Modification Form for Permit BIO-UWO-0149

Permit Holder: Jun Yang

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

Approved Personnel
(Please stroke out any personnel to be removed)

Tingjie Li

Nour Ghonaim

Qiuquan Guo

Binyu Yu

Additional Personnel
(Please list additional personnel and their Biosafety training dates here)

Jia, Pei Pei Oct 28 2011

Zhang, Teng Yuan ~~Zhang~~ Nov 15, 2012

Yang, Zhao Liang Nov. 15. 2012

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	Staphylococcus aureus, E. coli K12	MRSA, C. Diff, VRE A. niger, B. subtilis
Approved Primary and Established Cells	[Established] (Human): established cell line from Lonza.	
Approved Use of Human Source Material	Human Blood (whole)	
Approved Genetic Modifications (Plasmids/Vectors)		
Approved Use of Animals		
Approved Biological Toxin(s)		
Approved Gene Therapy		

Approved Plants and Insects		

As the Principal Investigator, I have ensured that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder:

Current Classification: 2 **Containment Level for Added Biohazards:**

Date of Last Biohazardous Agents Registry Form: Feb 8, 2010

Date of Last Modification (if applicable):

BioSafety Officer(s)*:

***For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.**

Chair, Biohazards Subcommittee: **Date:**

Bacterial strains and cultural conditions

Preparation of spores

Spores were prepared by growth on Duncan and Strong agar medium. Spores were stored at 4°C in sterile distilled water until use. Prior to testing, spore preps were confirmed by phase contrast microscopy and malachite green staining to be >99% dormant, bright-phase spores.

Microbiology

For vancomycin-resistant Enterococcus (***VRE***), Methicillin-resistant Staphylococcus aureus (***MRSA***), and ***C. difficile*** cultures, selective media included Enterococcosel agar (Becton Dickinson, Cockeysville, MD) containing 20 µg/mL of vancomycin, CHROMagar (Becton Dickinson) containing 6 µg/mL of cefoxitin, and cycloserine-cefoxitin-brucella agar containing 0.1% taurocholic acid and lysozyme 5 mg/mL (CDBA), respectively. Plates were incubated at 37°C for 48 hours. VRE and MRSA colonies with unique morphology were subjected to identification and susceptibility testing in accordance with Clinical Laboratories Standards Institute guidelines. As for C. difficile, the plates were then held aerobically at room temperature for 7 days. All colonies were harvested and placed into 5 mL of sterile distilled water and 5 mL of ethanol. After a half hour, 300 µL of the suspension was centrifuged, the supernatant was decanted, and the spores were resuspended in 300 µL of sterile distilled water and vortexed repeatedly until no visible clumps were present in the suspension. It was confirmed on the basis of typical odor and appearance of colonies and by a positive reaction using C. difficile latex agglutination (Microgen Bioproducts, Camberly, UK).

Bacterial strain culture for ***B. subtilis*** and ***A.niger***

B. subtilis

A loopful of a stock culture stored at -80°C in a 30% (vol/vol) glycerol solution of ***B.subtilis*** on Luria-Bertani agar (LBA) plates at 30°C for 24 h (pH = 7.0). A

colony of the strain was picked and suspended in 1 ml of sterile distilled water, and then a volume of 200 µl was spread for spore production on LBA agar for 7 days at temperatures at 30°C. After 7 days, the cultures contained > 90% free spores. Three 1 ml volumes of sterile demineralized water were poured onto the LBA agar plates. The agar surface was scraped with caution with a spreader to detach and suspend spores. The suspensions were pipetted and centrifuged for 15 min at 7,000 × g, and the pellet was then suspended again in 25 ml of sterile water. This operation was performed twice. The pellet was suspended again and centrifuged twice at 5,000 × g and twice at 4000 × g. After the last wash, the pellet was suspended in 2 ml of sterile distilled water. Immediately before use, the spores suspensions of B.subtilis were heated for 10 min at 70°C.

A.niger

A loopful of a stock culture stored at 20oC in a 30% (vol/vol) glycerol solution of A.niger was spread on malt extract agar (MEA; Sigma Aldrich, Steinheim, Germany) plates at 30oC for 5 days. The mycelium of these plates was then spread on malt extract agar and incubated for 7 days at 30oC. After 7 days, two 5 ml volumes of a sterile Tween 80 solution (0.1%.wt/vol) were successively poured onto the mycelium. Spores were detached by smooth agitation of the plates. The solutions containing the spores were pipetted, pooled and dispensed into tubes for storage at 4°C until use.

Sample preparation

One million spores in 10 ml of water were spotted in triplicate on sterile glass microscope slides, and the spots were air dried at 37°C for 15 min. Spores were then exposed to different treatments of UV radiation. After UV exposure, spores were recovered from the microscope slides as follows. Briefly, 0.1 ml of 10% (wt/vol) sterile polyvinyl alcohol (molecular weight, 30,000 to 70,000; Sigma Chemical Co., St. Louis, Mo.) was applied onto the dried spore spots and air dried at 37°C for 1.0 to 1.5 h. The resulting polyvinyl alcohol films containing the spores were then peeled from the microscope slides with a sterile scalpel and

forceps, resuspended in 1 ml of a freshly prepared sterile solution of 60 mM DPA and 60 mM $CaCl_2$ (pH 8), and germinated for 1 h at room temperature. Spores were then diluted serially 10-fold in phosphate-buffered saline and plated on Luria-Bertani agar, and colonies were counted after overnight incubation at 37°C. The survival percentage of spores (%S) was calculated by the following equation: %S 5 (Nt/N0) 3 100, where Nt and N0 stand for the numbers of CFU of the spores at the exposure time t and time zero, respectively. Spore UV resistances are expressed as the lethal dose of UV (in joules/square meter) required to inactivate 90% of the spore population (LD90) and are reported as averages 6 standard deviations. Differences in LD90s were analyzed for statistical significance by analysis of variance (ANOVA). Differences with a P value of #0.05 were considered significant.

ATCC

MATERIAL SAFETY DATA SHEET

MSDS FOR MICROBIAL CULTURES (Biosafety Level 1 or 2 or 3)

MATERIAL SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Microbial Cultures at Biosafety Level 1 or 2 or 3
ATCC Catalog #: Various

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating: N/A
NFPA Rating: N/A

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

ATCC recommends that all ATCC microbial cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al) and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories.** This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm .
Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DNA Molecules** at http://grants.nih.gov/grants/policy/recombinentdnaguidelines.htm.

SECTION 4 - FIRST AID MEASURES

Report to your Safety Office and Seek Medical Attention as Soon as Possible

Ingestion: If person is unconscious seek emergency medical attention; never give anything by mouth to an unconscious person. If the person is conscious wash mouth out with copious amounts of water and call a physician then administer three cupfuls of water. Do not induce vomiting unless directed to do so by a physician.

Inhalation: If person is unconscious seek emergency medical attention, if person is conscious remove to fresh air and call a physician.

Dermal exposure: Immediately wash skin with copious amounts of water followed by washing with soap and copious amounts of water. Remove all contaminated clothing.

Eye exposures: Flush eyes with copious amounts of water for at least 15 minutes with eyelids separated and call a physician.

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available

Suitable Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, Halon (where regulations permit), or appropriate foam.

Firefighting

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent inhalation, ingestion, skin and eye contact.

Specific Hazard(s): Responders should take into consideration the biohazard risk associated with responding to a fire in the area where the material may be stored or handled.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8. Wear laboratory coat, gloves and eye protection. Avoid all contact.Methods for Cleaning Up

Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.

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

Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:
Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use fume hood or other appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

Eye Contact: Data not available. Avoid eye contact.
Skin Contact: Data not available. Avoid skin contact.
Skin Absorption: Data not available. Avoid skin absorption.
Inhalation: Data not available. Avoid inhalation.
Ingestion: Data not available. Avoid ingestion.
Parenteral Exposure: Data not available. Avoid parenteral exposure.

Sensitization
Skin: Data not available
Respiratory: Data not available

Target Organ(s) or System(s): Data not available

Signs and Symptoms of Exposure
Skin and Mucous Membranes: Data not available
Respiratory: Data not available
Gastrointestinal: Data not available

Toxicity Data: Data not available
Effects of Long Term or Repeated Exposure: Data not available
Chronic Exposure–Teratogen: Data not available
Chronic Exposure–Mutagen: Data not available
Chronic Exposure–Reproductive Hazard: Data not available

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Decontaminate all wastes before disposal (steam sterilization, chemical disinfection, and/or incineration).

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

Contact ATCC for regulatory information.

SECTION 16 - OTHER INFORMATION

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

COLLECTION OF BACTERIA

ATCC NUMBER: 43300™

ORGANISM: *Staphylococcus aureus* subsp. *aureus*
Resistant to methicillin and oxacillin. Used in susceptibility testing, and evaluation of Mueller-Hinton Agar.

CITATION OF STRAIN:
If use of this strain results in a scientific publication it should be cited in that manuscript in the following manner: *Staphylococcus aureus* subsp. *aureus* ATCC® 43300™.

ATCC MEDIA:
#18 Broth: Tryptic Soy Broth (BD 211825)
#18 Agar: Tryptic Soy Agar (BD 236950)

CONDITIONS:
Temperature: 37°C
Atmosphere: Aerobic

BIOSAFETY LEVEL: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

ATCC WARRANTY:
The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

DISCLAIMERS:
This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

PROPAGATION PROCEDURE:
1. Open vial according to enclosed instructions.
2. Using a single tube of #18 broth (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a Pasteur or 1.0 ml pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #18 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 hours.

NOTES:
Colonies on #18 agar are entire, glistening, circular, smooth, opaque, white, and low convex. Both β-hemolytic and non-hemolytic colonies are observed.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

REFERENCES:
1. NCCLS. Protocols for evaluating dehydrated Mueller-Hinton agar. Wayne, PA: NCCLS; NCCLS M6-A, 1996.
2. NCCLS. Performance standards for antimicrobial susceptibility testing. Wayne, PA: NCCLS; NCCLS M100-S8, 1998.
3. Baker CN, Tenover FC. Evaluation of Alamar colorimetric broth microdilution susceptibility testing method for staphylocci and enterococci. J. Clin. Microbiol. 34:2654-2659, 1996. PubMed: 8897159
4. NCCLS. Screening test for oxacillin-resistant staphylococci. Wayne, PA: NCCLS; NCCLS M7-A4.

Updated (11/05) MVB/SAL

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

ATCC®43596™
Clostridium difficile

BACTERIAL CULTURE PRODUCT SHEET

Strain Designation
545

Deposited Name
Clostridium difficile (Hall and O'Toole) Prevot

Intended Use
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Biosafety Level: 2

It is the responsibility of the investigator to determine appropriate safety procedures for use with this material. As a reference, laboratory safety is discussed in the publication *Biosafety in Microbiological and Biomedical Laboratories* and can be accessed by searching "BMBL" at www.cdc.gov.

Propagation

Growth Conditions
Min Temperature: 35.0°C
Temperature: 35°C to 37°C
Atmosphere: Anaerobic

Growth Media
Reinforced Clostridial medium (Oxoid CM149)
Trypticase soy agar with defibrinated sheep blood

*For additional information on media preparation, please visit our website at **www.atcc.org.**

Propagation Procedure
1. Open vial according to enclosed instructions.
2. Under anaerobic conditions, withdraw 0.5 mL of the recommended broth from a single test tube (5 to 6 mL) and rehydrate the entire vial contents.
3. Aseptically transfer this aliquot back into the broth. Additional tubes may be inoculated with 0.5 mL each from the suspension. Also, 0.1 mL may be inoculated onto a slant. Streak several blood plates to check for colonial morphology and purity.
4. Incubate tubes under an anaerobic atmosphere at 37°C. Incubate one agar plate anaerobically for colony formation, and one aerobically for aerobic contamination check.
5. Within 24 to 48 hours, growth should be evident by turbidity in the broth and by colonies on the anaerobic agar surfaces. No growth should occur on agar plates incubated aerobically.

ANAEROBIC CONDITIONS:
Anaerobic conditions for transfer may be obtained by either of the following:
1. Use of an anaerobic gas chamber, or
2. Placement of test tubes under a gassing cannula system connected to anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:
1. Loose screw caps on test tubes in anaerobic chamber,
2. Loose screw caps on test tubes in an activated anaerobic gas pack jar, or
3. Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.

Notes
Colonies on #260 agar plates are irregular, flat, spreading, erose and gray in color.
Presence of the genes for Toxins A and B confirmed by PCR. Toxin B production was confirmed by cytotoxicity assay using Vero cells (Cell line # CCL-81).
Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Citation Guideline
If use of this strain results in a scientific publication it should be cited in that manuscript in the following manner:
Clostridium difficile ATCC ® 43596™.

References

References and other information relating to this product are available online at **www.atcc.org.**

ATCC Warranty

The viability of ATCC® cultures is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on their product information sheet. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

For other ATCC products, ATCC warrants that they will meet the specifications listed on their product information sheet until the expiration date shown on the product label, if applicable. This warranty applies only if the product is stored and handled as described on the product information sheet.

Disclaimers

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Unless expressly stated, the purchase of this product conveys no license or grant of any intellectual property rights, whether express, implied, by estoppel or otherwise.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at **www.atcc.org.**

ATCC®700221™
Enterococcus faecium

BACTERIAL CULTURE PRODUCT SHEET

Intended Use
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* , from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

It is the responsibility of the investigator to determine appropriate safety procedures used with this material. As a reference, laboratory safety is discussed in the publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) and can be accessed at **www.cdc.gov/biosafety** .

Propogation Procedure

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Growth Media
Trypticase soy agar with defibrinated sheep blood
Brain heart infusion agar or brain heart infusion

PROPAGATION PROCEDURE
1. Open vial according to enclosed instructions.
2. Using a single tube of #44 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #260 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 hours.

Notes
Colonies on #260 agar are circular, entire, small, convex, gray, and glistening.
Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Citation Guideline
If use of this strain results in a scientific publication, it should be cited in that manuscript in the following manner:

References
References and other information relating to this product are available online at www.atcc.org.

ATCC Warranty
The viability of ATCC® cultures is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on their product information sheet. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

For other ATCC products, ATCC warrants that they will meet the specifications listed on their product information sheet until the expiration date shown on the product label, if applicable. This warranty applies only if the product is stored and handled as described on the product information sheet.

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This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains

on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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MYCOLOGY COLLECTION

ATCC® NUMBER: 9642™

ORGANISM: *Aspergillus brasiliensis*
Strain (designation) ID: SN 26
Genotype: Not Available

CITATION OF STRAIN:
If use of this strain results in a scientific publication it should be cited in that manuscript in the following manner: *Aspergillus brasiliensis* ATCC® 9642™.

PRODUCT DESCRIPTION:
An ampoule containing viable cells (may include spores and mycelia) suspended in cryoprotectant

BIOSAFETY LEVEL: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

PROPAGATION:
The information recommended in this section is to assist users in obtaining living culture(s) for their studies. The recommendation does not imply that the conditions or procedures provided below are optimum. Experienced researchers may initiate the growth of a culture in their own way.

Recommended Medium:
Users are encouraged to visit the web page of this ATCC product at www.atcc.org for additional, updated product information. The following web site allows for searching the formulae of an ATCC medium. http://www.atcc.org/TechnicalSupport/MediaFormulations/tabid/760/Default.aspx

ATCC Medium Number:
28 (Sabouraud Dextrose Agar),
200 (Yeast Mold Agar), or
336 (Potato Dextrose Agar)

For making a liquid medium, don't include agar.
Autoclave at 121°C for 15 minutes.

Recommended Incubation Temperature: 23 - 26 °C

Recommended Incubation Atmospheric Conditions:
Typical Aerobic

Recommended Procedure:
For **freeze-dried (lyophilized) ampoules**:

1. Open an ampoule according to enclosed instructions.
2. From a single test tube of **sterile distilled water** (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a sterile pipette and apply directly to the pellet. Stir to form a suspension.
3. Aseptically transfer the suspension back into the test tube of sterile distilled water.
4. Let the test tube sit at room temperature (25°C) undisturbed **for at least 2 hours**; longer (e.g., overnight) rehydration might increase viability of some fungi..
5. Mix the suspension well. Use several drops (or make dilutions if desired) to inoculate recommended solid or liquid medium. Include a control that receives no inoculum.
6. Incubate the inoculum at the propagation conditions recommended.
7. Inspect for growth of the inoculum/strain regularly. The sign of viability is noticeable typically after 1-2 days of incubation. However, the time necessary for significant growth will vary from strain to strain.

Notes:
Colonies initially white, mycelium growing rapidly (to cover a plate in 8-10 days), soon producing dense layer of erect smooth-stiped, thick-walled conidiophores terminated by globose vesicles bearing phialides (uniseriate) or (commonly) metulae with phialides (biseriate) which produce dry chains of conidia. Reverse of plate pale yellow or cream, often showing radiating ridges in mycelium. Spore heads radiate, sometimes dividing into columns with age, initially pale, becoming dark brown to black. Individual conidia spherical, mid-to-dark brown, highly roughened with ridges and blunt or pointed protuberances, 3.5 to 6 micrometers in diameter.

Will grow equally well up to at least 37 C. Sporulation may be inhibited in plates sealed completely with tape or film. Colonies grown directly from rehydrated spores may exhibit sectoring, with areas of varying levels of sporulation.

Additional, updated information on this product may be available on the ATCC web site at www.atcc.org.

DNA SEQUENCE INFORMATION:
The ITS region of the nuclear ribosomal RNA gene cluster was sequenced from a recently produced lot. Its

ATCC (American Type Culture Collection)
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

sequence is as follows and it matched 100% to GenBank accession FJ195349.

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GTAGGTGAAC CTGCGGAAGG ATCATTACCG
AGTGCGGGTC CTTTGGGCCC AACCTCCCAT
CCGTGTCTAT TGTACCCTGT TGCTTCGGCG
GGCCCGCCGC TTGTCGGCCG CCGGGGGGGC
GCCTCTGCCC CCCGGGCCCG TGCCCGCCGG
AGACCCCAAC ACGAACCCTG TCTGAAAGCG
TGCAGTCTGA GTCGATTGTT TGCAATCAGT
TAAAACTTTC AACAATGGAT CTCTTGGTTC
CGGCATCGAT GAAGAACGCA GCGAAATGCG
ATAACTAATG TGAATTGCAG AATTCAGTGA
ATCATCGAGT CTTTGAACGC ACATTGCGCC
CCCTGGTATT CCGGGGGGCA TGCCTGTCCG
AGCGTCATTG CTGCCCTCAA GCCCGGCTTG
TGTGTTGGGT CGCCGTCCCC TCTCTCCGGG
GGGACGGGCC CGAAAGGCAG CGGCGGCACC
GCGTCCGATC CTCGAGCGTA TGGGGCTTTG
TCACATGCTC TGTAGGATTG GCCGGCGCCT
GCCGACGTTT TCCAACCATT CTTTCCAGGT
TGACCTCGGA TCAGG
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ISOLATION: Wireless radio equipment, New South Wales, Australia

REFERENCES:

Sakano Y, et al. Pullulan 4-glucanohydrolase from Aspergillus niger. Arch. Biochem. Biophys. 153: 180-187, 1972. PubMed: 4650606

Inoue Y, et al. Metabolism of 2-ketoaldehydes in mold: purification and characterization of glyoxalase I from Aspergillus niger. J. Biochem. 102: 583-589, 1987. PubMed: 3123469

ASTM International Standard Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi. West Conshohocken, PA:ASTM International;ASTM Standard Test Method D 4300-01.

ASTM International Standard Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi. West Conshohocken, PA:ASTM International;ASTM Standard Test Method D 4783-01e1.

ASTM International Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi. West Conshohocken, PA:ASTM International;ASTM Standard Test Method G 0021-96 (Reapproved 2002).

RTCA Environmental conditions and test procedures for airborne equipment -- Fungus resistance. Washington, DC:RTCA;RTCA DO-160C.

U.S. Department of Defense Insulation, electrical, synthetic-resin composition, nonrigid. Washington, DC:US Department of Defense;Military Specification MIL-I-631D, 1961

U.S. Department of Defense Wax, impregnating, waterproofing, for laminated paper tubes for small arms ammunition. Washington, DC:US Department of Defense;Military Specification MIL-W-10885B, 1956

U.S. Department of Defense Environmental test methods and engineering guidelines. Washington, DC:US Department of Defense;Military Standard MIL-STD-810F, 2000

Klausmeier RE. Results of the second interlaboratory experiment of biodeterioration of plastics. Int. Biodeterior. Bull. 8: 3-7, 1972.

Schmidt E, French DW. Two-day mold testing using a contact agar method. For. Prod. J. 29: 39-42, 1979.

Standard Specification for Cellulosic Fiber Loose-Fill Thermal Insulation. West Conshohocken, PA:ASTM International;ASTM Standard Test Method C 0739-05be1.

Standard Specification for Self-Supported Spray Applied Cellulosic Thermal Insulation. West Conshohocken, PA:ASTM International;ASTM Standard Test Method C 1149-06e1.

Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings. West Conshohocken, PA:ASTM International;ASTM Standard Test Method C 1338-00.

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Varga J, et al. Aspergillus brasiliensis sp. nov., a biseriate black Aspergillus species with world-wide distribution. Int. J. Syst. Evol. Microbiol. 57: 1925-1932, 2007.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

(April 2010, JZ)

COLLECTION OF BACTERIA

ATCC® NUMBER: 6633™

ORGANISM: *Bacillus subtilis* subsp. *spizizenii*

Used in sterility testing and assay of rifampin [rifampicin, rifamycin AMP], neomycin, monensin, kanamycin, lasalocid [X-537A], mitomycin [mitomycin C], vancomycin, novobiocin, penicillin, framycetin, tobramycin, spiramycin, gentamicins [gentamicin], amoxicillin, bekanamycin [kanamycin B; aminodeoxykanamycin], hygromycin B, streptomycin, erythromycin, dactinomycin [actinomycin; actinomycin D], streptonigrin, lactam antibiotics [beta-lactam antibiotics], capreomycin sulfate [capreomycin], and dihydrostreptomycin sulfate [dihydrostreptomycin]. Susceptibility disc testing of rifampin [rifampicin; rifamycin AMP] and novobiocin. Produces restriction endonuclease *Bsu6633*I and subtilin.

CITATION OF STRAIN:

If use of this strain results in a scientific publication it should be cited in that manuscript in the following manner: *Bacillus subtilis* subsp. ***spizizenii*** ATCC® 6633™.

ATCC® MEDIA:

#3 Broth: Nutrient Broth (BD 234000)
#3 Agar: Nutrient Agar (BD 213000)

CONDITIONS:

Temperature: 30°C
Atmosphere: Aerobic

BIOSAFETY LEVEL: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current edition of *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

PROPAGATION PROCEDURE:

1. Open vial according to enclosed instructions.
2. From a single tube of #3 broth (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a Pasteur or 1.0 ml pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a second tube of broth, a slant and/or a plate.
5. Incubate all tubes and plate at 30°C for 24 hours.

NOTES:

Growth is poor in statically incubated broth, forming a thin pellicle and leaving the broth clear. The colonial morphology of this strain varies considerably depending on the temperature of incubation, medium used, and length of time incubated. Colonies on Nutrient Agar may be dull and dry to shiny, irregularly-shaped, opaque, flat, erose, and irregular. As they age, they appear to spread and become more uniform. Some colonies are adherent, making them difficult to remove from the agar surface. On Tryptic Soy Agar, the colonies are creamy, raised, erose, and mildly adherent with a soft sheen.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

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(6/09) LQ / MMA

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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR: Jun Yang
SIGNATURE:
DEPARTMENT: Mechanical and Materials Engineering
ADDRESS: SEB 3089
PHONE NUMBER: ext. 80158
EMERGENCY PHONE NUMBER(S):
EMAIL: jyang@eng.uwo.ca

Location of experimental work to be carried out: Building(s) SEB Room(s) 3074

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC, CIHR, OCE, UWO, MRI
GRANT TITLE(S): Blood-on-a-chip: multiscale transport phenomena in microcirculation; Biophysical studies of alpha4 Beta1 integrin-ligand interactions at a single molecule level and a single cell level; Microfluidics meets Microbiology

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Nour Gjonaim
Qiuquan Guo
Binyu Yu
Tingjie Li

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? Ø YES O NO
(including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? Ø YES O NO
If YES, please give the name of the species. E. Coli, S. aureus
What is the origin of the microorganism(s)? Cedarlane
Please describe the risk (if any) of escape and how this will be mitigated:
~~No risk they are~~ The risk is low. all experiments are conducted in Biosafety level 2 lab. &

Please attach the CFIA permit. Attached at the end
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli	Ø Yes O No	Ø Yes O No	O Yes O No	0.1 L	Cedarlane	O 1 Ø 2 O 3
S. aureus	Ø Yes O No	Ø Yes O No	Ø Yes O No	0.1 L	Cedarlane	O 1 Ø 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? Ø YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	Ø Yes O No	Endothelial cell from Lonza	Not applicable
Rodent	O Yes Ø No		
Non-human primate	O Yes Ø No		
Other (specify)	O Yes Ø No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	✓ Yes O No	endothelial cell line from Lonza	Lonza
Rodent	O Yes ✓ No		
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 ✓ 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ✓ YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	healthy donors ??	✓ Yes O No O Unknown		O 1 ✓ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes ✓ No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes ✓ No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Dec 8/09 email ??

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES ✓ NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ✓O NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES ✓O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES O NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ✓O NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 ✓O NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

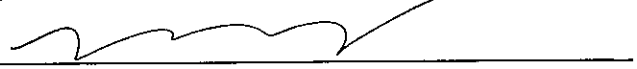
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ✓ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

✓ YES, permit # if on-campus Bio-UWO-0149

O NO, please certify

O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: N. Dec. 2. 2009

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

_____

_____

_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

As ~~suitable~~ required by UWO biosafety procedure

_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidd

Date: 8 Feb. 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: J Stanley

Date: Feb 7, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____

Date: _____

Approval Number: Bio-UWO-0149 Expiry Date (3 years from Approval): Feb 7, 2013

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Human aortic endothelial cell (HAEC) is cultured in both petri-dish and microchannel system. After cell comes confluent, it will be transferred to a small petri-dish (60mm) for atomic force microscopy (AFM) scan. The morphology and mechanical property will be measured. Critical dry is also performed for endothelial cells sometimes.

Human raw blood was drawn from a healthy donor in the university hospital and stored in a vacutainer containing anticoagulant (EDTA) at 4Â°C in the lab refrigerator. Blood sample was taken out with syringe and loaded into the Lab-on-a-CD device for blood separation. After experiment, the sample was wash with bleach. The waste was collected, and assorted into solid waste and liquid waste. They were stored in the labeled container for autoclave.

The antibacterial activities of TiO2 coating on silicon wafer obtained against S.aureus and E.coli were studied using the so-called antibacterial drop-test. S.aureus ATCC6538 and E.coli O157 were used as the experimental bacteria. E.coli, precultured in 15ml of nutrient broth (DifcoTM BD) at 37° C for 24h, were washed by centrifuging at 4000 rpm for 10min. After removing the supernatant, the cells were wash with phosphate buffer solution (PBS) twice and were resuspended and diluted to approximately 2×105 CFU/ml with PBS solution.The samples were placed in the sterilized petridishes. Then 100ul of PBS solution with bacteria was added dropwise onto the surface of each sample, and uncoated piece of silicon wafer was chose as a blank reference. The petridishes were sealed and were laid at incubator at 37° C with the humidity 46% for different time or laid at ambient temperature under UV-light for different time period.
After each time period the bacteria containing drops were washed from the surface of the sample by using 5ml PBS in the sterilized petri dish. The 100ul each of bacteria suspension was dispersed on the plate count agar. The number of surviving bacteria colony on the petri dishes were counted after incubation for 24 h at 37°C.

Disposable biohazard waste including: Bacterial aqueous solution, plastic pipets, plastic tubes, disposable inoculating loops, petri dish with Plate count agar.

received Jan 2010
by e-mail

Public Health Agency of Canada
Agence de la santé publique du Canada

Office of Laboratory Security
Bureau de sécurité des laboratoires

Centre for Emergency Preparedness and Response
Centre de mesures et d'interventions d'urgence
100 chemin Colonnade Road, Loc.: 6201A
Ottawa, Ontario, Canada K1A 0K9

WHO Collaborating Centre for Biosafety

Centre collaborateur OMS pour les techniques de biosécurité

Fax: (613) 941-0596 **Tel: (613) 957-1779**

DATE_____ **FROM / DE :** Marianne Heisz

TO / À : **Jun Yang & Edmond Leung**
University of Western Ontario
Mechanical & Materials Engineering

FAX: 519 - 661-3020 **TEL:** 519 - 661-2111 x. 80158 **PAGES TO FOLLOW / PAGES À SUIVRE :** 1

COMMENTS - COMMENTAIRES

Please see attached a letter for your attention. Original will follow through regular mail. Thank you.	Vous trouverez sous pli une lettre à votre attention. La copie originale suivra par le courrier régulier. Merci

Public Health Agency of Canada / Agence de la santé publique du Canada

Your file / Votre référence

Our file / Notre référence

Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3rd Ed., 2004*

This letter serves to confirm that the Office of Laboratory Security has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

Organiz. tion:	University of Western Ontario Mechanical & Materials Engineering
Attention:	Jun Yang & Edmond Leung
Address:	1151 Richmond Street N. SEB 3088 London, ON N6A 5B9
Laboratory Room Number(s):	SEB 3074
Type of work:	☒ *in vitro* only ☐ *in vitro* and *in vivo**
Compliance Letter expiry date:	NOVEMBER 1 5, 2010

To renew your compliance letter please complete a CL2 checklist and fax it to our office at (613) 941-0596. The checklist can be obtained from the following website: www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz
Chief, Importation and Regulatory Affairs

OCTOBER 2 7, 2008
Date

*The Office of Laboratory Security must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses.

Canada

------- Original Message -------
Subject:[Fwd: RE: Biohazardous Agents Registry Form: Yang]
Date: Tue, 08 Dec 2009 15:25:45 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

-------- Original Message --------
Subject: RE: Biohazardous Agents Registry Form: Yang
Date: Tue, 08 Dec 2009 14:34:53 -0500
From: Jun Yang <jyang237@uwo.ca>
To: 'Jennifer Stanley' <jstanle2@uwo.ca>
References: <4B1D4561.6080609@uwo.ca>
<A1AA2B9FC03E46029B71E8DD02A7FCB0@Bionano> <4B1D6944.7070006@uwo.ca>
<fc17e2b71e5f2.4b1e4ae6@uwo.ca>

Hi, Jennifer:

I think you can use the enclosed MSDS since endothelial cell lines are similar from one company to another.

Regarding blood, sorry I made a mistake. The blood is not infected, which is from health donors.

Best regards!

Jun

From: Jennifer Stanley [mailto:jstanle2@uwo.ca]
Sent: 2009年12月8日 12:48 PM
To: Jun Yang
Subject: Re: Biohazardous Agents Registry Form: Yang

Hi Dr. Yang

Perhaps if you give me the exact cell line name I will be able to find it on the Lonza site?

Also, I noticed on Table 3.2 that you are using human blood that is infected...can you tell me what it is infected with?

Regards

Jennifer

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Bacteria

ATCC® Number: 33807™ Order this Item
Price: $240.00

Organism: *Staphylococcus aureus* subsp. *aureus* Rosenbach
Designations: D0318
Isolation: clinical isolate
Depositor: JC Feeley
History: ATCC <<--JC Feeley<<--Wisconsin State Hlth. Dept
Biosafety Level: 2
Shipped: freeze-dried
Growth Conditions: ATCC medium3: Nutrient agar or nutrient broth
Temperature: 37.0°C
Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Comments: Does not produce pyrogenic exotoxin C

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All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.
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Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Bacteria

ATCC® Number: 29425™ Order this Item
Price: $195.00

Organism: *Escherichia coli* (Migula) Castellani and Chalmers
Designations: K12
Isolation: Basel, 1969 [185139]
Depositor: R Yuan
History: ATCC <<--R Yuan<<--W. Arber
Biosafety Level: 1
Shipped: frozen
Growth Conditions: ATCC medium3: Nutrient agar or nutrient broth
Temperature: 37.0°C
Duration: aerobic
Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
References: 185139: R Yuan, personal communication

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All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.
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invitrogen

Material Safety Data Sheet

Revision Date: 10-Jul-2007

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code C0065C
Product name HAEC, 500,000 cells/vial

Company/Undertaking Identification

INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
3 FOUNTAIN DRIVE
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
011 44 141 814 6100

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
1-800-263-6236

CASCADE BIOLOGICS
INVITROGEN CORPORATION
1341 S.W. CUSTER DRIVE
PORTLAND, OR 97219
++1 503-292-9521
++1 800-778-4770

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	7-13

The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview

Components of the product may be absorbed into the body through the skin

The product contains no substances which at their given concentration, are considered to be hazardous to health

Form

Suspension

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	Moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Rinse mouth.
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry powder. alcohol-resistant foam. The product is not flammable.
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material. Clean contaminated surface thoroughly. Take up mechanically and collect in suitable container for disposal.

7. HANDLING AND STORAGE

Handling Avoid contact with skin and eyes.
Storage Keep in properly labelled containers.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Suspension

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability Stable.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/
Potential Health effects

Eyes	Mild eye irritation.
Skin	Moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

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End of Safety Data Sheet