Clinical-mycologic diagnosis of oral yeast infections

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Conventional oral specimens for recovery of yeasts are swabs and smears. Oral rinses and imprint/impression cultures can also be used. Yeasts grow well at room temperature and may multiply in specimens under transport. Direct smears examined for blastospores, hyphae, and inflammatory cells ensure rapid presumptive diagnosis. Fungal identification requires culture, preferably on different media and at different temperatures to ensure recognition of all species present. YM agar supplemented with 0.01% aniline enables detection of *Candida albicans* and *C. parapsilosis* on primary plates through fluorescence. Microstix-Candida or Oricult-N slides can be read after culture at room temperature. Histologic sections for demonstration of yeasts require periodic acid-Schiff, Gridley, or Gomori's methenamine silver staining. Fungiqual staining enables non-specific diagnosis, also of rare oral mycoses, within 30 min, through fluorescence. Calcofluor white is even faster (<30 sec). Specific antibodies labeled with fluorescent stain enable more precise mycologic diagnosis. Mycologic findings should be interpreted together with clinical findings. □ *Fungal identification; mycology; oral candidosis*

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Several fungal diseases may appear in the oral cavity and the perioral regions. Infections caused by *Candida* species, candidoses, are those that the dental practitioner in Scandinavia meets most frequently. Rare mycoses, such as histoplasmosis, blastomycosis, and coccidioidomycosis, are occasionally seen in the oral cavity (1), often as manifestations of general infections, and particularly in immigrants or persons having visited areas of endemic mycoses or in immunocompromised patients. Therefore, rare fungal infections should be included as possible differential diagnoses for oral lesions of uncertain etiology.

Oral candidosis has several clinical manifestations (2), and it is important to distinguish these from similar non-fungal lesions. Usually, oral yeast infections are mild and localized. Under special circumstances, such as in patients with compromised immune defense, they can spread to the outer surroundings of the oral cavity or to the esophagus or become systemic. With systemic yeast mycoses the incidence of unrecognized infection is probably quite high.

In HIV-seropositive individuals not showing AIDS and in AIDS patients the oral cavity and also neighboring regions, such as the pharynx and esophagus, can be the first body sites affected by candidal infection, thus unfolding premonitory signs of this syndrome. The manifestation of an unexplained oral *Candida* infection in persons at risk of AIDS should be followed by aggressive screening for this syndrome. Dentists should also be able to diagnose yeast infections that already have invaded oral tissues so that proper systemic treatment can be implemented.

Optimally, a mycologic diagnosis of oral yeast infection should be made before antifungal treatment is instituted. This is particularly important when causes other than yeasts cannot be ruled out immediately. It would seem more reasonable to make a smear from oral lesions and denture for presumptive diagnosis than to prescribe an expensive antifungal drug cure for the patient, anticipating that yeasts are involved and regarding clinical improvement as proof of fungal etiology.

Diagnosis of oral yeast infections should

be based on a combination of mycologic approaches and assessment of clinical signs and symptoms. The present review will consider approaches that the dentist can use or require to arrive at a specific mycologic diagnosis.

Specimens

Any kind of clinical material (swabs, sputum, etc.) for microscopy or culture should be examined as quickly as possible. Yeasts grow well at room temperature and may multiply under transport or be overgrown by bacteria. Drying out of the material may cause loss of the viability of present yeasts. Since quantitative assessment is an important factor in the differentiation between carriers and infected patients, specimens must be dealt with immediately. If this is not possible, the specimen should be kept moist or in a transport medium and stored in a refrigerator. When mailing is necessary, transport must take place in sterile containers and postal regulations for contaminated material be adhered to.

Sampled patients may have infectious diseases such as tuberculosis, hepatitis, AIDS, etc. The diagnostic microbial laboratory must be informed so that specimens can be processed with caution and without risks for the technical staff. Laboratory regulations must be rigidly adhered to.

Swabs

Clinical material for culture is most frequently collected with swabs. Once received in the laboratory, swabs are seeded on Sabouraud agar (25°C or room temperature), on blood agar (35°C), on Pagano–Levin medium (35°C), or on Littmann's substrate (25°C). Incubation at 25°C is done to ensure recovery of species growing badly at 35°C (3). The commonest yeasts form colonies within 1–3 days of incubation.

Mixed yeast infections are seen in the oral cavity more frequently than previously thought, particularly in immunocompromised or debilitated patients (4). Sabouraud dextrose agar, frequently used as a primary culture medium in clinical laboratories, may not always be the best choice for distinguishing between multiple yeast species, especially not for the unexperienced. Pagano–Levin agar or Littmann's substrate can be useful supplements because they enable distinction of yeasts on the basis of differences in colony color. Samaranayake et al. (5) found Pagano–Levin medium superior to Sabouraud dextrose agar in the detection of multiple yeast species from a single oral sample. Failure to detect yeasts in specimens may have therapeutic and prognostic consequences, particularly if the undetected organisms have low sensitivity to the administered antifungal drug and the patient is immunocompromised or debilitated.

Microstix–Candida/Oricult-N

The outgrowth of yeasts from oral mucosa and denture can also be assessed by culture on Microstix–Candida (M–C) slides (Ames Co., Division Miles Lab., Inc., Elkhart, Ind., USA). A significant relationship between the concentration of yeasts assessed by microscopy of smears and by culture on M–C has been reported in denture stomatitis (6). However, false-negative cultures occurred. In all instances of such cultures the corresponding smears showed only a few yeast cells. The M–C test system had sufficient diagnostic sensitivity to serve as an alternative to the smear technique in establishing the diagnosis of *Candida*-induced denture stomatitis. It is read after 2 days at 37°C and after 5 days at room temperature and is a suitable chair-/bed-side method. A similar diagnostic system of possibly even higher sensitivity is Oricult-N (Orion Diagnostica, Helsinki, Finland) (Fig. 1). The Oricult-N dipslide technique provided false-negative results in 12% of the patients (7) and thus less than the 22% false-negatives provided with Microstix-Candida from denture samples and the 44% obtained from mucosal surfaces (6) when the same criteria of infection and growth were used in both

studies. A simplified culture technique has also been developed for the diagnosis of *Candida*-induced denture stomatitis on the basis of the acid-producing capacity of *Candida* and is displayed in color changes (8).

Imprint/impression cultures

The imprint culture technique uses a sterile foam pad (2 × 2 cm), dipped in peptone water and then placed on a restricted area of the oral mucosa for 30 sec (9). Thereafter, the pad is placed directly on Pagano–Levin or Sabouraud agar, and the growth of *Candida* is quantitated. This technique may be useful for assessing yeast growth in different areas of the oral mucosa and the denture.

Impression cultures can be provided by using alginate impressions of the palate and denture (10, 11). In denture wearers with non-inflamed palatal mucosa, *Candida* species were cultured more frequently from impressions or mouth washings than from oral swabs (for a review, see Ref. 12). There was a close relationship between the outgrowth of yeasts on Sabouraud agar models of the maxilla and the localization and intensity of the palatal inflammation in patients with denture stomatitis (10).

Paper points

In a recent study nearly one-third of severe periodontitis patients harbored yeasts in their periodontal pockets (13). All subjects had sites non-responding or 'refractory' to conventional periodontal therapy, and most of them had received one or more courses of broad-spectrum systemic antibiotics. *C. albicans* has previously been detected in high numbers in the subgingival flora or in the gingival tissues of acute periodontal abscesses (14, 15), advancing periodontitis of AIDS patients (16), juvenile periodontitis (17, 18), and leukemic patients undergoing myelosuppressive chemotherapy (19). Before sampling of the periodontal pocket, supragingival plaque should be removed from isolated teeth with a sterile curette or cotton balls. An absorbent sterile point is then inserted to the depth of the pocket and kept there for 10 sec (Fig. 2). Caution should be exerted during point manipulation to evade deposition of cellulose fibers, which may induce perpetuating inflammatory reactions (20). Points are transferred to a 2-ml vial containing Möller's VMGA III transport medium, which also facilitates survival of facultative and anaerobic bacteria (13). A similar technique was used to recover yeasts from root canals (21).

Saliva

It has been suggested that carriers of yeasts and patients with overt candidosis can be distinguished with 95% confidence limits on the basis of quantitative cultures of *C. albicans* from saliva (22). Individuals with <400 colony-forming units (CFU)/ml of saliva could be classified as carriers and those with >400 CFU/ml listed as either having acute or chronic candidosis. It was held that quantitative saliva cultures represent a practical aid in the diagnosis of oral candidosis when considered as part of an overall assessment of each individual.

The usefulness of rinses for quantification of oral yeasts was also evaluated by Samaranayake et al. (9). A 10-ml sample of phosphate-buffered saline was swirled in the mouth for 1 min, then expelled, and inoculated onto Sabouraud agar using a spiral plater. The resultant growth could easily be quantitated. Lamey & Samaranayake (23) recently recommended oral rinse culture or imprint culture for differentiating between commensal yeast carriage and clinical candidal infection.

In assessing the significance of quantitative saliva cultures, fluctuations in counts during the day and daily must be considered, and counts should be taken over several days and at the same time of the day (24).

Smears

Detection of yeasts in a clinical specimen should start with direct microscopic examination of unstained smears from the lesion.

This does not enable species identification but does enable presumptive yeast diagnosis and clues to which cultural and biochemical tests to run for subsequent species identification. Smears are taken from the infected oral mucosa, rhagades, and the fitting side of the denture, preferably with wooden spatulas. The material is pressed between two glass microscope slides or distributed evenly on either slide and then fixed immediately in ether/alcohol 1:1 or with Sprayfix (Histo-Lab, Bethesda Trading Ltd., Gothenburg, Sweden). Samples to be sent to the laboratory for staining of yeasts are placed in sterile, screw-capped vials. Fragments of membranous coatings should be collected if present. After fixation, one slide is stained with Gram and the other with periodic acid-Schiff (PAS). Yeast cells appear dark blue after Gram staining and red in PAS preparations (Fig. 3). The reliability of smear microscopy for diagnosing candidal infection is relatively high. It has been found equal to that of histologic examination in leukoplakias (25). Smears may also be treated without fixation with 10–20% KOH, although this is usually reserved for skin scrapings. KOH preparations, although rapid, may have inherent artifacts caused by protein denaturation (26). PAS or Gram stain is therefore recommended for all KOH-negative smears.

Pseudohyphae rather than blastospores have been associated with candidal infection. The facts that hyphae can be abundant in denture plaque of clinically healthy denture wearers (27) and be present in oral mucosa smears from healthy subjects (28) have suggested that this phase of the dimorphic *Candida* is not pathognomonic for oral yeast infection. The presence of large amounts of blastospores and hyphae is indicative of candidal infection, although hyphae may be more dominant than blastospores in smears from clinical lesions. It should be realized that *C. (Torulopsis) glabrata*, which has been reported as the second most frequent yeast isolated from oral candidosis (11), does not usually form hyphae. Another indication of a *Candida*-infected lesion is the presence of large accumulations of inflammatory cells in direct smears (29).

Fluorescence techniques

Several fluorescent dyes with specificity for fungal cell wall polysaccharides have been reported effective in screening clinical specimens and tissue sections for fungi. These include Calcofluor white, Blankophor P flüssig, and Fungiqual. Calcofluor white (CW) (Calcofluor, Polysciences, Inc., Warrington, Pa., USA), a water-soluble, colorless dye, can be used for histopathologic diagnosis of oral candidosis (26, 30). The planar transform of the molecule fluoresces light blue when exposed to ultraviolet light at 345 to 365 nm. The technique is extremely rapid (takes <30 sec) and is inexpensive. Its sensitivity is comparable to those of frozen sections and exfoliative cytology for diagnosing candidal infections (30). CW staining does not interfere with subsequent use of Gomori's methenamine silver (GMS) or PAS. Differential staining of fungi in clinical specimens from both mucosa and skin with fluorescent whitening agent (Blankophor P flüssig) (BP) (Bayer AG) is also possible (31). A new fluorescence staining technique, Fungiqual staining (Ciba-Corning Diagnostics GmbH, Fernwald-Annerod, FRG), has been found superior to both CW and BP (32). It provides a reliable, rapid (within 30 min), simple, and economic means of diagnosing fungus infections, whether superficial or systemic (33). Fungiqual can be used for rare mycoses with oral manifestations (1) such as aspergillosis, blastomycosis, coccidioidomycosis, cryptococcosis, geotrichosis, histoplasmosis, rhinosporidiosis, and sporotrichosis (33). *Candida* species other than *C. albicans* can cause false-negative results. Elastic fibers may display an unspecific fluorescence that can be quenched by counterstaining.

The use of specific antibodies labeled with fluorescent stain permits causative organisms to be diagnosed accurately within minutes. However, the preparation of specific antisera and purified polyclonal or monoclonal antibodies entails a much more extensive technical outlay, so the application of these reagents need only be considered when a very precise diagnosis is of therapeutic consequence (33).

YM agar (Difco) supplemented with 0.01% aniline blue enables direct detection of *C. albicans* and *C. parapsilosis* from oral samples on primary agar plates through fluorescence at 365 nm (34). In critically ill patients the direct rapid isolation and identification of opportunistic fungi such as *C. albicans* may be important to the outcome of the disease.

Histologic samples

Because yeasts may be part of the normal oral flora and may proliferate rapidly in vitro, histologic specimens for culture should be processed soon after collection. Fixed histologic sections constitute the most reliable way to diagnose an invasive infection. In leukoplakias they can reveal hyphal infestation of the superficial hyperkeratotic epithelial cell layer (Fig. 4). Demonstration of fungi in biopsy specimens may require several serial sections to be cut (25). Furthermore, histologic sections detect yeasts from a restricted area of the oral cavity only, thus contrasting with swabbing and other procedures that can cover large areas. Special stains for fungi such as GMS, and the PAS and Gridley's procedure are invaluable when searching for small numbers of organisms and for determining their morphology. The relatively poor color differentiation provided by the conventional PAS method may be significantly increased by application of a 0.1% aqueous solution of light green as a counterstain (Fig. 4). GMS is claimed to be the best histologic stain for demonstrating fungi because it provides high contrast with minimal background (35). When GMS-stained sections are counterstained with hematoxylin-eosin, the host reaction towards the fungus can be evaluated simultaneously.

In vivo hybridization

For specific identification of yeasts within tissue, in situ hybridization can be carried out. As an example, the fungus *Pneumocystis carinii* was identified in lung tissue by in situ hybridization with biotinylated *Pneumocystis*-specific oligonucleotides (36). Oligonucleotides corresponding to the regions of *Pneumocystis*-like ribosomal (r)RNA with least sequence identity with known 16S-like rRNA were first synthesized. Dot-blot analysis demonstrated that the oligonucleotides hybridized to RNA from several preparations of *Pneumocystis* trophozoites but failed to hybridize to uninfected rat lung RNA or to RNA from various bacterial and fungal pathogens. Biotin-dUTP was then added to the oligonucleotides by terminal transferase. The biotinylated oligonucleotides were used to probe tissue sections of *Pneumocystis*-infected human lung.

DNA probes

DNA probes, particularly biotin-labeled non-radioactive probes, have potential application in clinical and epidemiologic evaluations of outbreaks of nosocomial candidosis (37). New DNA polymorphisms in this gene family arise at high rates. As a consequence, DNA probes will readily distinguish strains from different patients in the same hospital and from various sites in individual patients (38).

Latex agglutination test for candidosis

The detection of *C. albicans* antigens in vaginal secretions has been reported as a useful method for rapid diagnosis of vaginal candidosis (39, 40). A new commercial slide latex particle agglutination test (The Candidate Super SLA test (Mercia Diagnostics, U.K.)) for rapid (2 min) diagnosis of vaginal candidosis was evaluated and compared with conventional methods (41). The test was positive in 15 of 23 women (65.2%) with clinical signs of vaginal candidosis, the incidence of a positive test increasing in direct proportion with the amount of yeasts isolated. The test's sensitivity, specificity, and predictive values were comparable to those of microscopy and culture. To our

knowledge, the diagnostic value of this test or any other commercial slide agglutination test in oral candidosis has not yet been reported.

Serodiagnosis of *Candida* infection

Numerous techniques have been devised to detect *Candida* antibody or antigen in patient sera. They have so far been of little value in laboratory diagnosis of infections of mucous membranes. Serodiagnosis may be more justified when oral candidosis becomes systemic, and several promising techniques, such as reverse passive latex agglutination (42), have been developed. Most of the test systems for *Candida* serology have, however, remained research procedures. Most tests show the greatest accuracy in non-neutropenic patients (43). A fair degree of reliability of early diagnosis of deep-seated candidosis probably requires a synoptic evaluation of clinical, serologic, and cultural data (44, 45).

Chromatography

In cases of systemic candidosis gas–liquid chromatography may be of diagnostic value. A characteristic chromatographic profile was seen in sera from patients with invasive candidosis compared with controls (46). Pyrolysis gas–liquid chromatography for detection of *Candida* antigen has also been attempted in patients with disseminated candidosis (47). Besides, serum arabinitol, a metabolic product of *Candida*, has been used for diagnosing systemic candidosis (48). Normal human serum contains both D- and L-arabinitol but D-arabinitol is the enantiomer responsible for the high serum arabinitol levels in patients with candidosis. Enantioselective measurement of serum D-arabinitol might result in improved ability to diagnose and estimate the severity of candidosis (49).

Application of major diagnostic techniques to oral lesions

Lesions of pseudomembranous candidosis should be swabbed and remnants of the pseudomembrane smeared. The pseudomembrane consists of shedded epithelium, fibrin, food remnants, leukocytes, and bacteria and is attached to the underlying epithelium by numerous hyphae. Normally, a biopsy is not necessary to verify the clinical diagnosis.

In acute erythematous candidosis swabs and smears should be prepared from areas with remnants from shedded plaque, if present. Biopsies are inappropriate for establishing the diagnosis. The patient should take antimycotics together with the drugs that initiated the lesion until a negative mycologic diagnosis is achieved and until these drugs are abolished.

In chronic plaque-like and nodular candidosis (2) smears will show abundant mycelium and inflammatory cells together with shedded epithelial cells. Swabs will yield positive cultures. Biopsy is recommended because the condition may be premalignant with various degrees of epithelial dysplasia. There is invasion of hyphae in the superficial, parakeratotic epithelial layer and massive chronic inflammation of the subepithelial connective tissue together with acanthosis and sometimes dysplasia of the epithelium (50).

Patients with *Candida*-induced denture stomatitis should have their oral mucosa and the fitting side of the denture swabbed and smeared. There will be an abundance of hyphae, blastospores, and inflammatory cells in smears from the palate, tongue dorsum, and denture. The latter will carry more yeasts than the palatal mucosa and should *always* be sampled. Biopsies are unsuitable for *Candida*-induced denture stomatitis, since normally the affected tissue is not invaded by yeasts. Swabs and smears moistened with sterile water or saline should be taken from rhagades. The reservoirs of the angular organisms should be determined, such as dentures for *Candida* species and anterior nares for *Staphylococcus aureus*.

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Fig. 1. Growth of colonies of *Candida* on Oricult-N dipslide.

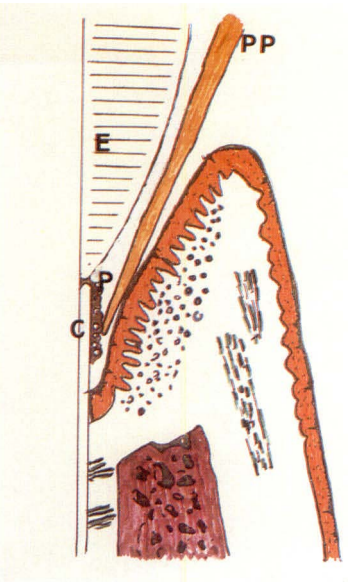

Fig. 2. Diagrammatic illustration of paper point in situ for sampling of subgingival yeasts. C = cementum on root; E = enamel on crown; P = plaque; PP = paper point.

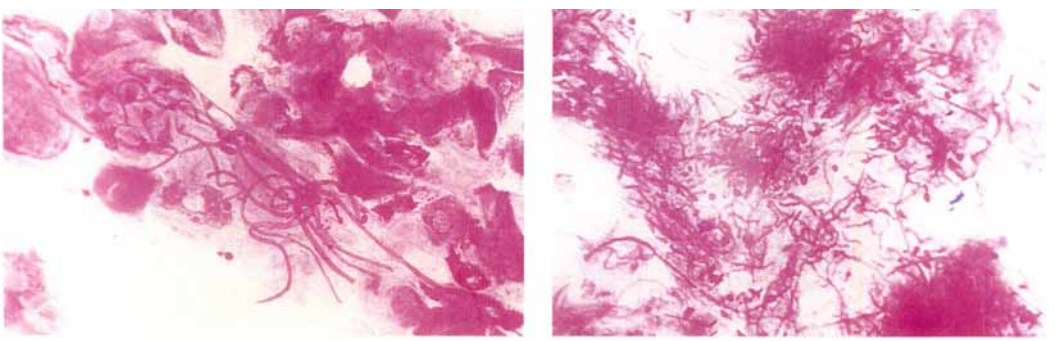

Fig. 3. PAS-stained *Candida* hyphae and blastospores in palatal smear (*left*) and in smear from the fitting side of the denture (*right*).

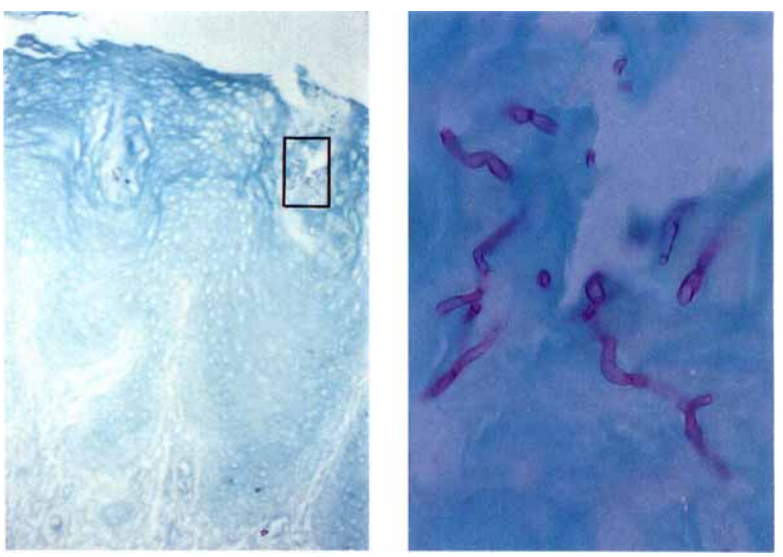

Fig. 4. PAS-light green-stained biopsy from *Candida*-infected oral leukoplakia. Inset area of Fig. 4 *left* is presented in detail in Fig. 4 *right*. (Courtesy of Professor Hanna Strømme Koppang.)

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