

1. Information about the proposed experiment, including technical information about the proposed transfer of drug resistance (e.g., the vector(s), gene(s) encoding the resistance, degree(s) of resistance, cross-resistance to other drugs etc).

Successful transformation via electroporation was recently reported in the closely related *C. psittaci* (Binet and Maurelli, 2005). We propose to construct two different plasmid vectors to electroporate *C. trachomatis* under similar electroporation conditions using a tetracycline resistant cassette. Both constructs will be constructed in *E. coli*. The first plasmid will contain ~8kb of PCR amplified *C. trachomatis* chromosomal DNA encoding the *trpRBA* regulon plus ~3-5 kb of flanking DNA. A *tetR* cassette will then be inserted into the *trpB* ORF creating a deletion/insertion (i.e. a *trpB* null allele). Previous work in our laboratory has shown this gene is not essential for chlamydial growth *in vitro* (Fehlner-Gardiner et al., 2002). We will then electroporate *C. trachomatis* with this plasmid construct, selecting for allelic exchange in the presence of tetracycline. The construction of this plasmid will utilize the Invitrogen cloning vector pCR-XL-TOPO. The second vector will utilize a shuttle vector previously constructed in our laboratory (pBRCT) and approved for use by the RML Biosafety Committee (RD#06-105). This plasmid construct contains all 8 ORFs encoded by the naturally occurring cryptic plasmid of *C. trachomatis* inserted into the *E. coli* vector pBR322. The chlamydial plasmid insert disrupts the *tetR* gene found on pBR322. In our proposed construct, we wish to replace the *bla* (AmpR) gene with a *tetR* cassette. In addition, we will insert a red fluorescent cassette (mCherry) elsewhere on the plasmid. Tetracycline resistance will be the selectable marker and red fluorescence screened in electroporated *C. trachomatis*. In theory, this plasmid will be stably maintained extrachromosally in *C. trachomatis*. Diagrams of the proposed constructs are attached (**Attachment 1**).

Suchland et al. (2009) recently demonstrated the interspecies lateral gene transfer of a naturally occurring *tetR* marker in *C. suis* to *C. trachomatis*, conferring resistance to tetracycline at a concentration of 8 µg/ml. While the *tetR* cassette we propose to use is not identical to the one they used, it shares 98% identity at the amino acid level so we expect it will give similar levels of resistance. As this cassette also confers resistance to doxycycline in *E. coli*, we also expect it to also confer doxycycline in *C. trachomatis*.

2. Rationale for why the work should go forward, including an assessment of how the scientific and public health benefits outweigh the potential risks for humans, animals or agriculture.

There is currently no genetic system available for use to genetically manipulate *C. trachomatis*, though several laboratories have worked diligently on this for several years. One of the major

problems is the identification of a selectable marker that (1) does not give a high background of resistance as the result of spontaneous point mutations (i.e. rifampicin) or (2) has a minimum inhibitory concentration (MIC) that is prohibitively high (i.e. chloramphenicol and kanamycin). While tetracycline has a low MIC (0.3 µg/ml), *tetR* cassettes have been prohibited from use in previous attempts to transform *C. trachomatis* as tetracycline is a frontline antibiotic used in treating chlamydial infections, particularly in third world countries where blinding trachoma is the world's leading cause of preventable blindness.

Recently, azithromycin has replaced tetracycline as the recommended antibiotic treatment for trachoma and urogenital infections (Workowski & Berman, 2002; Reveneau et al., 2005; Geisler, 2007), though doxycycline and erythromycin are still recommended for treating LGV infections (CDC guidelines, 2006). Moreover, the use of a naturally occurring *tetR* marker was approved for lateral gene transfer studies (Recombinant DNA Advisory Committee minutes, 6/19-21/07) and was successfully transferred into *C. trachomatis* (Suchland et al., 2009). Therefore it is our desire to create plasmid constructs containing a *tetR* cassette and electroporate these plasmids into the *C. trachomatis* strain L2(25667R) to further our attempts to define the conditions required to transform *C. trachomatis*.

- 3. A discussion of whether there are alternative approaches to this research that would not involve conferring resistance to a drug that has utility in the treatment of the organism in question. This should include a statement as to whether the submitting investigator or others have pursued any alternatives.**

We have previously attempted transforming *C. trachomatis* with an allele of *rpoB* that confers rifampicin resistance (*rifR*). A laboratory acquired spontaneous *rifR* mutant was isolated and the *rpoB* gene sequenced to identify the nucleotide substitution. For use in our transformation experiments, the *rifR* allele was PCR amplified and two flanking, silent nucleotide substitutions were introduced to allow the molecular differentiation between our *rifR* cassette versus the original spontaneous *rifR* allele. Constructs were made to transform rifampicin sensitive strains to *rifR* through allelic exchange as well as through the use of the previously mentioned shuttle vector. All attempts to transform *C. trachomatis* to *rifR* have been unsuccessful.

In addition, attempts to transform *C. trachomatis* with a chloramphenicol resistance cassette also employed a shuttle vector (Tam et al., 1994). However, the chloramphenicol resistance expression was transient and the transformants were lost after successive passages. Moreover, the use of chloramphenicol resistance cassettes is complicated by the fact that the bacterial minimum inhibitory concentration of the antibiotic is toxic to the eukaryotic host cell. Prolonged exposure to

the high levels of chloramphenicol required for the plaque purification of transformants results in the death of the eukaryotic host cell prior to plaque formation. As such, its utility as a selectable marker for chlamydial transformation is limited at best.

The *tetR* marker we will employ in our study originates from the *E. coli* vector pBR322. While it is not the same tetracycline resistance cassette employed by Suchland et al. (2009), it exhibits 99% identity at the nucleotide level with the published *C. suis tet(C)* markers (Dugan et al., 2004), thus enabling the ability to trace the origin of a possible (though unlikely) laboratory acquired tetracycline-resistant *C. trachomatis* infection. In addition, the *C. trachomatis* LGV strain [L2(25667R)] strain, which will be used for transformation, lacks the cryptic plasmid and is highly attenuated (400 fold increase in its ID₅₀ compared to the plasmid positive L2 parent) (Carlson et al., 2008) thereby further reducing the risk of infection by a *tetR* transformant.

4. **A description of the proposed risk mitigation strategies that will be implemented to both minimize risk to laboratory personnel as well as the public.**

All experimentation will be conducted in a BSL2 lab (RML Bldg. 2, Rm 2205) following BSL-3 practices and procedures as described in the attached draft SOP “**Standard Operation Procedures for Chlamydiae.**” (**Attachment 2**) Workers not familiar with these SOPs will be trained. As the primary route for laboratory acquired LGV infections is via inhalation (Bernstein et al., 1984), respiratory protection will be worn when working with these organisms. No human-to-human transmission has been reported, making a secondary infection unlikely. Any tetracycline resistant *C. trachomatis* transformants will not be transferred to any other laboratory within or outside RML. No work with other serovars of Chlamydia which cause ocular disease will be performed when work with tetracycline is being conducted.

Prior to the start of work, a medical surveillance program will be established by the RML Occupational Medical Services based on the recommendations of the RAC (refer to the relevant sections of the RAC minutes from the 6/19-21/07 meetings). See enclosed draft document “Medical Services for Researchers Working with Tetracycline-Resistant Chlamydia”. (**Attachment 3**)

References

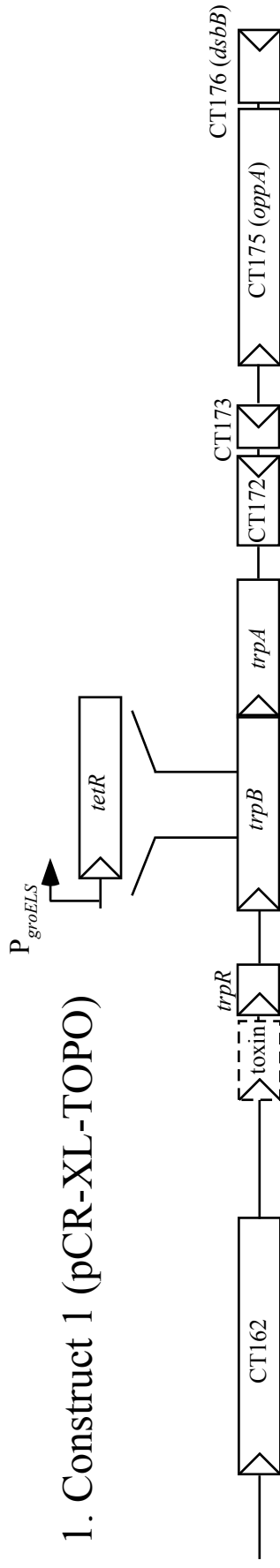
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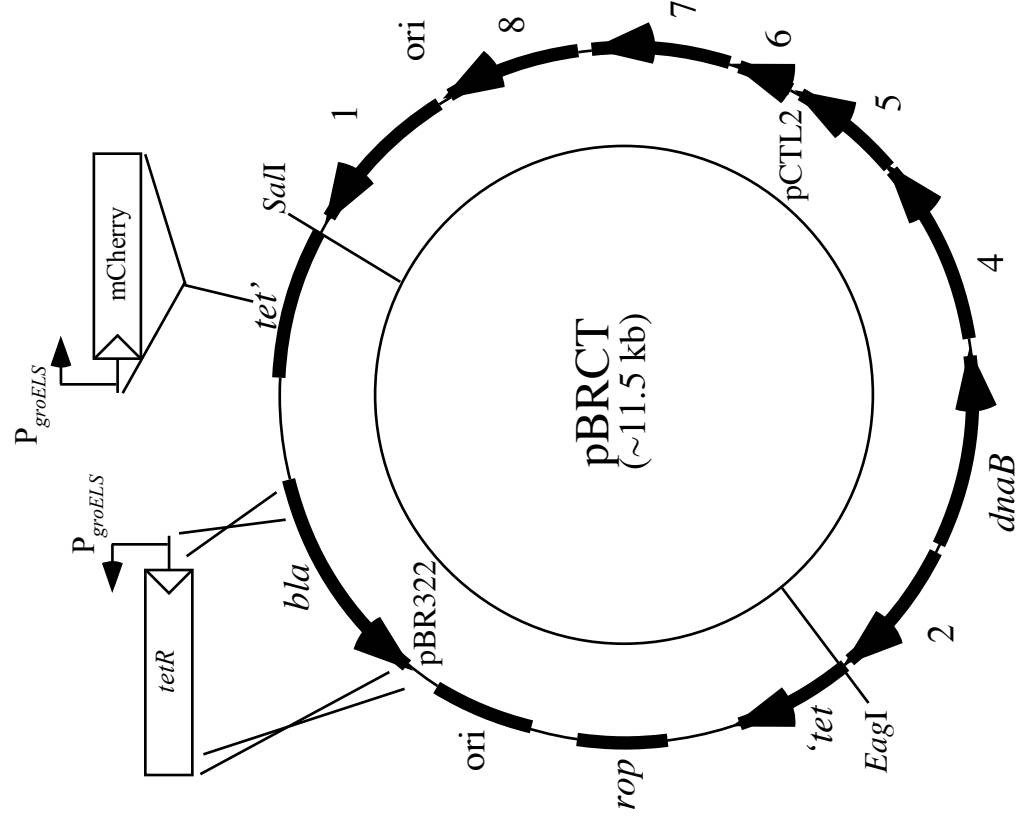
Attachments

1. Map of 1) plasmid PCR-XL-TOPO and 2) plasmid pBRCT
2. DRAFT: Standard Operating Procedures for Chlamydiae
3. DRAFT: Medical Services for Researchers Working with Tetracycline-Resistant Chlamydia

1. Construct 1 (pCR-XL-TOPO)



2. Construct 2 (pBRCT)



A. General Procedures

1. The following procedures will be performed exclusively in Room 2205:
 - i. Harvesting and Purification
 - ii. Experiments involving Tet^R and *C. trachomatis* strain L2(25667R)
2. **Any** illness of questionable origin or any incident must be reported immediately to the Laboratory Supervisor or RML Occupational Health Nurse. In the event that none of these are available, seek medical attention immediately at either the hospital emergency room or through a personal physician. Notify the Laboratory Supervisor of any unexpected absence - regardless of cause. Laboratory members should also notify the Laboratory Supervisor if colleagues are unexpectedly absent and check by phone if necessary. The Laboratory Chief should be apprised of any infections and corrective actions.

Reporting: Any significant research-related accident or illness involving rDNA will be reported by the RML Biosafety Officer to the NIH Office of Biotechnology Activities within thirty days.

3. Access
 - i. All RML personnel who require access to room 2205 will be designated by the Chief, Laboratory of Intracellular Parasites (LICP). Only staff with approved proximity access and documented training will be granted access to room 2205.
 - ii. During harvesting and purification, a sign will be placed on the outside of the lab door informing others that these procedures are taking place and that access to 2205 is restricted to the person performing the procedures.
 - iii. Access for Cleaning, Maintenance, and Repair Personnel
As per facility Standard Operating Procedures (SOPs), laboratory staff is responsible for performing routine laboratory cleaning. To facilitate routine or emergency repairs or maintenance in room 2205, members of the Office of Research Facilities (ORF) have been authorized by the Biosafety Officer to enter the designated area. However, laboratory personnel working in the laboratory must secure all infectious material prior to allowing entry of **any** support personnel such as maintenance employees. All laboratory components (sinks, countertops, etc.) and equipment scheduled for repair or servicing will be thoroughly decontaminated by research personnel prior to initiation of the work. A "Certification that Property is Free from Hazards" NIH form 2683 will be affixed to equipment scheduled for repair prior to the

start of work. A staff member authorized to access the laboratory and who is familiar with the operation of the laboratory shall be present whenever maintenance or repair work is conducted.

4. Standard Operating Procedures

- i. This Standard Operating Procedure will be revised as necessary. Any revisions will be reviewed by the RML Biosafety Officer who will determine if approval by the RML Institutional Biosafety Committee is required prior to implementation.

5. Medical Surveillance

- i. All laboratory personnel must be enrolled in the relevant RML medical surveillance program.
- ii. Serological analysis is critical to diagnosis of laboratory-acquired chlamydial infections. In order to monitor and support the health of employees working with the organism, serum samples will be collected annually on a voluntary basis from those at highest risk of Chlamydia infection, including those performing harvesting and purification procedures. This serum will be archived at RML and only analyzed in case of a laboratory exposure or accident. In these instances additional serum specimens may also be collected for testing.

6. Personal Protective Equipment (PPE)

- i. Upon entry, staff will don gloves and a disposable laboratory coat. This is the minimum PPE to be worn in the laboratory.
- ii. During harvesting and purification of chlamydiae, disposable lab coats, gloves, and a liquid-aerosol-proof respirator (N100) are to be worn.
NOTE: Personnel must be properly fitted, trained in the use of respirators, and receive a medical evaluation before respirator assignment. See the RML Occupational Safety and Health Manager (Paul Carlson, X39431) prior to using a respirator.
- iii. All PPE will be removed prior to exiting the laboratory.

7. Training

- i. All personnel will be trained and instructed by the Laboratory Supervisor or designee prior to performing work in Room 2205. Part of the training process will include reading this SOP and signing the associated training certification.

8. Facility Access Control

- i. Access to room 2205 is controlled using a proximity card reader.

9. General Safety Procedures

- i. Centrifuge rotors are only to be opened in the Biological Safety Cabinet (BSC).

- ii. All steps other than centrifugation are to be done on ice in the BSC.
- iii. Good sterile technique is to be used throughout all procedures.

10. Spill Response

- i. See Appendix A.

B. Special Procedures for Experiments involving Tet^{TR} and *C. trachomatis* strain L2(25667R)

1. Any work with the Chlamydia servovars which cause the ocular disease trachoma will not be conducted at the same time as experiments in which tetracycline resistance is being introduced into *C. trachomatis* L2(25667R).
2. All interfacility transfers of tetracycline resistant *C. trachomatis* are coordinated and approved through the RML Biosafety Officer. The laboratory is responsible for preparing the agent in appropriate packaging, affixing appropriate shipping labels, markings and documentation in accordance with International Air Transport Authority (IATA) regulations. **Recipients must be authorized to possess these materials.**

C. Laboratory Procedures

1. Experiments involving Tet^R and *C. trachomatis* strain L2(25667R)
 - i. The following procedures will be performed in room 2205 according to established protocols: transformation or electroporation of purified plasmid DNA into *C. trachomatis*; storage of transformants/electroporant stocks in SPG.
2. Harvesting and Purification
 - i. Media, centrifuge bottles and tubes should be chilled and kept on ice. Add 1 ml HEPES (1M) per 100 ml HBSS to guard against radical pH changes. Make 1000 ml of HBSS (two 500 ml bottles) and 200 ml aliquots of 30, 40, 44 and 54% RenoCal-76 (Bracco Diagnostics) in water, prior to beginning this procedure.
 - ii. Place all bottle and tube discards into a water bath containing 2% or more of bleach and commercial powdered soap for several hours, before subsequent washing and autoclaving procedures.
 - iii. Decant culture media from infected cells (usually 20-24 150 cm² TC flasks for large preparation) into a bleach bath in BSC. Do 3-4 flasks at one time and move onto the following steps.
 - iv. Add 5 gm (or about 1/3 of 6 dram vial) of sterile 4 mm borosilicate glass beads (Kimble) to each flask. No need to chill the beads.
 - v. Add 9-10 ml ice-cold HBSS to each flask and tighten the cap.

- vi. Remove the cell monolayer into the HBSS by rolling the beads over the monolayer, first around the margins of the flask (10 X) then perpendicular across the bottom (10 X). Stack the flasks so that 3 or 4 are done at once.
- vii. Remove the HBSS with 10 ml serological pipette and place into a 250 ml polycarbonate centrifuge bottle (Corning) that is on ice. (Have 4 bottles pre-chilled in BSC).
- viii. Add more HBSS to the above flasks, tighten caps and repeat steps 4, 5 and 6 two more times. Pool the washes. Usually each centrifuge bottle will ultimately contain about 150-180 ml of pooled HBSS and cells (total washes from 5-6 flasks).
- ix. Tighten caps and discard flasks that have been rolled and washed 3Xs, beads and all. Then repeat above procedure with 3 or 4 more flasks until all HBSS washes are pooled into the centrifuge bottles. This procedure usually takes between 1-1.5 hours to complete for a large preparation. THEREFORE, IT IS ESSENTIAL TO KEEP THE POOLS COLD.
- x. Sonicate each centrifuge bottle for 20 seconds using a sterilized medium probe (B.Braun Labsonic U) on Low setting (65-100 amps). Place the probe halfway into the cell suspension (to avoid foaming but generating maximum effect). During the sonication process, slowly rotate the probe through the suspension at this level by moving the bottle in a circular motion. Repeat process one more time (2X total). If cavitation bubbles are present, the sonication process is working. SONICATION IS VERY IMPORTANT FOR OPTIMAL YIELDS – BE CAREFUL!
- xi. Centrifuge the sonicated cell suspensions at 270 x g (1500 rpm Beckman Coulter J-25) for 15 min (4°C) in a fixed-angle rotor. Discard any resulting pellets (small or no pellets indicate successful sonication).
- xii. Decant the supernatants into a similar set of centrifuge bottles, balance and centrifuge at 22,000 xg (13.5 K rpm in Beckman Coulter J-25 with a fixed-angle rotor at 4°C) for 30 min. Decant the supernatant into the bleach bath in the BSC.
- xiii. Resuspend the pellets in their respective centrifuge bottles in 3 ml of cold SPG (Sucrose Phosphate Glutamine) with a 6 inch 18 gauge sterile cannula attached to a 10 ml sterile plastic syringe. Start by washing the side of one bottle above the pellet 3 or 4 times with the SPG, then gently dislodge the pellet and aspirate it into the syringe. Gently break up the pellet into a smooth suspension by refluxing the pellet and SPG through the cannula against the bottom of the bottle 10-12X. Avoid splattering up the sides of the bottle. Repeat the process with the other bottles (3) and combine the suspensions into 1 bottle for a total of 12 ml of suspension. Keep bottles cold by standing in ice bucket throughout process.

- xiv. Wash the other 3 bottles with 10 ml of SPG each using a 10 ml serological pipette. Combine these washes with the suspension in the first bottle (on ice). Then wash these bottles with 3 successive 10 ml volumes of SPG (10 ml in the second bottle, transfer to the third and then to the fourth. Repeat 2 more times). Pool these washes with the original suspension to bring the total volume up to 72 ml. Discard the 3 washed bottles and sonicate the 72 ml suspension with a medium probe as described in STEP 10 above. Keep cannula and syringe sterile by placing in a sterile tube for safekeeping.
- xv. Place 12 ml of the suspension into each of 6 Ultra-Clear centrifuge tubes (25 x 89 mm, Beckman), wash the centrifuge bottle with 12 more ml of SPG and combine 2 ml of this wash with the suspension in each Ultra-Clear tube. Discard the bottle and underlay the suspension in each tube with 10 ml of 30% RenoCal, using the same cannula and syringe described in STEP 13. You must underlay RenoCal by using slow steady pressure without mixing. The RenoCal should exhibit a clear and clean separation from the suspension as the suspension is lifted upwards in the tube. (In total, 60 ml of 30% RenoCal are necessary for this procedure).
- xvi. Centrifuge the 30% gradients at 40,000 x g (24K rpm in Beckman Coulter Optimal L-70 Ultracentrifuge) in a swinging bucket rotor (SW-28, Beckman) for 30 min at 4°C.
- xvii. At this point, while the 30% gradients are spinning, make the density gradients (DG) for purifying EBs if desired. To make DG, set up a new cannula and syringe. Add 5 ml of 40% RenoCal into the bottom of 6 Ultra-Clear tubes each with the cannula. Underlay the 40% RenoCal with 12 ml of 44% RenoCal in each tube using a slow careful steady pressure. Finally, using the same cannula, underlay the 44% RenoCal with 8 ml of 54% RenoCal in each tube by the same fashion. On visual observation, each tube should have 3 distinct layers with two lines of separation, one at the 54-44% level (towards the bottom) and the other at the 44-40% level (towards the top). Carefully place the DG in a refrigerator until they are ready to be used (STEP 18). (In total, 30 ml of 40%, 72 ml of 44% and 48 ml of 54% RenoCal are necessary for this procedure).
- xviii. Using the same cannula and syringe from STEP 13, aspirate and discard the host cell layer at the 30% RenoCal interface. Then discard the rest of the supernatant, leaving the cell pellet at the bottom of each tube. With the same cannula and syringe resuspend all cell pellets into 2 ml SPG. Do this by adding 2 ml of SPG into first tube, resuspend the pellet by raking and gently refluxing against the bottom of the tube as before for 10 times. Avoid splattering. Transfer this suspension to the second tube and repeat the process and so forth (keep tubes on ice) until all six pellets are resuspended together as a smooth suspension. Place this suspension into a 15 ml conical centrifuge tube on ice. Wash all six Ultra-Clear tubes with successive three 2

ml washes and add to the original suspension on ice. Discard the Ultra-Clear tubes and sonicate the suspension for 4 two sec bursts using a sterilized microprobe. Do not let the suspension heat up. (If it feels warm, the sonication is excessive). At this point the suspension can be q.s. to desired volume (usually 15-20 ml of SPG), aliquoted into labeled tubes as a 30% preparation (0.2-0.1 ml with 1:10 dilution to determine IFU/ml) which contains all stages of chlamydial growth cycle with little cell debris and frozen (-80°C of liquid nitrogen). If purified EBs are preferred, then q.s. the suspension to 12 ml total with SPG and mix well (by vortex or inversion).

3. Preparation of EBs

- i. Briefly centrifuge the 15 ml tube containing 12 ml of sonicated 30% suspension in a regular centrifuge to about 1000 rpm and back down in order to lightly pellet material that was not broken up by the sonication process.
- ii. Layer 2 ml of the 30% suspension onto the top of each density gradient (6) by gentle pipetting using a serological pipette with no mixing. Centrifuge the density gradients at 40,000 xg in a swinging bucket rotor for 45 min at 4°C .
- iii. Following centrifugation, remove the flocculent, white band at the 44-54% RenoCal interface of each tube with a new sterile cannula and syringe by aspiration. Place each band in a chilled 50 ml Oak Ridge centrifuge tube (6) (harvest each band in 10 ml of volume in a circular manner around the circumference of the DG tube first, then move towards the middle of the band), add 20 ml of cold SPG to each tube (30 ml total), mix well by inversion and centrifuge (JA 25) at 31,000 x g (16 K rpm) in a fixed angle rotor (JA 25.50, Beckman) for 30 min.
- iv. Discard the supernatant, (take off the last little bit from each tube with the cannula and syringe used in STEP C3 after draining). Resuspend all 6 cell pellets into one 15 ml or 50 ml conical tube (depending on the volume) with cannula and syringe using 2 ml of SPG. Do three successive washes of the tubes as before and q.s. to desired total volume (usually 15-18 ml) with cold SPG. Discard the washed Oak Ridge tubes (5), vortex combined DG prep until a uniform suspension is attained. Aliquot suspension into labeled tubes (usually 0.2 ml aliquots). Make a 1:10 dilution (50 ul into 450 ul of SPG in one tube for IFU titration) and freeze all tubes at -80°C in a labeled box. A drop or two of the final preparation can be placed on a pre-warmed sheep blood agar plate and incubated for a day or two to check for sterility of the preparation. The DG preparation contains highly purified EBs with little RBs or cell debris. Discard the last Oak Ridge tube, do the IFU titration after at least overnight freezing to determine the IFU/ml of the preparation.

4. NOTES:

- i. All *C. trachomatis* non-LGV strains can be successfully grown at a MOI of 1-3.0, with DEAE-DEX treatment, a HBSS wash before infection with 2.5 ml

SPG-EBs/T150 flask and 2 hr at 37°C with gentle rocking. Refeeding with media containing 1 ug/ml of cycloheximide is the usual procedure to enhance inclusion growth and maturation.

- ii. LGV strains do not require DEAE-DEX treatment or a HBSS wash before infection. A MOI of 1-3.0 will work well with 2 hr of rocking at 37°C.
- iii. *Chlamydia psitticae* strains do not require DEAE-DEX or washing with HBSS prior to infection. A MOI of 1.0 is usually successful with 2 hr of rocking at 37°C.
- iv. *Chlamydia trachomatis* mouse pneumonitis strain (MoPn) is an exception to the above strains. An MOI of 1.0 or beyond may result in the destruction of the cell monolayer before maturation and harvesting. Generally a MOI of 0.5 to 0.75 is used for maximum production. Treatment with DEAE-DEX and washing is required with MoPn as described above for *C. trachomatis* non-LGV strains.

Appendix A

Spill Response

Any spill that results in overt or potential exposure to infectious materials must be reported to the laboratory supervisor and the Biosafety Officer. In ALL cases, you must report the incident to the Biosafety Officer or Occupational Health Nurse if someone is injured! Also, report the incident to the Biosafety Officer if you feel that it occurred as the result of poor practice or equipment failure.

Spills inside biological safety cabinets (BSCs):

- When a spill occurs, the operator should immediately cover the affected area with absorbent pads, tissues or towels to contain the incident and prevent further aerosolization.
- The absorbent material should then be soaked with Roccal.
- No work should then proceed within the cabinet for at least 30 minutes to allow the cabinet exhaust system to remove aerosols and give sufficient contact time for the germicide to act.
- At the end of this period, the operator should don a second pair of gloves and place all clean up materials (broken tubes, plates, absorbent towels, etc.) into a biohazard bag. All sharp objects or broken materials should be placed in Sharps receptacle prior to bagging.
- Any disposable plastic-ware or tubes that were within the cabinet at the time of the incident should be disposed of as above.
- Any other non-disposable items should be carefully decontaminated with Roccal.
- The entire cabinet interior (including grills at front and rear) should be wiped down with disinfectant.
- All biohazard bags should then be autoclaved.

Spills outside a biological safety cabinet:

- Small spills (e.g. drips) occurring within the laboratory and where there has been NO significant splashing or personnel contamination should be dealt with as follows –
 - Wearing the appropriate protective equipment (gloves, lab coat, etc.) cover the spill with disinfectant-soaked absorbent toweling or other material. Allow at least 30 minutes to give sufficient contact time for the germicide to act.
 - Using paper toweling, wipe up the spill working from the outside edges toward the center.
 - Be careful to avoid cuts with broken glass. To eliminate the potential for cuts use tongs, dust pan, or some other device for pickup and carefully discard into an approved sharps container.
 - Clean the spill area again with fresh disinfectant.

- Place all used materials into Biohazard bags and autoclave.
- Larger spills, or any spill which has caused extensive splashing or personnel contamination, should be dealt with as follows –
 - Avoid breathing in any aerosols, remove contaminated PPE, and immediately evacuate the laboratory.
 - Prevent others from entering the laboratory by placing a "DO NOT ENTER-BIOLOGICAL SPILL" sign on the door.
 - Report the spill to the Bio-safety Officer and give as much information as possible (nature of incident, agent involved, location, approximate volume of spill if known, your telephone extension).
 - Depending upon the agent involved and the nature of the incident, the Bio-safety Officer may elect to inspect the spill and to arrange clean up or may direct you to attend to it.
 - If approved by the Bio-safety Officer, and after at least 30 minutes have elapsed (to allow aerosols to disperse), don protective clothing (respirator, gowns, rubber boots and gloves), enter the laboratory and cover the bulk of the spill with disinfectant-soaked towels to assist in decontaminating the fluid. Pour disinfectant onto the towel and leave 30 minutes to neutralize the infectious material.
 - Mop up the spill using absorbent paper (try to avoid walking in the liquid) by working from the outside edge to the center. Carefully pick up any broken glass or other equipment. Place the waste into appropriate containers (sharps disposal containers, autoclave bags or tote-boxes) for autoclaving. Finally, decontaminate splashed equipment and furniture with disinfectant and paper towels. .

Spills in laboratory centrifuges

Failure of tubes and rotors during centrifugation can be the cause of significant aerosol production. If a failure is suspected during a centrifuge run –

- Immediately switch the machine off and allow the rotor to come to rest.
- DO NOT OPEN THE CENTRIFUGE.
- Avoid breathing any aerosols and evacuate the immediate area.
- Proceed as above for spills outside of a BSC.

If a spill is discovered after opening the centrifuge, or is visible through a transparent lid:

- Avoid breathing any aerosols and immediately close the centrifuge if open.
- Evacuate the immediate area and proceed as above for spills outside of a BSC.

Training Certification

I certify that I have viewed, read and understood the above requirements and procedures and agree to follow the standard operating procedure specified. I understand that failure to abide by the appropriate safety procedures may result in written reprimand and/or loss of privilege within this facility.

Signature

Date

Printed Name

SUPERVISOR'S APPROVAL

Harlan Caldwell, Ph.D.
Laboratory Supervisor
Chief, Laboratory of Intracellular Parasites

Date

IV-20

MEDICAL SERVICES FOR RESEARCHERS WORKING WITH TETRACYCLINE-RESISTANT CHLAMYDIA

I. Purpose – The purpose of this procedure is to provide individuals working with tetracycline-resistant *Chlamydia trachomatis* (*C. trachomatis*) with relevant counseling at their preplacement medical evaluation and appropriate medical care for potential occupational exposures to the organism.

II. Relevant OMS Procedure Manual Sections

- A. Exposure Control Plan. Chapter I Section 3
- B. Workers' Compensation. Chapter I Section 10
- C. Occupational Injuries and Illnesses. Chapter III Section 20
- D. Respirator Clearance. Chapter III Section 27
- E. Serum Storage Program. Chapter III Section 29
- F. Wound Care Guidelines. Chapter III Section 35

III. Attachments

A wallet card listing signs and symptoms suggestive of infection with *Chlamydia trachomatis* (Attachment I)

IV. Background Information

- A. *C. trachomatis* is an intracellular bacterium that is transmitted from person to person and causes infections of the genital tract and eyes. Three of its serovars (L1, L2 and L3) are associated with lymphogranuloma venereum (LGV) and proctocolitis. LGV is primarily an infection of lymphatics and lymph nodes. The infections are rarely asymptomatic.
 - 1. *C. trachomatis* gains entrance through breaks in the skin, or it can cross the epithelial cell layer of mucous membranes. The organism travels from the site of inoculation down the lymphatic channels and multiply within mononuclear phagocytes of the lymph nodes it passes.
 - 2. There is an initial inflammatory response to an infection. This is followed by tissue infiltration with lymphocytes, macrophages, plasma cells, and eosinophils. Eventually, a granuloma and small abscesses may develop.
- B. The *C. trachomatis* LGV strain [L2(25667R)] is used in the research. This strain lacks the cryptic plasmid and is highly attenuated (i.e., there is a 400 fold increase in its ID50 compared to the plasmid positive L2 parent).
- C. Diagnostic Testing
 - 1. A complement fixation test measures antibodies against group-reactive antigens. Virtually 100% of individuals with LGV have complement-fixing antibody titers of >1:16.
 - 2. A microimmunofluorescence test is a second serologic study that is more sensitive than the complement fixation test and can differentiate among

the various species of Chlamydia; however, it is not commercially available.

3. Nucleic acid amplification tests (NAATs) that target the cryptic plasmid for amplification will be of limited value, as the strain of *C. trachomatis* used in these experiments lacks the cryptic plasmid. NAATs that utilize transcriptase-mediated amplification of chlamydial ribosomal RNA (rRNA) may be useful. NAATs are considerably more sensitive than cultures and nearly as specific.

D. Treatment

1. Azithromycin 1 gm PO (single dose), and/or
2. Ofloxacin 400 mg bid PO or levofloxacin 500 mg daily for 7 to 14 days

V. Participation

- A. Federal employees with access to NIH laboratories containing tetracycline-resistant *C. trachomatis* are required to participate in this program.
- B. Non-federal workers with access to NIH laboratories containing tetracycline-resistant *C. trachomatis* are required to report work related injuries to the nearest OMS clinic and are eligible for evaluation and treatment of suspected occupational exposures as described in Sections VIII and IX.

VI. Identification of Eligible Workers – Principal Investigators working with tetracycline-resistant *C. trachomatis* are responsible for identifying individuals who enter their facilities and ensuring that they are enrolled in, and comply with, this program.

VII. Enrollment Evaluation

- A. Medical history
 1. The applicant is questioned regarding a personal history for illnesses, medications and allergies.
 2. The worker completes the initial Respirator Clearance questionnaire to determine whether or not there are any medical contraindications to the use of a respirator.
- B. Laboratory testing
 1. A baseline complement fixation assay for *C. trachomatis* is performed.
 2. A blood sample is obtained for serum storage.
- C. Counseling – workers are instructed to:
 1. Report all work related injuries and accidents (e.g., puncture injuries, splashes, spills).
 2. Initiate first aid measure at the workplace, following a potential exposure to a human pathogen.
 3. Be alert for the earliest clinical manifestations of infection with the specific *C. trachomatis* and promptly report signs or symptoms suggestive of an occupational infection to OMS.
- D. Supplies – workers enrolling in this program are provided with a wallet-sized card that identifies physical signs and symptoms suggestive of an infection with

C. trachomatis, and advice to prevent transmission to others and how to contact OMS for evaluation.

VIII. Post-Exposure Evaluation

- A. Worker's responsibility - in the event of a potential exposure to tetracycline-resistant *C. trachomatis* resulting from either a splash or a percutaneous injury, the worker:
 - 1. Immediately initiates first aid at the work site and notifies the supervisor of the occurrence.
 - 2. Reports the occurrence to the nearest OMS clinic, as soon as first aid has been completed. If the OMS clinic is closed, the worker pages the on-call OMS physician by dialing 301-496-1211.
- B. Supervisor's responsibility
 - 1. Assures any released biologically active material is contained and that the workspace is decontaminated properly.
 - 2. Alerts OMS of the occurrence and the need for immediate evaluation.
 - 3. Alerts the DOHS safety specialist of the accident and initiate an accident investigation.
- C. OMS clinician's initial responsibilities
 - 1. Confirms that DOHS safety specialist is aware of the incident and involved in the accident investigation.
 - 2. Consults with the supervisor as needed to clarify the significance of the accident.
 - 3. Utilizes standard (universal) precautions during the evaluation. They include:
 - a. Careful attention to hand hygiene before and after all contact with the injured worker and
 - b. Careful handling of potentially contaminated items.
 - 4. Administers first aid and determines whether any additional wound care is indicated. First aid is performed regardless of whether first aid was performed at the workplace, provided that the injury is reported within four hours of the occurrence.
- D. The OMS physician
 - 1. Obtains a history for the injury history, that includes at a minimum:
 - a. Circumstances just prior to the event such as the procedure, equipment, biologic material being used, and identities of others working in the area at the time of the accident.
 - b. How the injury occurred.
 - c. Laboratory equipment involved in the accident.
 - d. Quantity of fluid involved and any measures taken to minimize its infectivity before the accident.
 - e. Route of exposure (e.g., percutaneous, spill, aerosol)
 - f. Personal protective equipment utilized at the time of the injury.
 - g. First aid efforts initiated at the workplace including: the cleansing agent used, how it was used, and the duration of use.

2. Reviews the worker's current medical conditions and related treatment.
3. Reviews the worker's social history including: contacts at home, involvement in hobbies, and activities at home and work.
4. Consults with infectious disease specialists. Treatment decisions and plans for follow-up evaluation are made in consultation with the infectious disease specialists.
5. Obtains a blood sample for purposes of serum storage and possible future testing.
6. Records the incident is recorded in the normal OMS format and issues the appropriate Workers' Compensation paperwork.
7. Returns the worker to full duty.
8. Advises the worker to return to OMS every two to three days for two weeks to check the wound and draining lymph nodes and question the worker regarding symptoms suggestive of an occupational infection.

IX. Illness Evaluation

- A. If a worker with access to a lab containing tetracycline-resistant *C. trachomatis* lab develops a fever >100.4°F, conjunctivitis, a granuloma, abscess, ulceration, or lymphadenopathy suggestive of an occupational infection, the worker immediately notifies an OMS physician and his supervisor. Similarly, the worker notifies an OMS physician and his supervisor, if he develops severe pharyngitis, dyspnea or other respiratory symptoms following a potential aerosolized exposure while wearing insufficient personal protective equipment.
- B. Upon learning of the illness, the supervisor:
 1. Immediately alerts the OMS physician of the concern,
 2. Provides the OMS physician with contact information for the worker, and
 3. Investigates potential laboratory exposures in the preceding three weeks in consultation with the DOHS safety specialist.
- C. The OMS physician
 1. Obtains a medical history that includes at a minimum:
 - a. A description of the evolution of the physical findings,
 - b. The time of onset, progression, and severity of any related symptoms,
 - c. Treatment tried, if any, and its apparent effect,
 - d. Review of personal health history, medications, and allergies, and
 - e. Review of systems by organ system.
 2. Inquires about the worker's work experience for the past three weeks, including a detailed description of:
 - a. The individual's work responsibilities,
 - b. Work procedures followed,
 - c. Barrier protection and other safety practices used,
 - d. Equipment used,

- e. The work others are performing in the lab, and
- f. Any possible exposure to biologically active material.
- 3. Obtains a social history including a description of the worker's personal contacts at home and their health in the preceding three weeks, involvement in hobbies, and activities at home and work.
- 4. If not already done, speaks with the principal investigator to clarify his understanding of the work conducted in the laboratory in the past three weeks, potential occupational exposures, and the identities of others working in the lab.
- 5. Performs a targeted physical exam.
- 6. Consults with infectious disease specialists to determine a course of action and discusses the consultants' advice with the worker.
- D. Diagnostic testing and treatment
 - 1. Decisions for testing and treatment are made in consultation with the infectious disease specialists.
- E. Contact study – depending upon the circumstances of the case, consideration may be given to identifying contacts, evaluating and monitoring them for evidence of disease. See OMS Contact Study Guidelines for additional details.
- F. Follow-up
 - 1. Additional medical care is provided as clinically indicated and as recommended by the consulting infectious disease specialists.
 - 2. If the testing is suggestive of an occupationally acquired infection, the worker is provided with the applicable Workers' Compensation Claim forms.
- X. Periodic Review – this procedure is reviewed and revised annually, or as appropriate based upon relevant scientific discoveries.
- XI. References
 - A. Carlson JH, Whitmire WM, Crane DD, et al The *Chlamydia trachomatis* Plasmid Is a Transcriptional Regulator of Chromosomal Genes and a Virulence Factor. *Infect. Immun.* 2008 Jun;76:2273-2283.

Signs and Symptoms Suggestive of Infection with Tetracycline-Resistant *Chlamydia trachomatis* (*C. trachomatis*)

All illnesses involving the following signs and symptoms may be evidence of a work-related infection and must be reported immediately to your supervisor and an OMS physician.

1. A fever >100.4°F,
2. Conjunctivitis (redness of the white of the eye with or without pus or crusting)
3. A tender nodule or abscess (a red, raised bump),
4. Skin ulceration,
5. Tender swollen lymph nodes, or
6. Severe sore throat, cough or difficulty breathing following a possible respiratory exposure.

Precautions to Avoid Transmission to Others

If you develop conjunctivitis, a tender skin nodule or abscess, skin ulceration, or tender swollen lymph nodes, you should:

1. Avoid touching the area of concern.
2. Wash your hands frequently.
3. Avoid skin-to-skin contact with others.
4. Properly dispose of tissues and other items contaminated with body fluids.
5. Alert an OMS physician.

Monday through Friday from 7:30 to 5:00 EST call 301-496-4411.

Other hours: call the NIH page operator 301-496-1211.