Hiroshima Journal of Medical Sciences
Vol.36, No.3, 289~294, September, 1987
HIJM 36-40

A Simplified Culture for the Diagnosis of Denture Stomatitis

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(Received May 27, 1987)

Key words: Denture stomatitis, Candida, Culture

ABSTRACT

The relationship between the clinical features of denture stomatitis and the number of *Candida* colonies determined by swabs from the palatal mucosa has been proved to be close. In daily practice, if the number of *Candida* organisms can be assessed present without both difficulty and need for special equipment, it might be of benefit. Therefore, a simplified culture has been developed for the diagnosis of denture stomatitis in the dental office, which is based on the acid-producing capacity of *Candida* species and is displayed in color changes. The coefficient of correlation between the culture reaction and the clinical assessment of denture stomatitis was $r=-0.64 \sim -0.86$. It has also been helpful in instructing patients about denture cleanliness.

The relationship between the clinical features of denture stomatitis (DS) and the number of *Candida* colonies determined by swabs from the palatal mucosa has been proved to be close. In daily practice, if we are able to assess the number of *Candida* organisms present without both difficulty and need for special equipment, it might be of benefit.

In gynaecology, many simplified media for *Candida* have been prepared and are on the market, e.g., Mizuno-Takada Medium® (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), Clinicult (Canditect)® (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), Microstix® -Candida (Miles-Sankyo Co. Ltd., Tokyo, Japan) and CA-TG Medium® (Hokuiken Co. Ltd., Sapporo, Japan). The basic components of these media are almost identical with the special character of each being due to the addition of small amounts of other ingredients to the Sabouraud's medium.

In the dental field, many research projects have been performed in the field of dental caries susceptibility and microbiological testing of root canals. Budtz-Jørgensen[2] reported the use of the Microstix® -Candida for the microbiological investigation of DS. At the research level, the use of the Sabouraud's media has been described by many researchers. However, in the dental office, ease of handling is one of the most important and practical factors. Therefore, a simplified culture for the diagnosis of DS in the dental office has been developed, which is based on the acid-producing capacity of *Candida* species and is displayed in color changes (red-yellow).

MATERIALS AND METHODS

1. Preliminary experiment on test solution for the diagnosis of DS

1) Composition of test solution

The test solution contained a carbon source, a nitrogen source, a nutrient source and a pH-indicator as principal elements together with chloramphenicol to provide specificity for *Can-*

This paper is based upon work presented at the IADR meeting in Sydney, August, 1983, Abstract #289.

dida and preserve the test solution. Sucrose, glucose, galactose, maltose and lactose were used as carbon sources. One % polypepton (Daigoeiyou Co. Ltd., Osaka, Japan) was used as nitrogen and nutrient sources. Each of resazurin, methyl red, alizarin, bromcresol green and chlorphenol red as a pH indicator was prepared to be 0.006% in the test solutions. The concentration of chloramphenicol was 0.1 mg/ml. The pH of each test solution was adjusted to 5.80 with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid.

2) Microorganism

C. albicans, which was taken from the mouth of a person with DS and identified, was used as a test microorganism.

3) Preparation of cell suspensions

The test microorganism was cultured in the Sabouraud's medium at 37°C for 10-18 hr, harvested and washed with 0.01M phosphate-buffered saline (pH7.2, PBS) for three times. That was diluted to give a suspension containing 10^5 cells/ml and was inoculated into the test medium described in 4).

4) Medium for viable counts

The Sabouraud's medium containing 0.1 mg/ml chloramphenicol was used.

2. Basic studies on simplified culture for the diagnosis of DS

1) Simplified culture for the diagnosis of DS (Stomastat®)

Stomastat® consisted of 4.0% glucose, 1.0% polypeptone, 0.01% chloramphenicol and 0.006% chlorphenol red. The total volume was 2 ml in which the initial pH was adjusted to 5.80.

2) Microorganisms

C. albicans IFO 1385, *C. tropicalis* IFO 1400, *C. krusei* IFO 1395, *C. parapsilosis* IFO 1396, *C. guilliermondii* IFO 0566, *Torulopsis glabrata* IFO 0622 and *T. inconspicua* IFO 0621 were obtained from the Institute for Fermentation Osaka and were used as test microorganisms. *Streptococcus mutans* E-49, FA-1, OMZ-176, K1R, *Escherichia coli* NIHJ C-2, *Lactobacillus casei* 4646, *L. fermenti* 14731 and *L. plantarum* 8014 were obtained from Osaka University Dental School, and were used as control microorganisms.

3) Relation between the pH and color change in Stomastat® and various microorganisms

Seven species of *Candida* were cultured in the Sabouraud's media and eight species of microorganisms except *Candida* were cultured in Trypticase Soy Broth(Microbiology Systems, BBL, Cockeysville, USA). They were centrifuged off, diluted to the same turbidity ($O.D._{\lambda=600nm}=1.0$) and used as a suspension of microorganisms. The pH drop and color change of Stomastat® inoculated with the suspension, at 37°C, were observed after 24 hr.

4) pH change in Stomastat® by initial cell numbers

Candida was cultured in the Sabouraud's medium at 37°C for 16-18 hr and then centrifuged off. That was diluted to several concentration from 10^2 to 10^7 cells/ml and inoculated in Stomastat® . The pH of Stomastat® was measured by pH meter (Corning Glass Works, Model 125, Ma, U.S.A.) after 24 hr at 37°C.

3. Clinical studies on Stomastat®

1) Subjects

Forty-nine patients with removable upper dentures (most of them complete dentures) in the National Sanatorium (56-93 years) and 13 outpatients of Hiroshima University Dental Hospital (52-85 years).

Swabs were taken from both the palatal mucosa and the fitting surface of the upper denture, put into the Stomastat® and incubated for 24 hr at 37°C. The pH change and color change were recorded.

2) Clinical assessment of DS

Clinical assessment of DS was performed by 2 dentists. The criteria of assessment under denture base were as follows.

+ + +	:	marked inflammation
+ +	:	defined inflammation
+	:	inflammation
±	:	slight inflammation
−	:	no inflammation

RESULTS

1. Preliminary experiment on the test solution for the diagnosis of DS

1) Examination of sugars as a carbon source

C. albicans (5×10^5 cells/ml) were inoculated into the test solutions which contained 5% of each of the following sugars: sucrose, glucose, galactose, maltose and lactose. The pH change of the test solutions after 24 hr incubation at

37°C is shown in Table 1. The drop in pH occurred in all test solutions except one with lactose. In particular, the pH dropped to 4.16 after 24 hr in the test solution with glucose, which was the greatest change.

Table 1. Incubation pH of various test solution after 24 hr inoculated with the suspension of *Candida albicans*.

Carbon source	Final pH after 24hr
Sucrose	5.39
Glucose	4.16
Galactose	5.64
Maltose	5.10
Lactose	5.80

Initial pH : 5.80
Initial cell numbers : 5 × 10^5 cells/ml

2) Examination of glucose concentration

C. albicans (5 × 10^5 cells/ml) were inoculated in each of six test solutions in which the glucose concentration was 0.5, 1.0, 2.0, 4.0, 5.0 and 10.0%. As a result, the pH of the 2.0, 4.0 and 5.0% glucose solutions dropped remarkably but only slightly in the 1.0 and 10.0% solutions. The pH change of the test solutions after 24 hr incubation at 37°C is shown in Fig.1. As a result of these findings, the test solution with 4.0% glucose was used in the following experiments.

3) Examination of pH-indicator

A suspension of *C. albicans* was inoculated into the test solutions which contained 4.0% glucose as a carbon source and various pH-indicators. The color change was recorded after 24 hr incubation at 37°C. As a result, when resazurin or methyl red was used, the reduction and decolorization in incubation failed to produce a recognizable color change. On the other hand, obvious color changes were observed with alizarin (pink to light pink), bromcresol green (blue to light blue), and chlorphenol red (red to yellow). Of all the pH-indicators in use, the color change of chlorphenol red was possibly most easily judged by the naked eye. In addition, the order of its color change was from red to orange to yellow with time. So chlorphenol red was used as a pH-indicator in the following experiments.

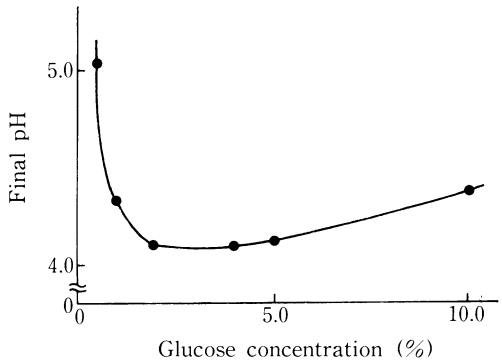


Fig. 1. Effect of glucose concentration on the incubation pH after 24 hr.
Initial pH : 5.80
Initial cell numbers : 5 × 10^5 cells/ml

4) Examination of incubation temperature

In order to study the effect of incubation temperature, the test solutions in which 5 × 10^5 cells/ml of *C. albicans* was inoculated were incubated for 24 hr at 20, 27, 30, 37 and 40°C. The relation between the various temperatures and pH is shown in Fig.2. The optimum temperature for drop in pH was 30-37°C.

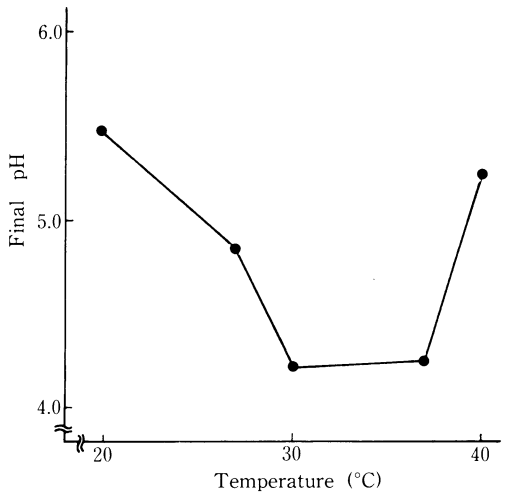


Fig. 2. Effect of temperature on the incubation pH after 24 hr.
Initial pH : 5.80
Initial cell numbers : 5 × 10^5 cells/ml

2. Clinical studies on the simplified culture for the diagnosis of DS

1) The relation between the pH and color change in Stomastat®, and various microorganisms

The results shown in Table 2 indicate the pH

Table 2. Incubation pH and color change of Stomastat® after 24 hr inoculated with various microorganisms.

Microorganisms	Final pH	Color change
Candida		
albicans IFO 1385	4.48	+
tropicalis IFO 1400	4.92	+ ~ ±
krusei IFO 1395	4.42	+
parapsilosis IFO 1396	5.56	±
guilliermondii IFO 0566	5.58	±
Torulopsis		
glabrata IFO 0622	5.16	+ ~ ±
inconspicua IFO 0621	5.60	±
Lactobacillus		
casei 4646	5.79	−
fermenti 14731	5.80	−
plantarum 8014	5.77	−
Streptococcus mutans		
E-49	5.77	−
FA-1	5.78	−
OMZ-176	5.76	−
KIR	5.72	−
Escherichia coli NIHJ C-2	5.77	−

Initial pH : 5.80
Initial cell numbers : 5 × 10^4 cells/ml

and color changes in Stomastat® inoculated with the suspensions of various microorganisms containing 5 x 10^4 cells/ml after 24 hr incubated at 37°C. The color change was to red if −, orange if ± and yellow if +. The pH dropped to 4.48 and 4.42, and the color change indicated + for *C. albicans* and *C. krusei*. For *C. tropicalis* and *T. glabrata*, the pH dropped to 4.92 and 5.16, and the color change indicated + ~ ±. However, the pH and color only changed slightly for *C. parapsilosis*, *C. guilliermondii* and *T. inconspicua*. For *S. mutans*, *L. casei*, *L. fermenti*, *L. plantarum* and *E. coli*, the pH and color did not change.

2) pH change in Stomastat® by initial cell numbers

The relation between the initial cell numbers of the various microorganisms and the pH change after 24 hr is shown in Fig.3. Regardless of *Candida* species, the tendency was observed for the degree of the pH drop to be in proportion to initial cell numbers. However, the acid-producing potential of *C. albicans* and *C. krusei* was higher than that of *C. tropicalis* and *T. glabrata*. The acid-producing potential of *C. parapsilosis*, *C. guilliermondii* and *T. inconspicua* was the lowest.

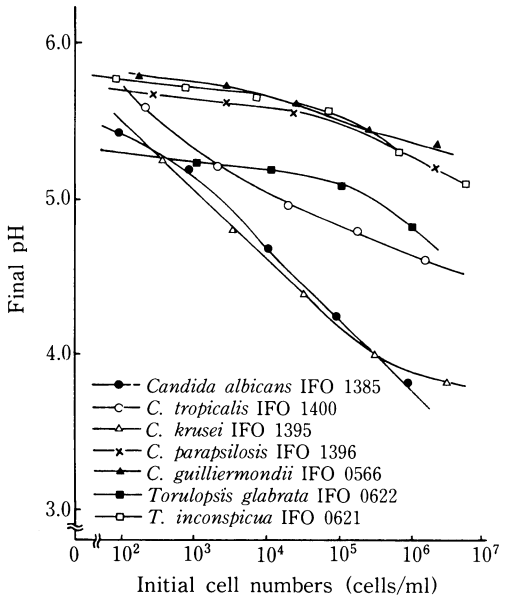


Fig. 3. Relation between the initial cell numbers and the incubation pH after 24 hr.

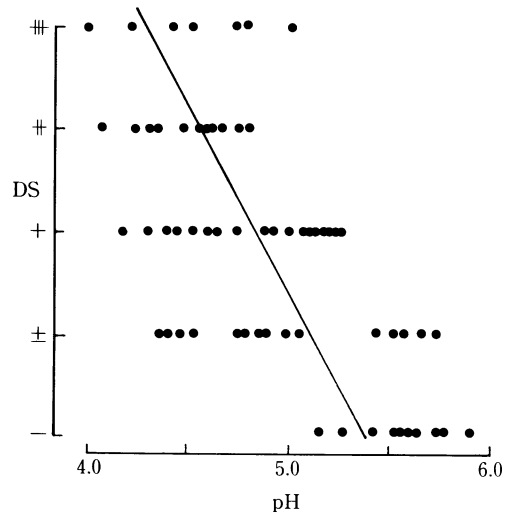
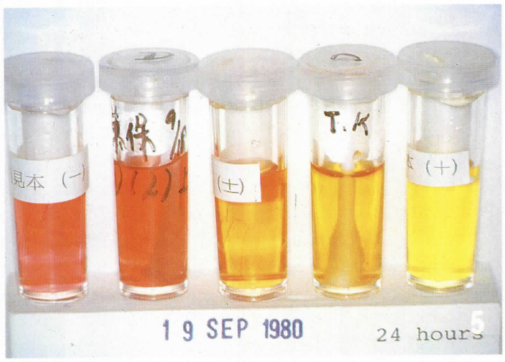


Fig. 4. Relation between Denture Stomatitis (DS) and pH change of Stomastat® ($r=-0.64$, $p<0.001$).

3. Clinical studies on Stomastat®

The relation between the clinical assessment(+ + + ~ −) of the inflammatory reaction of the mucous membrane under the denture base and the pH change in Stomastat® is shown in Fig.4 ($r=-0.64$, 63 persons). For about 25 pa-



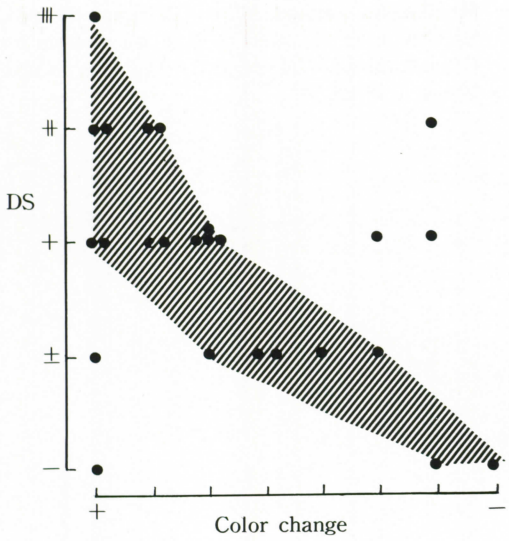


Fig. 5. Color changes in Stomastat® with reference to standard color samples (−, ±, +) (top) and relation between Denture Stomatitis (DS) and color change of Stomastat® (hatched area $r=-0.86$, $p<0.001$, bottom).

tients in the National Sanatorium, the assessment of the inflammatory reaction of the mucous membrane and that of the color change in Stomastat® , with reference to a standard color sample, is shown in Fig.5. The high correlative relation could be recognized in about 20 of the 25 patients (80%, $r=-0.86$).

DISCUSSION

The association of DS with dirty dentures is high. *C. albicans* is most commonly concerned with such cases but *T. glabrata*, *C. tropicalis* and *C. krusei* are also occasionally present (Olsen[4]).

C. albicans can be detected in mouths with natural dentitions. However, there is a marked difference in the frequency and quantity of *Candida* organisms between persons with natural dentitions and persons with dentures, especially patients with DS.

C. albicans is not the only aetiological factor in DS, however, *C. albicans* and related species have been proved to be important in this condition. Actually, in the majority of the cases, a strong correlation could be recognized between the color change of the medium and clinical symptoms. The literature suggests that the cases with a poor correlation might have a multiple aetiology including allergy, denture trauma and decrease of systemic resistance. Deficiency diseases of iron and folic acid have been proved to be such systemic factors[3].

In gynaecology, a simplified medium for *Candida* is used in daily practice (Mizuno et al[5]). A simplified diagnostic medium for oral candidosis was also reported[1]. The medium has the merit of simplicity, however, it is inadequate for a more detailed examination. In this case, *Candida* can be judged by the basic method of using the Sabouraud's medium or biochemical characters. The basis of the medium for *Candida* is to make it specific by addition of an antibiotic to the Sabouraud's medium. This medium contained glucose as a substrate, chloramphenicol and chlorphenol red as a pH-indicator.

The incubation temperature in the present study was 37°C. As Fig.2 shows, the growth of *C. albicans* and the pH change of the medium were examined. The growth after 24 hr was large at 27, 30 and 37°C, and the pH dropped remarkably at 30 and 37°C.

The contamination of microorganisms with *Candida* is inevitable in a sample by swabs from the palate. Therefore, the pH and color changes of microorganisms which have a comparatively high possibility of contamination were examined (Table 2). The pH and color changes were positive for *Candida* and *Torulopsis*, however, for *Lactobacillus*, *S. mutans* and *E. coli* they were negative.

In one clinical case, the pH change was slight in a sample of plaque adhering to teeth.

Contamination from the air in the clinic or sickroom when putting the swab into the container was examined. Nothing was found to influence the color change.

In patients with DS (53 persons), no one had subjective symptoms related to the condition of the mucous membrane under the denture base. This contrasts with denture-sores caused by mechanical irritation. The assessment of DS is indicated as +++, ++, +, ± and −, with reference to the degree of mucosal redness, difference of mucosal color and surface condition by inspection. Whether DS is serious or not cannot be judged at first sight. A more accurate judgement can be made by following the progress of treatment.

Inflammation of the mucous membrane under the denture base may occur by the interaction of factors such as systemic disorders, allergy and microorganisms. So, it cannot be explained by only the culture reaction. However, in about 80% of the cases examined, it was found that the degree of DS was closely correlated to the microbiological aspects.

Stomastat® (Sankin Industry Co. Ltd., Osaka, Japan) and Candida Yellow Medium® (Fujiseiyakukogyo Co. Ltd., Tokyo, Japan) on the market are based on this research.

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