised by autoclaving at 15lbs pressure (121°C) for 15 minutes.

#### Preparation of sugars:

2% of sugars are prepared -2gms of the respective sugar (Glucose, Maltose, Sucrose and Lactose) is added to 100ml of distilled water and incubated at 100°C for 15 minutes.

#### Preparation of CHROMagar Candida:

42.72gms of CHROMagar candida is suspended in 1000ml of distilled water and heated up to boiling to dissolve the medium completely. Cooled to 45 - 50°C and poured into sterile petri plates.

The oral cavities of all the subjects were examined for the presence or absence of candida by visual examination and were given 10ml of phosphate-buffered saline and requested to rinse thoroughly for 60 seconds. These rinses are collected in

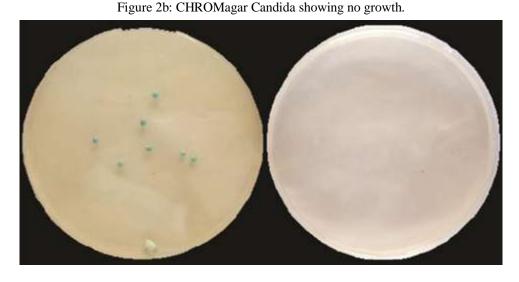
sterile sample containers. Out of which 10µl is plated on CHROMagar plate and incubated at 37°C for 24 to 48 hours. After incubation colonies were identified based on the colour exhibited by the species.

The carbohydrate fermentation test was performed in liquid media and is based on a demonstration of acid and/or carbon dioxide production. The test was carried out in test tubes using particular carbohydrates viz. glucose, sucrose, lactose and maltose. 3ml of basal media containing phenol red indicator and 2% test sugar is added to each test tube. Inverted Durham tubes are placed in test tubes sealed and stored at 4°C. Before use, they are brought to room temperature. One drop of the sample was inoculated in each tube and incubated at 25–30°C for 7 days.

Figure 1a: CHROMagar Candida showing- C. albicans, C. tropicalis, C. krusei and C. glabrata. Figure 1b: CHROMagar Candida showing- C. krusei and C. glabrata.



Figure 2a: CHROMagar Candida showing- C. albicans.



Positive fermentation is indicated by the turbidity and accumulation of gas (CO2) in the Durhams' tube. As a change in the colour of the indicator (Phenol Red) from red to yellow (acid production) signifies carbohydrate assimilation, the production of gas is necessary to indicate fermentation, while only acid production was taken as carbohydrate assimilation<sup>11</sup>.

In our study, several precautions were implemented during the preparation and handling of CHROMagar culture media and for performing Carbohydrate fermentation tests to minimize the risk of contamination as much as possible, thereby supporting the reliability and reproducibility of our results. Stringent sterile techniques were employed, including using laminar flow hoods, sterile disposables, and personal protective equipment with media preparation protocols such as autoclaving and disinfecting work surfaces.

#### 3. RESULTS

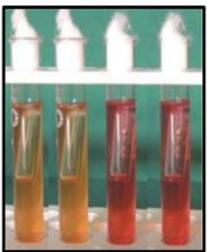
The present study was conducted to assess the efficacy of the CHROMagar method over the conventional method (carbohydrate fermentation test) in candidal speciation and to assess the sensitivity and specificity of both methods. In the present study, the total number of 40 HIV patients with males was 16 and females was 24, total number of patients above 40 years was 22 and below 40 years was 18. Of these, 20 patients are taking HAART therapy and 20 are not. We recorded the CD4+ T-cell counts for all patients to see how different Candida species relate to CD4 counts and HAART therapy. (Table-1)

Figure 3a: Carbohydrate Fermentation Test showing- Positive fermentation and assimilation of Glucose and Negative Maltose, Sucrose and lactose- Indicating C. krusei/ C. glabrata.

Figure 3b: Carbohydrate Fermentation Test showing- Positive fermentation and assimilation of Glucose and Maltose, Negative Sucrose and lactose- Indicating C. albicans.

Figure 3c: Carbohydrate Fermentation Test showing- Positive fermentation and assimilation of Glucose, Maltose and Sucrose and Negative lactose- Indicating C. tropicalis.





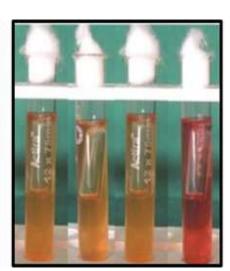


Table 1: Clinical data of the total HIV samples (by, Descriptive statistics).

	AGE		GENDER		CD4 COUNT		HAART	
	BELOW 40YEARS	ABOVE 40 YEARS	MALES	FEMALES	<200	>200	WITH HAART	WITHOUT HAART
TOTAL	18 (45%)	22(55%)	16(40%)	24(60%)	17(42.5%)	23(57.5%)	20(50%)	20(50%)
SAMPLES (n=40)								

All 40 HIV-positive patients have shown mixed Candida species of which the most common species isolated by using the CHROMagar method was C. albicans (66.6%) followed by C. glabrata (53.3%), C. krusei (50%) and C tropicalis (46.6%) whereas, in the control group (n=10), the species identified were C. albicans (40%) and C. tropicalis (10%). (Table-2)

Table 2: Distribution of Candida species by CHROMagar method in HIV-positive patients and Control group (by, Descriptive statistics).

SPECIES	HIV Patients		Control Group		
	N=40		N=10		
	N	%	N	%	
C.albicans	26	66.6	4	40	
C.tropicalis	16	46.6	1	10	
C.glabrata	22	53.3	0	0	
C.krusei	20	50	0	0	

The most common species isolated in HIV patients by using the Carbohydrate fermentation test was C. albicans (60%) and the second most common species was C. tropicalis (33.5%) followed by C. glabrata (7.5%) and C. krusei (5%). Among the control group (n=10) the only species identified was C. albicans (30%) (Table-3)

Table 3: Distribution of Candida species by Carbohydrate Fermentation Test method in HIV-positive patients and Control group (by, Descriptive statistics).

SPECIES	HIV P	atients	Control Group		
	N=	<b>=40</b>	N=10		
	N	%	N	%	
C.albicans	24	60	3	30	
C.tropicalis	13	33.5	0	0	
C.glabrata	3	7.5	0	0	
C.krusei	2	5	0	0	

Sensitivity and specificity are important metrics used to evaluate the performance of a diagnostic test. Here's how they are calculated by using formulas such as,

Sensitivity=True Positives (TP)/True Positives (TP)+False Negatives (FN)

Specificity=True Negatives (TN)/True Negatives (TN)+False Positives (FP)

In this study, the sensitivity of the CHROMagar method was 100%, with a specificity of 45.45%, a positive predictive value of 76%, and a negative predictive value of 100%. In contrast, the sensitivity of the conventional carbohydrate fermentation test was 63.64%, specificity was 25%, and positive predictive value was 70%. The negative predictive value was 20% showing that the CHROMagar method is more accurate with 80% accuracy when compared with the carbohydrate fermentation test with 53.3% accuracy. (Table-4)

Table 4: Sensitivity and Specificity of CHROMagar method and Carbohydrate

METHODS	SENSITIVITY	SPECIFICITY	POSITIVE PREDICTIVE VALUE	NEGATIVE PREDICTIVE VALUE	ACCURACY
CHROMagar method	100%	45.5%	76%	100%	80%
Carbohydrate fermentation test	63.64%	25%	70%	20%	53.3%

## 4. DISCUUSSION

Oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection in HIV-infected patients, with over 90% affected at some stage of their disease. <sup>12</sup> It is a sign of impaired local or systemic defence mechanisms. <sup>13</sup> HIV infection

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is not only associated with increased colonization rates but also with the development of overt disease. Factors like tissue adhesion, phenotypic switching, biofilm formation and production of extracellular hydrolytic enzymes play an important role in the invasion and colonization of host tissues<sup>14</sup>.

Although the introduction of highly active antiretroviral therapy (HAART) has reduced the incidence of OPC, it remains prevalent. OPC presents in two forms: pseudomembranous, characterized by removable white lesions and detectable pseudo hyphae, and erythematous, marked by macular erythema without visible hyphae. It can lead to oesophageal candidiasis, impacting nutrition and overall health.

Resistance to antifungal azoles often occurs when CD4+ counts drop below 200 cells/mm³, resulting from the selection of resistant Candida albicans strains or infection by inherently resistant species. The effectiveness of antiretroviral therapy in reducing OPC incidence is hindered by factors like poor adherence, toxicity, resistance, and limited treatment access in developing countries.

Candida albicans, the primary cause of candidiasis, is a diploid dimorphic fungus commonly found in about 40% of healthy individuals but can cause serious infections in immunocompromised hosts. <sup>12</sup> Candida albicans has long been the predominant species isolated from the oral cavities of both immunocompromised and immunocompetent individuals, accounting for 60%-80% of cases. However, the emergence of non-albicans Candida (NAC) species, which are more resistant to azole antifungals, poses a public health concern, particularly in resource-limited settings. Common NAC species linked to oral candidiasis include C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei (Issatchenkia orientalis). Few studies reported that HIV-infected individuals showed significantly higher rates of colonization by NAC species compared to non-HIV-infected individuals, likely due to their compromised immune systems. Those with low CD4 counts among HIV-infected individuals are at an increased risk of invasive infections from these predominant pathogens, associated with various clinical types of Candidiasis. Its isolation from clinical specimens can no longer be ignored as a non-pathogenic isolate or dismissed as a contaminant. Further research is needed to explore the role of NAC species in invasive fungal infections among immunocompromised patients. <sup>15</sup> The identification of Candida species is crucial as it allows for tailored antifungal therapy to combat specific pathogens causing oral candidiasis. Antifungal medications like azoles and topical agents, such as nystatin, miconazole, clotrimazole, and econazole, are commonly prescribed for the treatment of oral candidiasis.

External factors most notably antifungal resistance can influence the accuracy and reliability of candidal identification. Antifungal-resistant strains may exhibit altered growth patterns, atypical colony morphology, or modified biochemical profiles that deviate from expected norms. Such changes can complicate identification using traditional methods like CHROMagar or carbohydrate fermentation tests, which rely on predictable phenotypic characteristics. Consequently, these external influences may necessitate the use of additional confirmatory techniques (e.g., molecular assays or antifungal susceptibility testing) to ensure precise identification and to guide effective treatment strategies.<sup>17</sup>

All 40 HIV-positive patients have shown mixed species of which the most common species isolated by using the CHROMagar method was C. albicans (66.6%) followed by C. glabrata (53.3%), C. krusei (50%) and C. tropicalis (46.6%) whereas the control group (n=10) has shown only C. albicans (40%) and C. tropicalis (10%). The presence of C. albicans in the control group could be attributed to its presence as a normal commensal organism of the oral cavity. The results indicate that there is an increased Candida colonization in HIV patients particularly Non-albicans Candida (NAC) species. In the present study, the most common Non-albicans Candida (NAC) species isolated was C. glabrata. The study's findings matched the results of Mulu et al. (2013)<sup>18</sup> in which C. albicans (78.5%) was the most predominant species followed by C. glabrata (22.5%), C. tropicalis (14.1%), and C. krusei (5.6%). This variation in the percentage could be due to the size of the sample. The sample size in the study conducted by Mulu et al was 215 HIV-positive patients whereas the sample size in this study was 40. Similar findings were also observed in the studies done by Birhan Moges et al. (2016)<sup>19</sup>, Goulart LS et al. (2018)<sup>20</sup> and, Elham Aboualigalehdari et al. (2020)21. Among the control group (n=10) the only species identified by using the Carbohydrate fermentation test was C. albicans (30%) whereas among HIV patients the most common species isolated was C. albicans 24 (60%) and the second most common species was C. tropicalis 13 (33.5%) followed by C. glabrata 3 (7.5%) and C. krusei 2 (5%) (Table-5, Graph-3). This is inconsistent with the study done by Kali et al. (2015)<sup>22</sup> in which C. albicans (n=14) was the most common isolate followed by C. tropicalis (n=12), C. glabrata (n=2) and C. krusei (n=1). However, one of the limitations is that differentiation of C. glabrata and C. krusei cannot be done with a Carbohydrate fermentation test as both the species are positive only for glucose. An additional Urease test has to be done to differentiate between these two species, in which C. krusei shows urease positive and C. glabrata is negative. This urease test has not been done in the present study suggesting that there is an increased chance of getting false positive results with carbohydrate fermentation test.

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Another disadvantage of this test is time-consuming. The time taken for the carbohydrate fermentation test in this study was 7 days whereas the time taken for the CHROMagar method was 24-48 hours. This is in agreement with the study done by Sidhartha Giri and Anupma Jyoti Kindo (2015)<sup>23</sup>. Therefore, the carbohydrate fermentation test is labour-intensive and takes a longer time to determine the diagnosis and judge the proper antifungal agent. When compared to the carbohydrate fermentation test, CHROMagar is a simple, rapid, and inexpensive method used in the identification of yeasts from mixed cultures.

In the present study, the sensitivity of the CHROMagar method was 100%, specificity was 45.45%, positive predictive value was 76% and negative predictive value was 100% whereas the sensitivity of the conventional carbohydrate fermentation test was 63.64%, specificity was 25%, positive predictive value was 70% and the negative predictive value was 20% showing that CHROMagar method is more accurate with 80% accuracy when compared to carbohydrate fermentation test with 53.3% accuracy. This is similar to a study done by M.V. Pravin Charles et al., <sup>24</sup> in which the Chrome agar method had higher sensitivity and specificity compared to the carbohydrate fermentation test. However, in another study done by Nayak S et al., <sup>25</sup> CHROMagar methods showed 100% specificity and 100% sensitivity when compared to conventional methods. This variation in the specificity rate could be due to the variation in the sampling population. Nayak S et al. conducted the study among denture and non-denture wearers whereas the present study was conducted among HIV patients.

The study reveals that both CHROMagar and Carbohydrate fermentation tests exhibit a high degree of sensitivity in identifying Candida species in HIV patients, with similar results obtained for commonly isolated Candida species such as Candida albicans and Candida glabrata. However, the key findings suggest that CHROMagar demonstrated a higher specificity compared to the Carbohydrate fermentation test, particularly in the identification of less common Candida species. This may be due to CHROMagar's ability to provide visual identification of colonies, reducing the potential for misinterpretation.

The results of this study will not only help in understanding the accuracy of CHROMagar and the conventional carbohydrate fermentation test but also provide a basis for recommending the most effective and efficient method for identifying Candida species in HIV patients. This is crucial for appropriate management of Candida infections, improving diagnostic accuracy, optimizing laboratory workflow, and enhancing patient care, as timely and accurate identification of the infecting species can guide appropriate antifungal therapy and help reduce morbidity and mortality in HIV patients.

Our findings emphasize the utility of CHROMagar and carbohydrate fermentation tests as reliable, rapid, and cost-effective diagnostic tools for Candida identification. In many clinical settings especially in developing regions or smaller hospitals where access to advanced molecular diagnostics is limited, these methods provide a critical means of guiding timely and appropriate antifungal therapy. The study demonstrates that, despite their simplicity, these conventional techniques offer sufficient accuracy to inform clinical decisions, thereby optimizing patient management and potentially curbing the spread of antifungal resistance. Ultimately, the implementation of such diagnostic strategies can improve both therapeutic outcomes and infection control practices, ensuring that even facilities with limited resources can deliver high-quality patient care.

In conclusion, CHROMagar proves to be an expedient and reliable method for the isolation and speciation of Candida in HIV patients when compared to the traditional carbohydrate fermentation test. The preliminary study yields promising results; however; a key limitation of this study is the variation in sample size, age, and gender distribution between the test and control groups, which may influence the outcomes. To better understand and validate the findings, further research with a larger sample size is necessary. This will help ensure reliable conclusions about the optimal identification of candida in immunocompromised patients in contemporary clinical practice. Such future studies will help establish CHROMagar as a reliable and consistent alternative for Candida diagnostics in various clinical and research settings.

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