

Modification Form for Permit BIO-UWO-0155

Permit Holder: John McCormick

Approved Personnel

(Please stroke out any personnel to be removed)

~~Peter-Bastedo-~~
 Katherine Kasper
 Nur Rahman
 Christine Herfst
 Delfina Mazzuca Siroen
 Mohammad-Hussain-
 Lindsay-Bowman
 Shipa Gupta
 Stacey Xu
 Brent Armstrong
 Kelcey Patterson
 Jingru-Li-
 Fraser-Pollard

Additional Personnel

(Please list additional personnel here)

Lorea Baroja
 Joseph Zeppa

Please stroke out any approved Biological Agent(s) to be removed

Spelling error(s) / Typo(s)

Approved Microorganisms

E. coli, Streptococcus pyogenes, Staphylococcus aureus, Lactobacillus reuteri, Steptococcus gordonii

Listeria monocytogenes (see mod appendix 1)
 Streptococcus agalactiae (see mod appendix 2)

Approved Primary and Established Cells

Human (primary) peripheral blood mononuclear cells. Rodent (primary) splenocytes, hepatocytes, Lymph Nodes, Thymocytes, Bone marrow derived dendritic cells, Nasal associated lymph node tissues.

Approved Use of Human Source Material

Human blood (whole) or other body fluid, Human blood (fraction) or other body fluid: Healthy volunteers.

Approved Genetic Modifications (Plasmids/Vectors)

E.coli [plasmids]: pET28a, pBluescrip, pGghost, pMAD, pAmilux. S. pyogenes [plasmid]: pGghost. S. aueus [plasmid]: pMAD, pAmilux. Phage 80alpha [vectors]: pMAD, pAmilux.

Approved Use of Animals

Mouse

Approved Biological Toxin(s)

Superantigens

* 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. see attached description 1 (Modification 1) September 7, 2011

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: John Wilson 7 Sept 2011

Current Classification: 2 Containment Level for Added Biohazards: 2

Date of Last Biohazardous Agents Registry Form: Jun 21, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ Date: _____

MODIFICATION 1 (September 7th, 2011) McCormick

Listeria monocytogenes will be used to test a new ELISA protocol for detection of this species by Dr. Michael Reider's group. *L. monocytogenes* will be manipulated and grown under Level II conditions, and stored frozen at -80°C. Disposal will be done by standard UWO procedures for autoclaving.

Streptococcus agalactiae will be used to test for secretion of a signaling compound to up-regulate gene expression in *Staphylococcus aureus*. *S. agalactiae* will be manipulated and grown under Level II conditions, and stored frozen at -80°C. Disposal will be done by standard UWO procedures for autoclaving.

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mod appendix 1

Canada

Home > Laboratory Biosafety and Biosecurity > Biosafety Programs and Resources > Pathogen Safety Data Sheets and Risk Assessment > Listeria monocytogenes - Material Safety Data Sheets (MSDS)

Listeria monocytogenes - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Listeria monocytogenes*

SYNONYM OR CROSS REFERENCE: Listeriosis, Listerella

CHARACTERISTICS: Gram-positive, non-spore forming, aerobic bacilli; hemolytic and catalase positive; tendency to form chains and palisades, growth at 4° C, intracellular; food-borne human pathogen usually caused by serovars 1/2a, 1/2b and 4b

SECTION II - HEALTH HAZARD

PATHOGENICITY: Opportunistic pathogen manifested in the elderly, in neonates and or among immunocompromised individuals as meningoencephalitis and/or septicemia; inapparent infection at all ages with consequence only during pregnancy; perinatal infections occur transplacentally and can result in abortion, stillbirth; meningitis, endocarditis, septicemia, and disseminated granulomatous lesions in adults

EPIDEMIOLOGY: Uncommonly diagnosed infection; typically sporadic; few recent outbreaks associated with food; nosocomial acquisition; 40% of clinical cases occur in infants; in adults infection occurs mainly after age 40; European studies have disclosed large numbers of human carriers; case fatality rate in newborns is 50%

HOST RANGE: Mammals, birds, fish, crustaceans and insects

INFECTIOUS DOSE: Not known

MODE OF TRANSMISSION: In neonates, transmission from mother to fetus *in utero* or during passage through infected birth canal; direct contact with infectious material or soil contaminated with infected animal feces can result in papular lesions on hands and arms; ingestion of contaminated food (vegetables and dairy products have been reported); venereal contact and inhalation of the organism is possible; nursery outbreaks via hands of medical staff

INCUBATION PERIOD: Variable, outbreak cases have occurred 3-70 days following a single exposure to an implicated product, median incubation is estimated at 3 weeks

COMMUNICABILITY: Mothers of infected newborn infants may shed the agent for 7-10 days after delivery; infected individuals can shed organism in the stool for several months

SECTION III - DISSEMINATION

RESERVOIR: Infected domestic and wild mammals, fowl and humans; infection of foxes produces

an encephalitis simulating rabies; asymptomatic fecal carriage in man (5%) and animals; frequently found in free-living water and mud; seasonal use of silage as fodder is frequently followed by an increased incidence of listeriosis in animals

ZOONOSIS: Yes, all domestic and wild animals are susceptible; proper precautions by farmers and veterinarians in handling aborted fetuses are recommended

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to penicillin, ampicillin, aminoglycosides, tetracyclines (resistance has been observed), chloramphenicol

SUSCEPTIBILITY TO DISINFECTANTS: Moderately susceptible to disinfectants - 1% sodium hypochlorite, 70% ethanol, glutaraldehyde

PHYSICAL INACTIVATION: Sensitive to moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour); able to grow at low temperatures (-0.4 to -0.1° C); sensitive to short wave UV and gamma irradiation

SURVIVAL OUTSIDE HOST: Survives well in soil, water, food, feces

SECTION V - MEDICAL

SURVEILLANCE: Found in feces, CSF, blood; routine smear from all newborn infants examined for *L. monocytogenes*

FIRST AID/TREATMENT: Antibiotic therapy, penicillin or ampicillin alone or together with aminoglycosides; resistant to cephalosporins including third generation cephalosporins

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Not a common laboratory-associated infection; 2 reported infections

SOURCES/SPECIMENS: Cerebrospinal fluid, blood, placental or fetal tissue, genital tract secretions, amniotic fluid

PRIMARY HAZARDS: Experimentally infected animals are a risk factor to laboratory workers; ingestion is the common mode of exposure, however may cause eye and skin infection following direct exposure; parenteral inoculation, ingestion, exposure to highly concentrated aerosols

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for all activities involving clinical materials or cultures; biosafety cabinets should be used for activities likely to generate aerosols

PROTECTIVE CLOTHING: Laboratory coat; gloves and eye protection when direct contact with infectious materials is unavoidable

OTHER PRECAUTIONS: Pregnant women should avoid contact with infected materials

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wear protective clothing; gently cover spill with paper towels 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: March, 2001

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: 2011-02-18

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Home > Laboratory Biosafety and Biosecurity > Biosafety Programs and Resources > Pathogen Safety Data Sheets and Risk Assessment > Streptococcus agalactiae - Material Safety Data Sheets (MSDS)

Streptococcus agalactiae - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Streptococcus agalactiae*

SYNONYM OR CROSS REFERENCE: Group B streptococci

CHARACTERISTICS: Gram-positive cocci, ~2.0 µm occurring in pairs and short chains, facultatively anaerobic, beta hemolysis on blood agar

SECTION II - HEALTH HAZARD

PATHOGENICITY: Associated with diseases of the newborn; 90% of cases have septicemia, 40% have pulmonary involvement, and 30% have meningeal involvement; early onset disease acquired in utero or during passage through the birth canal and can have a case fatality rate of 50%; late onset disease with onset from 1 week to 3 months after birth have a case fatality rate of 20% and are probably acquired from the environment: survivors of meningitis cases can be left with hearing loss, blindness, cerebral palsy, mental retardation and/or epilepsy; adult infections include pneumonia, urinary tract infection, peritonitis, meningitis, endocarditis, osteomyelitis and rarely pharyngitis

EPIDEMIOLOGY: Worldwide; mainly causes diseases in infants <3 months of age with low birth weight and in the elderly; predispositions include diabetes mellitus, cancer, HIV

HOST RANGE: Humans, cattle (mastitis), other animals

INFECTIOUS DOSE: Not known

MODE OF TRANSMISSION: The manner of acquisition varies by age; 10-30% of pregnant women harbor Group B streptococci in the genital tract; approximately 1% of their offspring develop symptomatic infection within 6 days of birth; source of infection in older infants, children and adults are not well established

INCUBATION PERIOD: One to seven days for early onset disease, seven days to months for late onset disease

COMMUNICABILITY: Humans carry organisms in throat and vagina; attempts to eradicate genital tract group B streptococci in women during pregnancy with oral antibiotics only partially successful due to reinfection from rectal carriage of the organism or by reacquisition from culture-positive sexual partners

SECTION III - DISSEMINATION

RESERVOIR: Humans, cattle, horses, dogs, rabbits, guinea pigs, mice

ZOONOSIS: Possibly through direct or indirect contact with infected animals (mostly livestock)

workers); strains causing disease in humans are usually biochemically, metabolically or serologically different than those causing disease in animals; if animal transmission to humans does occur it is rare and of little significance

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to penicillin or ampicillin; some strains penicillin tolerant and require treatment with an aminoglycoside as well

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite and 70% ethanol, formaldehyde, glutaraldehyde, iodines

PHYSICAL INACTIVATION: Sensitive to moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour)

SURVIVAL OUTSIDE HOST: Dust - 20 to 30 days; contaminated cows feces - 21-63 days; litter - 20-30 days; paper contaminated with infected milk 4 days; urine 2-6 days; wood - 11 days

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm bacteriologically

FIRST AID/TREATMENT: Antibiotic therapy

IMMUNIZATION: None available

PROPHYLAXIS: Administration of penicillin or ampicillin at the onset and throughout labor to women who are colonized with group B and who are at high risk of delivering an infected infant (premature)

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 78 recorded cases of *Streptococcus* spp. up to 1976

SOURCES/SPECIMENS: Blood, genital specimens, feces, urine, throat swabs and respiratory specimens

PRIMARY HAZARDS: Accidental parenteral inoculation; ingestion: inhalation of infectious aerosols, direct contact

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for all activities involving known or potentially infected clinical materials or culture; animal biosafety level 2 facilities for studies utilizing infected animals

PROTECTIVE CLOTHING: Laboratory coat; gloves when contact with infectious materials in 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: April, 2001

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: 2011-02-18

Subject: Re: Modification Form: McCormick

From: John McCormick <john.mccormick@schulich.uwo.ca>

Date: Thu, 08 Sep 2011 14:44:00 -0400

To: Jennifer Stanley <jstanle2@uwo.ca>

CC: Delfina Mazzuca Siroen <dmazzuca@uwo.ca>, Michael J Rieder <mrieder@uwo.ca>

Hello Jennifer,

We will kill the *Listeria* by heating at 70C for 30 minutes (just put the tube in a water bath) and then a 100 microliter aliquote will be plated on Brain-Heart Infusion agar to insure proper killing. We would do this initially to make sure the heat is sufficient but pasteurization kills Lm no problem so this should be fine.

Best regards,

John K. McCormick, Ph.D.

Associate Professor and Undergraduate Chair, Microbiology and Immunology
Schulich School of Medicine and Dentistry, The University of Western Ontario
Scientist, The Lawson Health Research Institute

OFFICE/LAB: Siebens-Drake Research Institute Room 133

OFFICE PHONE: (519) 661-3309 (X83309)

LAB PHONE: (519) 661-2111 X80951

FAX: (519) 661-3499

EMAIL: john.mccormick@schulich.uwo.ca

WEB: <http://publish.uwo.ca/~jmccorm8/>

MAILING/COURIER ADDRESS:

Department of Microbiology and Immunology
Schulich School of Medicine and Dentistry
Dental Sciences Building Room 3014
University of Western Ontario
1151 Richmond St.
London, Ontario, Canada N6A 5C1

On 2011-09-08, at 11:26 AM, Jennifer Stanley wrote:

HI there

Also, how do you verify the the *Listeria* is killed?

Subject: Re: Modification: McCormick lab
From: Delfina Siroen <dmsiroen@gmail.com>
Date: Fri, 11 Nov 2011 14:30:01 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: John McCormick <john.mccormick@schulich.uwo.ca>

New Info

Hello Jennifer,

Embedded in the email below please see responses to your questions .

Hi there -

Your form was discussed at a recent Biohazards Subcommittee meeting. Please address the following questions:

The committee needs more information on the quantity of the bacteria that will be grown.

No more than 3 tubes of 50 mLs at a time will be grown.

In the project description it says that "*L. monocytogenes* will be manipulated" – this should be edited as bacteria are not manipulated.

The bacteria will not be manipulated. They will be grown.

The form should say "grown in Level 2 conditions" (instead of "grown under Level 2 conditions")

Ok. Please change form to say "grown in Level 2 conditions"

Per the e-mail provided in hardcopy (dated September 8th), the *Listeria* will be heat killed. Is this then provided to the Reider lab? Since the Reider lab has a commercial source of heat killed *Listeria*, what is the purpose of getting it from the McCormick lab?

With respect to the heat killing question, the Reider lab has autoclaved *Listeria* which likely destroyed the antigens he is trying to detect. We will kill at 70oC for 30mins, 100C for 15 mins and autoclave for 15 min to test this.

Re: Modification: McCormick lab

Thank you,
Cheers,
Delfina

Regards
Jennifer