

Short reports

Immunocytochemical detection of *Candida albicans* in formalin fixed, paraffin embedded material

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Table 1 Reaction of antibody 1B12 with strains of seven *Candida* species suspended in agarose blocks

<i>Candida</i> isolate	Source	Staining with 1B12 antibody
<i>C albicans</i> Y01.554	HIV*	Positive
<i>C albicans</i> 359/97	CHC	Positive
<i>C albicans</i> 121/94	CHC	Positive
<i>C tropicalis</i> Y09.60	HIV*	Negative
<i>C tropicalis</i> 340/94	Dry mouth	Negative
<i>C tropicalis</i> 723/93	CHC	Negative
<i>C krusei</i> Y02.48	HIV*	Negative
<i>C krusei</i> 22/94	Sjögren's syndrome	Negative
<i>C krusei</i> SU6	Unknown†	Negative
<i>C glabrata</i> Y33.149	HIV*	Negative
<i>C glabrata</i> 59/94	CHC	Negative
<i>C glabrata</i> 644/94	CHC	Negative
<i>C guilliermondii</i> Y02.02	HIV*	Negative
<i>C guilliermondii</i> 657/93	Sjögren's syndrome	Negative
<i>C parapsilosis</i> NCPF 3104	Laboratory reference strain	Negative
<i>C parapsilosis</i> 937A	Unknown†	Negative
<i>C parapsilosis</i> 190/94	Coated tongue	Negative
<i>C kefyr</i> Y06.01	HIV*	Negative
<i>C kefyr</i> Y06.07	HIV*	Negative
<i>C kefyr</i> 93/1290	HIV‡	Negative

*Courtesy of Pfizer Ltd; †SmithKline Beecham Pharmaceuticals; ‡Guy's and St Thomas' Hospital, London. CHC, chronic hyperplastic candidosis.

candidosis will help to clarify the role of individual *Candida* spp in this important form of oral candidosis.

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Mycological confirmation of the presence of oral candidosis generally involves sampling of the mucosal surface for the culture and subsequent identification of *Candida* spp. However, chronic hyperplastic candidosis (CHC) differs from the other forms of oral candidosis in that *Candida* spp invade the superficial layers of the epithelium. As a result the organisms are often only recovered in small numbers or not at all following sampling, a feature that makes it difficult to diagnose CHC on the basis of candidal culture alone. Therefore, biopsy of CHC lesional tissue and microscopic examination for the presence of structures consistent with fungal hyphae is essential for diagnosis.

Human soft tissue biopsy material is routinely preserved by fixation in formalin and subsequent paraffin wax embedding. Staining with periodic acid-Schiff (PAS) or methenamine-silver stains can detect hyphal structures consistent with *Candida* spp in fixed tissue sections. However, these stains do not permit identification of individual candida species. The association of CHC with the

Table 2 Reaction of antibody 1B12 in 16 FFPE tissues

Tissue	Origin of tissue	Staining with 1B12 antibody
1	CHC	Positive
2	CHC	Positive
3	CHC	Positive
4	CHC	Positive
5	CHC	Positive
6	CHC	Positive
7	CHC	Positive
8	CHC	Positive
9	CHC	Positive
10	CHC	Positive
11	CHC	Positive
12	CHC	Positive
13	Fibroepithelial polyp	Positive
14	Fibroepithelial polyp	Positive
15	CHC	Positive
Biomen control	Kidney (HIV patient)	Positive

CHC, chronic hyperplastic candidosis.

development of squamous cell carcinoma,¹ the reported differences in the ability of *Candida* spp to generate carcinogenic metabolites,² and the variable sensitivity of *Candida* spp to antifungal agents makes identification of the infecting species important. Previous studies

have identified *Candida* spp in CHC biopsies using a polymerase chain reaction (PCR) specific for candidal ribosomal DNA sequences.³ However, at present the use of PCR based approaches may not be practical in many routine clinical laboratories. Immunohistological identification of *Candida* spp has been hindered by the absence of species specific antibodies that are applicable to formalin fixed material. Recently, a mouse monoclonal antibody (1B12) against the high molecular weight mannoproteins of *C albicans* has become commercially available (BioGenex; distributed in the United Kingdom by Biomen Diagnostics). This antibody is reported to be species specific and suitable for use with formalin fixed, paraffin embedded material.⁴ Our aim in this present study was to assess the specificity of monoclonal antibody (Mab) 1B12 and use it to confirm the identity of hyphae in biopsy material from CHC mucosa.

Methods

CANDIDAL STRAINS

Twenty isolates of *Candida* comprising seven species were examined (table 1). All strains were initially identified using the API-20C carbohydrate assimilation system (bioMérieux) and the germ tube test.⁵ All isolates were cultured in horse serum for three hours at 37°C to stimulate hyphal growth, before embedding in 0.4% molten agarose. After solidifying, the embedded agarose material was treated in the same manner as test tissue.

FORMALIN FIXED, PARAFFIN EMBEDDED TISSUE

Fifteen mucosal biopsy specimens from separate patients (table 2) were originally fixed in 10% formal saline for 24 hours before processing through graded alcohol and xylene and embedding in paraffin wax. All tissues were shown by PAS stain to have fungal hyphae penetrating the stratum corneum of the epithelial layer (fig 1) and 13 had the histological features characteristic of CHC. Positive control material was sections of *C albicans* infected kidney tissue (Biomen Diagnostics).

IMMUNOCYTOCHEMISTRY

Serial sections (5 µm) were cut from formalin fixed, paraffin embedded tissue blocks and placed on SuperFrost microscope slides (Merck). The slides were kept for 24 hours at room temperature before further treatment. Sections were initially equilibrated in citrate buffer (0.01 M; pH 6.5) before microwave treatment (specimens heated at maximum power (650 W) to boiling and then allowed to simmer for 30 minutes at 455 W). Following microwave treatment, the immersed slides were allowed to cool to room temperature. All subsequent staining procedures were performed at room temperature in a humid box to prevent drying of sections. Sections were covered with 3% hydrogen peroxide in phosphate buffered saline (PBS; pH 7.2; 0.01 M; 10 minutes) to block endogenous peroxidase activity, washed in PBS (3 × 2 minutes) and overlaid with goat serum (Biomen Diagnostics) for 10 minutes to block non-specific protein

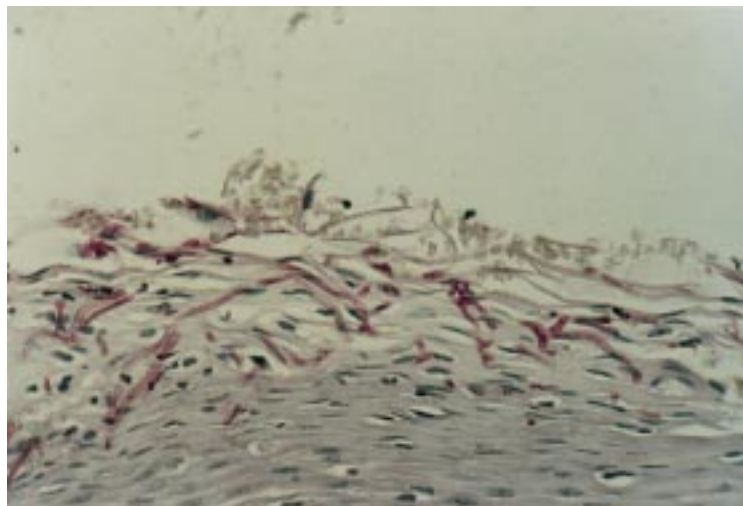


Figure 1 Periodic acid-Schiff staining of oral mucosa from a case of chronic hyperplastic candidosis, showing hyphal structures penetrating epithelium (×111).

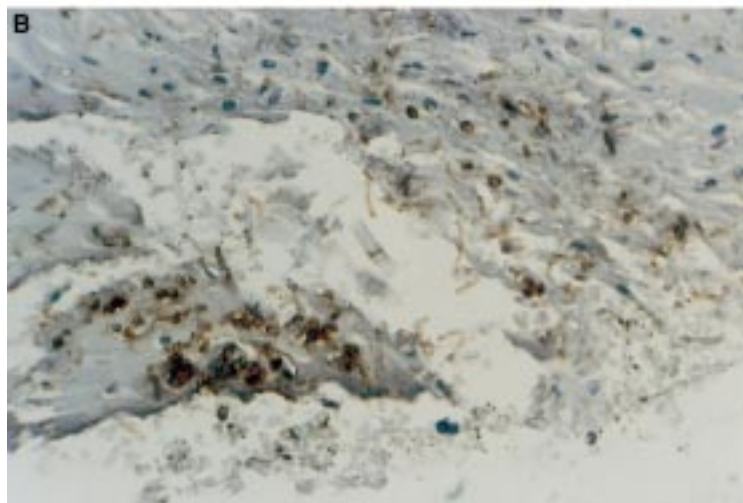


Figure 2 (A) Positive 1B12 antibody staining of *Candida albicans* in agarose block section. (B) Tissue section from case of chronic hyperplastic candidosis (×111).

binding sites on the section. Excess serum was tipped off and supersensitive Mab 1B12 (Biomen Diagnostics) applied to the wet section. After overnight incubation the sections were washed in PBS (3×2 minutes) and overlaid with biotin labelled goat anti-mouse/rabbit immunoglobulin (20-fold diluted in PBS; Biomen Diagnostics) for 20 minutes. Sections were washed in PBS (3×2 minutes) and then overlaid with peroxidase labelled streptavidin (20-fold diluted in PBS; Biomen Diagnostics) for 20 minutes, washed in PBS (3×2 minutes), and covered with freshly prepared diaminobenzidine reagent (Sigma; 5 minutes). Sections were then washed in tap water for 2 minutes and overlaid with copper sulphate solution (0.5% wt/vol in saline; 5 minutes), washed in water, and counterstained with Meyer's haematoxylin for 30 seconds. After washing in tap water, sections were dehydrated in alcohol, cleared in xylene, and mounted with Canada balsam (Fisons).

Results

Of the 20 isolates of *Candida* embedded in agarose, positive staining was evident only with those previously identified as *C albicans* (table 1; fig 2A). Positive staining with 1B12 antibody occurred in all 16 tissue sections where PAS positive hyphae had previously been detected (table 2; fig 2B).

Discussion

In recent years, it has become evident that *Candida* spp and strains vary in their production of putative virulence factors, pathogenicity in animal systems, and antifungal susceptibility. *Candida* spp are frequently associated with superficial infection of mucosal membranes, although fatal systemic infection in debilitated individuals can also occur. Chronic hyperplastic candidosis is a particularly important form of oral candidosis as it is associated with the development of epithelial dysplasia⁶ and intraoral squamous cell carcinoma. Sampling

methods for culture of *Candida* spp are limited to collection from the tissue surface and do not permit the recovery of organisms from within the lesions. Previous studies involving identification of *Candida* spp by PCR in fixed tissues from CHC lesions have indicated the presence of *C albicans*,³ although at present this technique is unlikely to be available in the majority of routine diagnostic laboratories.

Our present investigation has shown that monoclonal antibody 1B12 stained all isolates of formalin fixed, paraffin embedded *C albicans* tested, while cross reactivity with other *Candida* spp was not evident. Subsequent staining of oral mucosal tissue from CHC and fibroepithelial polyps indicated the presence of *C albicans* in all specimens examined. The ability to identify candida organisms penetrating the lesional tissue in cases of CHC provides an opportunity to further our knowledge on the role of individual *Candida* spp in this important form of oral candidosis. It would, however, be of interest to stain these tissues with antibodies specific for other *Candida* spp in an effort to identify mixed infection. However, to date suitable commercial antibodies do not appear to be available.

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