

Modification Form for Permit BIO-UWO-0235

Permit Holder: *Walter Siqueira*

Approved Personnel

(Please stroke out any personnel to be removed)

Additional Personnel

(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Oral Bacteria, Oral fungus	<i>Candida Albicans</i> <i>Streptococcus mitis</i>
Approved Cells		
Approved Use of Human Source Material	Human Saliva, Acquired enamel pellicle	
Approved GMO		
Approved use of Animals		
Approved Toxin(s)		

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 2

→ no biological safety cabinet

Date of last Biohazardous Agents Registry Form: Apr 3, 2009

Signature of Permit Holder: WALTER SIQUEIRA 

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Fungal and Bacterial Growth Inhibition Assay

Candida albicans will be grown on sabouraud dextrose agar (SDA) plates for 48 h at 30°C and subcultured in 5 ml of diluted sabouraud dextrose broth (SDB). After 24 h of incubation at 30°C, the suspensions will be diluted in SDB to yield 1×10^4 CFU/ml (OD620 = 0.002). A serial dilution of salivary-protein complex will be prepared in SDB in 96-well microtitre plates to a final volume of 50 µl/well. To each well, 50 µl of the diluted yeast suspension will be added. The plates will be incubated at 30°C for 48 h, then cells in each well resuspended, and OD620 determined using a microtitre plate reader. Values will be corrected for the absorbance of diluted broth only, and growth inhibition curves will be generated using Excel software.

Streptococcus Mutans growth inhibition, it is planned to use the method described by Mackay et al., 1984, which bacterial growth is monitored spectrophotometrically at 675 nm in a Todd-Hewitt dialysate broth.

Fungal and Bacterial Killing Assay

Candida albicans as well as *Streptococcus Mutans* will be picked from a SDA plate (<1 week old), suspended in 5 mM potassium phosphate buffer, pH 7.0, and grown to a final OD620 of approximately 0.35. From this suspension, 50 µl will be added to 50 µl of a serially diluted series of salivary protein complex in a 96-well microtitre plate. After 1.5 h of incubation at 37 °C, 50 µl from selected wells will be diluted 180-fold in phosphate buffered saline and a 25 µl aliquot of the diluted suspension will be plated on SDA. After 48 h of incubation, cell viability will be assessed by colony counting, using comparisons with the number of cells in a control sample incubated without any salivary protein.



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Candida albicans - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Candida albicans*

SYNONYM OR CROSS REFERENCE: Candidiasis, Thrush, Moniliasis

CHARACTERISTICS: Oval, budding yeast, produces pseudohyphae in culture and in tissues and exudates

SECTION II - HEALTH HAZARD

PATHOGENICITY: Mycosis of superficial layers of skin or mucous membranes (oral thrush, vulvovaginitis, paronychia, onychomycosis, intertrigo); ulcers or pseudomembranes in esophagus, gastrointestinal tract or bladder; hematogenous dissemination may produce lesions in kidney, spleen, lung, liver, prosthetic cardiac valve, eye, meninges, brain

EPIDEMIOLOGY: Worldwide

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Endogenous spread (part of normal human flora); by contact with excretions of mouth, skin, and feces from patients or carriers; from mother to infant during childbirth; disseminated candidiasis may originate from mucosal lesions, unsterile narcotic injections, catheters

INCUBATION PERIOD: Variable

COMMUNICABILITY: Communicable for duration of lesions

SECTION III - DISSEMINATION

RESERVOIR: Humans (normal human flora)

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to nystatin, clotrimazole, ketoconazole, fluconazole, amphotericin B for invasive candidiasis

DRUG RESISTANCE: Resistant strains have been described for all the above antifungal drugs

SUSCEPTIBILITY TO DISINFECTANTS: Sensitive to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde; only moderately sensitive to 70% ethanol (phenolic may be substituted)

PHYSICAL INACTIVATION: Inactivated by moist heat (121°C for at least 15 min)

SURVIVAL OUTSIDE HOST: Survives outside of host, especially in moist, dark areas

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; microscopic demonstration of pseudohyphae and/or yeast cells in infected tissue or fluid; confirmation by culture

FIRST AID/TREATMENT: Administer antibiotic therapy as required

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 2 reported laboratory-acquired infections with *Candida*

SOURCES/SPECIMENS: Sputum, bronchial washings, stool, urine, mucosal surfaces, skin or wound exudates, CSF, blood

PRIMARY HAZARDS: Accidental parenteral inoculation, exposure of mucous membranes to droplets and aerosols, ingestion

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for the manipulation of this organism

PROTECTIVE CLOTHING: Laboratory coat; gloves when contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection, incineration

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

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Date Modified: 2001-01-23

testing [92305] [92307] [92402] [92403]
 testing fungicides [92443] [92784] [92789] [92802] [92824] [92831] [92836] [92837]
 produces farnesic acid, an autoregulatory substance capable of regulating morphological transition [53041]
 This strain is recommended by ATCC for use in the tests described in ASTM Standard Test Method E979-91 where only the taxon is specified.
 For sterility testing, not more than five passages from the ATCC culture should be used.
 growth and invasiveness in mouse [19748]
 steroid interference with antifungal activity [19749]
 Cell wall hydrophobicity enhances corticosterone incorporation. [20319]
 ultraviolet microscopy [20321]
 Calcification [20476]
 morphology and physiology of strain sectors [20157]
 use of impedance for preservative efficacy testing [1968]
 fungitoxicity of alcohols and fatty acids [16096]
 esterase activity [19297]
 lipid composition [20072]
 effect of antineoplastic drugs [19796]
 genomic DNA:ATCC 10231D-5

Comments:**Related Products:****Subcollection:****References:**

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Bacteria

ATCC® Number: 700610™ [Order this Item](#)
Organism: *Streptococcus mutans* Clarke

Designations: UA159 [UAB577]

Depositor: PW Caulfield

Biosafety Level: 1

Growth Conditions: ATCC medium 269; Trypticase soy agar with defibrinated sheep blood

Temperature: 37.0°C

Duration: aerobic

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Antigenic Properties:

serotype c [39021]

Cross References:

Nucleotide (GenBank) : AE014133Streptococcus mutans UA159 complete genome.

Applications:

sensitive to erythromycin [39021]
 sensitive to rifampin rifampicin, rifamycin AMP [39021]
 sensitive to spectinomycin actinospectacin [39021]
 sensitive to streptomycin [39021]
 transformation host [39021]
 purified DNA:ATCC 700610D-5

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References:

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110815: Page W Caulfield, personal communication

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Re: Containment level request - S. mutans

Subject: Re: Containment level request - S. mutans
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
Date: Fri, 08 May 2009 11:05:21 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

There is nothing special with this strain, regular CL-2 requirements is sufficient. You can use the MSDS for the "regular" Streptococcus.

Have a nice day.

Genevieve

Jennifer Stanley <jstanle2@uwo.ca>
2009-05-06 09:56 AM

To
Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
cc

Subject
Containment level request - S. mutans

Hello there

Do you have any advice for the use of Streptococcus mutans? I know that there are MSDS for other Steptococcus strains on your website, any thoughts on this one?

Thanks
Jennifer