Detection and quantification of Candida spp. from subgingival areas

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ABSTRACT

Detection and quantification of Candida spp. from subgingival areas using two methods- sterile periodontal curette and sterile paper points.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Petrovic SM, Radunovic M, Barac M, Pficer JK, Pavlica D, Arsenijevic VA, Pucar A (2019) Subgingival areas as potential reservoirs of different *Candida* spp in type 2 diabetes patients and healthy subjects. PLoS ONE 14(1): e0210527. doi: 10.1371/journal.pone.0210527

PROTOCOL STATUS

Working

MATERIALS

NAME	CATALOG #	VENDOR
Disposable gloves, nitrile		
Periodontal probe	PCPNT2W	
G. Hartzell & Son Model # S4L/4RHSS; Heavy Duty Columbia Curette One End # S4L One End # 4RHSS	126-S4L/4RHSS	
Tab Top Microcentrifuge Tube Sorenson SioSience Inc. 1.5ml	16479	
Sabouraud Dextrose Broth	M033	
Sabouraud dextrose agar	MH063-100G	
Candida differential agar	M1297A-100G	

SAFETY WARNINGS

1 1. The first day- clinical periodontal examination (full mouth examination at six sites per tooth) in order to diagnose chronic periodontitis and detect the deepest periodontal pocket (with the highest PPD value).

2 -Record plaque index (Silness-Löe), bleeding on probing (BOP), periodontal pocket depth (PPD) and clinical attachment loss (CAL). Samples should not be obtained the first day, to avoid contamination by blood.

3 2. The second day- sampling. Isolate sampling tooth with cotton rolls.

4 -Remove the supragingival plaque by a curette and sterile gauze.

5 -Place two sterile paper points (#30) into the pocket/sulcus until a mild resistance appeares and keep for 30 seconds. Paper points contaminated by blood should be excluded.

6 -Collect paper points and place into 1mL of Sabouraud dextrose broth.

7 - Use sterile curette to obtain samples of the complete subgingival biofilm and inoculated in separate sterile plastic tubes containing 1 mL of Sabouraud dextrose broth.-

8 - Send samples to microbiologic laboratory.

9 3. The second day -microbiological protocols. Vortex samples for 60 seconds.

10 - Streak 20µl of suspended broth on SDA and ChromAgar using sterile plastic micro pipette.

11 -Incubate at 25°C for 48 hours

12 4. The fourth day. Count the number of Colony Forming Units per sample.

13 -Multiply the obtained number by 50 in order to obtain CFU/ml.