

Ecology of Waterborne Pathogens

Instructor:

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Office hours: Wednesdays, 10:30-11:30 or by appointment. **Location:** McCarty Hall A, RM 2181

Course website: UF SAKAI e-learning portal <https://lss.at.ufl.edu/>

Log in using your gatorlink

Prerequisites: MCB3020 (Basic Biology of Microorganisms), or MCB3023 or MCB4203 (Bacterial and Viral Pathogens) or equivalent.

Course Objectives and Goals

The *central objective* of this course is to foster students' ability to solve problems related to microbiological quality and safety of drinking and recreational waters. The development of problem-solving skills will require an in-depth understanding of microbial ecology concepts and methodologies for identifying and characterizing pathogens and their behaviors outside of their human hosts.

The *goals* of this course are:

- 1) To define strategies used by human waterborne pathogens for colonization and survival within alternate hosts (plants, invertebrates) and non-host environments (biofilms, soils, rhizosphere).
- 2) To compare and contrast culture-, nucleic acid-based and immunological methods for detecting human waterborne pathogens.
- 3) To analyze environmental factors contributing to the rise of emerging and re-emerging environmentally-transmitted pathogens.
- 4) To foster the development of critical skills for the analysis of primary literature.

Course Description

The course consists of lectures presented by the instructor, several guest lectures presented by experts in a particular field, student-led on-line discussions of primary research literature and a case study of an outbreak. Seven Sakai sessions are scheduled for students to directly interact with the instructor and each other in a virtual classroom.

Student Evaluation and Grading

| SWS4307 | | SWS5308 | |
|---------------------|-----|------------------------|-----|
| First exam | 20% | First exam | 10% |
| Pre-exam questions | 10% | Pre-exam questions* | 10% |
| Second exam | 25% | Second exam | 25% |
| Case Study | 20% | Case study | 20% |
| On-line discussions | 10% | On on-line discussions | 10% |
| Final exam | 15% | Final exam | 25% |

* pre-exam questions are expected to demonstrate Application, Analysis and Synthesis (see Appendix A, below).

The **First Exam** will contain multiple-choice questions; some questions may require short answers/essays (3-5 sentences).

Pre-exam questions: before the **Second Exam**, each student is expected to prepare 20 multiple-choice exam questions (*guidelines for preparing pre-midterm questions are in Appendix A, below*). The instructor will also prepare and share 20 exam questions. Some of these questions in a modified form may be incorporated into the midterm exam.

There are two potential formats for the **Second** and **Final** exams:

- 1) proctored multiple-choice questions; some questions may require short answers/essays (3-5 sentences).
- 2) take-home open book; all questions will require answers in the form of an essay. *The take-home version is available only to those who attended at least 75% of lectures and accumulated at least 75% of the participation points available before the exam date.*

Each student may attempt either or both formats, however, only one submission per student will be graded.

Case Study:

There will be two potential formats of the case-study:

- a) self-paced with a web-based module.
- b) cooperative investigation.

For the students participating in the Cooperative Investigation:

The class will divide into pairs of Reporters and Investigators. Each team will consist of 4 people (2 reporters + 2 investigators).

The Reporters will:

- a) Conceive a scenario of an outbreak and discuss it with the course instructor (example: An outbreak of salmonellosis associated with the consumption of raw shellfish on a cruise ship).
- b) Following the discussion of the outbreak scenario with the instructor or a TA, the Reporters will post a “news release” of the outbreak on the message board of Distance Education website (e.g. as relates to the example above: “Fifteen people on a cruise ship XX reported vomiting and diarrhea ten days after leaving from the port of Miami”).
- c) Will specifically address the questions from the Investigators (please see below).

The Investigators will:

- a) Ask 5 specific questions to narrow down the epidemiology of the outbreak, it will be the responsibility of the Reporters to provide accurate answers to the questions
- b) Within the following 2-3 weeks, the Investigators will make specific requests to culture samples on detection media and then develop primers or probes for the nucleic acid-based identification of the pathogens. The Investigators will develop specific experiments (including controls) to identify the cause of the outbreak.
- c) Upon completion of the case study, the Investigators will present a short (10-15 minutes) power point presentation which explains the rationale for the experimental approach.

The Case Study will take 5 weeks, it will take place outside the classroom, questions and answers will be posted by the investigators and reporters on the message board. The Investigators will make only one post with request per week, and the Reporters are expected to attend to the request within a week.

The case study will be evaluated based on technical accuracy (i.e. description of symptoms, correct use of culture-based and nucleic acid-based tools for identifying microbes) and progression of logic (i.e. as more information becomes available on the causal organism, the techniques should become more focused). Professional demeanor is expected of all participants. The message board will be monitored and moderated daily by the instructor and the TA.

A sample case study from a previous year is attached (please see Appendix B).

The final grade is a reflection of the individual student’s mastery and comprehension of the subject material presented during the semester. The grading will not be based on a bell curve. UF Grading policies are followed, more information on the UF policies is here:

<https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx>

| Passing Grade | A | A- | B+ | B | B- | C+ | C | C- | D+ | D | D- | E |
|---------------|-----|------|------|-----|------|------|-----|------|------|-----|-----|----------------|
| Grade Points | 4.0 | 3.67 | 3.33 | 3.0 | 2.67 | 2.33 | 2.0 | 1.67 | 1.33 | 1.0 | .67 | 0.66 and below |

Participation. These points are not automatic and will be assigned based on the following criteria: (1) participation in class and the on-line discussions. Each student is expected to meaningfully contribute to discussions, either in class or on-line. In on-line discussions, a post that restates somebody else’s opinion or simply concurs with another student’s opinion will not be accepted for credit. (2) timely (within 36 hrs of the post) responses to your peers’ specific questions. To receive full credit, each student is expected to develop thoughtful posts in response to at least 70% of the questions posted by the instructor/discussion leader or in Sakai.

Topical Outline

See attached

Policy on Class Attendance. It is the instructor’s expectation that each student will keep up with posted lectures, readings and other assignments. It is the student’s responsibility to adhere to the agreed upon timeline. While attendance of lectures is not mandatory, students who missed over 25% of lectures will not be eligible to take the “take-home” versions of the second exam and the final (see above)

Policy on Make-up examinations and Assignments. Late assignments will not be accepted for credit. Make-up examinations (midterm or final) will be administered only to the students who were not able to complete the exam due to a well-documented emergency or an illness. Pre-midterm questions can be submitted ahead of the deadline if an absence from class is anticipated.

Course Format: Web-based lectures and weekly web-based discussions via UF Distance Education Portal (Sakai). Seven Sakai chats are scheduled and students are required to participate in 5.

Accommodations for Students with Disabilities:

Students requesting classroom accommodation must first register with the Dean of Students Office. The Dean of Students Office will provide documentation to the student who must then provide this documentation to the Instructor when requesting accommodation.

Required Text and Reading Assignments

Environmental Microbiology. Second Edition

By Maier et al. Academic Press. 2009.

ISBN: 978-0-12-370519-8

Additional reading assignments will be distributed to the course participants by email.

Instructor's lectures are loosely based on the assigned readings from the textbook(s) and the research articles. Students are strongly encouraged to keep up with the assigned readings. All class participants will be prepared to engage in discussions of the reading assignments.

All PowerPoint presentations, instructor's lecture notes and students' presentations will be distributed to all course participants via the course website:

Academic Honesty:

In Fall 1995, the University of Florida student body enacted a new honor code and voluntarily committed itself to the highest standards of honesty and integrity. When students enroll at the university, they commit themselves to the standard drafted and enacted by the students.

Preamble: In adopting this honor code, the students of the university of Florida recognize that academic honesty and integrity are fundamental values of the university community. Student who enroll at the university commit to holding themselves and their peers to the high standard of honor required by the honor code. Any individual who becomes aware of a violation of the honor code is bound by honor to take corrective action...

The Honor Code: *We, the members of the University of Florida community, pledge to hold ourselves and our peers to the highest standards of honesty and integrity.*

On all work submitted for credit by students at the University of Florida, the following pledge is either required or implied: *On my honor, I have neither given nor received unauthorized aid in doing this assignment.*

UF Counseling Services:

Resources are available on-campus for students having personal problems or lacking clear career and academic goals. These resources include 1.) University Counseling Center, 301 Peabody Hall, 392-1575, personal and career counseling; 2.) SHCC Mental Health, Student Health Care Center, 392-1171, personal counseling; 3.) Center for Sexual Assault/Abuse Recovery and Education (CARE), Student Health Care Center, 392-1161, sexual assault counseling; and 4.) Career Resource Center, Reitz Union, 392-1601, career development assistance and counseling.

Software Use:

All faculty, staff and students of the University are required and expected to obey the laws and legal agreements governing software use. Failure to do so can lead to monetary damages and/or criminal penalties for the individual violator. Because such violations are also against University policies and rules, disciplinary action will be taken as appropriate.

How to write questions for multiple choice exams.

Based on: <http://cit.necc.mass.edu/atlt/TestCritThink.htm>

Here is some background on learning, which will help you think about strategies for developing your multiple choice questions.

There are five cognitive Knowledge Levels:

1. **Recalling memorized information.** Involves remembering specific facts and/or complete theories, but all that is required is the restatement of memorized information. Represents the lowest level of learning outcomes in the cognitive domain.

Learning objectives at this level: know common terms, know specific facts, know methods and procedures, know basic concepts, know principles. Question verbs: Define, list, state, identify, label, name, who? when? where? what?

2. **Comprehension.** The ability to a) grasp the meaning of material, b) interpret original data, c) translate material from one form to another (words to numbers), d) estimate trends (predicting consequences or effects). Goes one step beyond the simple memorization of material, and represent a level of understanding.

Learning objectives at this level: understand facts and principles, interpret verbal material, interpret charts and graphs, translate verbal material to mathematical formulae, estimate the future consequences implied in data, justify methods and procedures. Question verbs: Explain, predict, interpret, infer, summarize, convert, translate, give example, account for, paraphrase x?

3. **Application.** The ability to use learned material in new and concrete situations. Applying rules, methods, concepts, principles, laws, and theories. Learning outcomes in this area require a higher level of understanding than those under comprehension.

Learning objectives at this level: apply concepts and principles to new situations, apply laws and theories to practical situations, solve mathematical problems, construct graphs and charts, demonstrate the correct usage of a method or procedure. Question verbs: How could x be used to y? How would you show, make use of, modify, demonstrate, solve, or apply x to conditions y?

4. **Analysis.** The ability to break down material into its component parts, identify parts, analyze relationships between variables, recognize hypotheses and rationale for studies. Learning outcomes here represent a higher level than comprehension and application because they require an understanding of both the content and the structural form of the material (e.g. rationale and hypotheses).

Learning objectives at this level: recognize unstated assumptions, recognizes logical fallacies in reasoning, distinguish between facts and inferences, evaluate the relevancy of data, analyze the organizational structure of a work (art, music, writing). Question verbs: Differentiate, compare / contrast, distinguish x from y, how does x affect or relate to y? why? how? What piece of x is missing / needed?

5. **Synthesis.** The ability to critically evaluate and synthesize information, and to integrate new information into the existing framework of knowledge. How does x relate to y? why? how?

What piece of x is missing / needed? Learning outcomes in this area stress creative behaviors, with major emphasis on the formulation of new patterns or structure.

Learning objectives at this level: write a well organized paper, give a well organized speech, write a creative short story (or poem or music), propose a plan for an experiment, integrate learning from different areas into a plan for solving a problem, formulate a new scheme for classifying objects (or events, or ideas). Question verbs: Design, construct, develop, formulate, imagine, create, change, write a short story, etc.

Practical Suggestions for Writing Multiple-Choice Questions

General Suggestion

Do not write the test in one day. Spread the work out over time. Questions demanding high-level thinking take longer to craft-professional item writers often write only 3 or 4 per day. Write one or two questions after each class, so it becomes a simple matter of assembling them into an exam.

Be fair! Make sure that questions represent concepts that were extensively discussed in class. Avoid relying on obscure information that appears as footnotes.

Avoid True/False questions. Rarely a scientific statement is absolutely correct or absolutely false.

Writing the stem (“body of the question”)

1) Phrase stems as clearly as possible -- confusing questions can generate wrong answers from students who do understand the material. For example, a confusing stem like: "According to Tuckman's model, groups develop through several stages over time. Furthermore, it contradicts Poole's activity-track model which has groups switching among several different linear sequences. Which of the following is not one of the stages identified in Tuckman's model?" could be cleaned up to read: "Tuckman's model of group development includes: [Select all that apply]"

2) Avoid verbosity in the stem. Some think extraneous details make a question more complex. However, they most often just add to the reading time. This reduces the number of questions you can put on a test, therefore reducing the reliability of the test. For example, in the Tuckman question above, the information on Poole's model had nothing to do with the information sought by the question.

3) Include any language in the stem that you would have to repeat in each answer option. For example, a stem such as "Biology is defined as the scientific study of:" keeps you from having to repeat "is the scientific study of" at the beginning of each option.

Answer Options

1) Avoid lifting phrases directly from text or lecture. This becomes a simple recall activity for the student. Use new language as frequently as possible.

2) Most literature recommends writing the correct answer before writing the distracters. This makes sure you pay enough attention to formulating the one clearly correct answer.

3) Answer options should be about the same length and parallel in grammatical structure. Too much detail or different grammatical structure can give the answer away.

For example, the specificity and grammatical structure of the first option here are dead giveaways:

The term "side effect" of a drug: a) refers to any action of a drug in the body other than the one the doctor wanted to drug to have. b) is the chain effect of a drug. c) additionally benefits the drug.

4) Limit the number of answer options. Research shows that three-choice items are about as effective as four-choice items. Four choice items are the most popular, and never give more than five alternatives.

5) *Distracters must be incorrect, but plausible.* If you can, include among the distracters options that contain common errors.

6) To make distracters more plausible, use words that should be familiar to students.

7) If a recognizable key word appears in the correct answer, it should appear in some or all of the distracters as well. Don't let a verbal clue decrease the accuracy of your exam.

For example, someone with no biology background would not have to think very hard to make a correct guess on this question:

Every organism is made of cells and every cell comes from another cell. This is the:

a) Relativity Theory b) Evolution Theory c) Heat Theory d) Cell Theory

8) Help students see crucial words in the question.

For example: "Which of the following is NOT an explicit norm?" Likewise, when you ask a similarly-worded question about two different things, always highlight the difference between the questions.

9) It is often difficult to come up with 3 or 4 plausible distracters, and teachers will sometimes add some that are not plausible, or even humorous. *Be careful.* If it is too easy to eliminate one or two options, then the question loses much of its measurement value. If energy or time is limited and you must come up with one more distracter, consider either offering a true statement that does not answer the question and/or a jargon-ridden option that is meaningless to someone who understands the concept.

Examples

These two pathogens are most likely to grow well in refrigerated foods.

- a) *Vibrio cholera.* and *Salmonella enterica*
- b) *Campylobacter spp.* and *Listeria monocytogenes*
- c) *Vibrio vulnificus* and EHEC *E.coli*
- d) *Campylobacter spp.* and *Legionella pneumophila*

vs

These two pathogens are most likely to grow well in refrigerated foods.

- a) *Vibrio cholera.* and *Salmonella enterica*
- b) *Campylobacter spp.* and *Listeria monocytogenes*
- c) *Vibrio vulnificus* and my mother-in-law
- d) Typhoid Mary and *Legionella pneumophila*

10) Use Rarely:

Extreme words like "all," "always" and "never" (generally a wrong answer). Vague words or phrases like "usually," "typically" and "may be" (generally a correct answer).

"All of the above" - eliminating one distracter immediately eliminates this, too.

"None of the above" - use only when the correct answer can be absolutely correct, such as in math, grammar, historical dates, geography, etc.. Do not use with negatively-stated stems, as the resulting double-negative is confusing. Studies do show that using "None of the above" does make a question more difficult, and is a better choice when the alternative is a weak distracter.

Some Techniques for Writing Multiple-Choice Items that Demand Critical Thinking

1) Premise - Consequence Students must identify the correct outcome of a given circumstance.

Example: If nominal gross national product (GNP) increases at a rate of 10% per year and the GNP deflator increases at 8% per year, then real GNP: a) Remains constant. b) Rises by 10%. c) Falls by 8%. d) Rises by 2%.

2) Analogy. Students must map the relationship between two items into a different context.

Example:

EDTA:nuclease is as

- a) protease inhibitor: protease
- b) antibiotics: *Salmonella enterica* DT104
- c) DNA:nuclease
- d) cholera toxin:protease

Bdellovibrio:*E.coli* is like

- a) *Giardia*: epithelial cells of human gut
- b) *Cryptosporidium*: epithelial cells of human gut
- c) *Bdellovibrio*: *Bacillus spp*
- d) *Vibrio cholera*:*Daphnia*

3) Case study. A single, well-written paragraph can provide material for several follow-up questions.

Example:

To begin characterization of bacteria in your water/soil sample, you chose to enumerate bacteria on a detection medium called "ZXZ". The medium contains lactose and a pH indicator. This medium will be most useful for:

- a) identification of pathogenic *E. coli* associated with humans and other mammals
- b) *all* bacteria that produce acid as a result of lactose utilization
- c) those bacteria that are unable to utilize lactose in acidic pH
- d) only bacteria that cannot use lactose, but produce acid

Examples of questions that will not be accepted for full credit for the following reasons:

Which one is not a cause of waterborne illness?

- a) viruses
- b) bacteria
- c) protozoa
- d) antigens

Reason: This question is too basic.

Public access to land with a high potential for public exposure shall be restricted for _____ after application of biosolids.

- a) 30 days
- b) 6 months
- c) 1 year
- d) 2 years

Reason: this question is a basic recall-type question

The ability to digest lactose, often used in selective media, definitively suggests which of the following for enteric bacteria?

- A) The bacteria is an anaerobe
- B) The bacteria was isolated from soil samples
- C) The bacteria has evolved to survive in an animal hosts
- D) The bacterium is a K strategist

Reason: The stem, the answer and distracters contain mistakes. Distracters are poorly chosen.

Why is soil important to the microbiologist

- A) Drug resistant bacteria commonly occur in soils
- B) Pathogens are predated upon by soil inhabitants
- C) Soils are nutrient rich environments in which many bacteria thrive
- D) Soils hold moisture which induce bacteria to rapidly divide

Reason: the stem and the answers are meaningless. The question was not designed to test anything discussed in the class.



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Waterborne Pathogens 4307/5308 CASE STUDIES

Case Study 2. Hospice outbreak

Max Teplitski

1/25/2009 22:38

Philadelphia PA, Hospice care center investigated after 42 patients complain of chronic diarrhea, vomiting and flu-like symptoms since a plumbing repair on Jan. 7. Four nurses were sent home on Jan. 22, complaining of debilitating nausea and confusion. Two children have also developed flu-like symptoms since they began spending the day with their grandparents at the center since Jan. 10.. Workers responsible for recent plumbing repairs have not been available for comment. Entire kitchen dish washing staff has returned today claiming to feel much better after missing work, however, complain of nausea after todays (1-26) shift. Three of the forty-two elderly patients are being transported to ICU for close monitoring

1/26/2009 19:52

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Two out of the three elderly patients in ICU have died of pneumonia, the third patient is responding to treatment and has stabilized. Four more patients admitted to the hospital with body temperatures over 103F. Twenty other elderly went home to family complaining of fatigue, headache and nausea. The remaining patients have no apparent symptoms and records indicate they have no medical conditions. The nurses still feel tired and they cough frequently during smoke breaks. The grandchildren have recovered from flu-symptoms. Kitchen staff are better, however, complain of confusion.

1/29/2009 19:34

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Based on the case details, we hypothesize that the agent causing this outbreak is Legionella. Thus, we ask the reporters to please collect the following information to test this hypothesis: 1. How many of the nurses, kitchen staff & patients who have been affected are smokers? 2. Are the patients who have been affected suffering from other conditions that may have impaired their immune system or lung function, such as cancer, diabetes or kidney problems? 3. What was the nature of the plumbing

repairs? Were any water tanks, especially hot water reservoirs, involved? 4. Have any other people in the households of affected individuals developed any symptoms? 5. Collect phlegm samples & culture on Buffered Charcoal Yeast Extract (BCYE) agar supplemented with L-cysteine and iron. Plates should be incubated for 5 days at 37°C. 6. Collect swabs from just inside all faucets used by all affected patients, nurses and the kitchen staff. Wash swabs using water collected from the same faucet to generate liquid samples, and plate these on supplemented BCYE plates as well. 7. As negative controls for the culture plates, please plate aliquots of all samples on MacConkey agar, and incubate as above. 8. Collect urine samples from all affected patients & use any commercially available Legionella Urinary Antigen Test Kit (e.g., Binax), including both the positive & negative controls specified by the manufacturer (most kits include these controls in the kits).

2/5/2009 23:07

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1. After surveying the smoking habits of the infected patients, nursing and dishwashing staff we can report the following: nine resident patients smoke, four nurses smoke and none of the five dishwashers smoke. 2. The first two resident patients who died are not suffering anymore, they had suffered from lung cancer. Twenty-eight of the patients were reported healthy, according to routine geriatric physical examination and blood chemistry profiles. Seven of the patients who went home, were known to have elevated kidney enzymes. The third patient originally admitted into the ICU has emphysema and was sent home on oxygen. The four later admitted to the hospital are considered to have impaired immune systems. Of those four, one patient who was receiving chemotherapy has died, another with impaired renal function went into acute kidney failure and recently passed. The other two are diabetic and now listed in stable condition. The children had no known medical conditions, however their grandparents are smokers and they once got Giardia, from their neighbors dog. The kitchen and nursing staff have no known medical condition. 3. The plumbing repair was a main-supply line break after a landscape crew planted a tree in the wrong location. To replace the pipe, the soil was removed and a fifteen-foot piece installed and backfilled with top-soil from a nearby site. Water tanks, hot water heaters, shower heads and drinking water are supplied by this source. One of the landscapers has been admitted, three days after planting the trees, he applied mulch and watered in the trees on a hot day. The landscaper tested positive for tuberculosis. 4. None of the households of the infected individuals have reported symptoms. 5. Less than fifty percent of the individuals produced phlegm, when available phlegm

samples were collected from the patients complaining of adverse symptoms. Samples were plated on BCYE with L-cysteine and iron supplements. These samples were subsequently incubated in a slightly humidified atmosphere at 37°C for five days. The following inspection of the inoculated BCYE plates showed predominately white-grey and some blue-grey colonies. The colonies look crystallized and some are round and convex in shape. Some of the plates also showed growth of a few chalky white colonies that are also round in shape. Colonies with irregular margins and a smooth mucoid convex were also reported. Colonies with irregular margins are developing papilla on the surface. Gram-positive, lancet-shaped cocci elongated cocci with a slightly pointed outer curvature and glistening appearance. 6. Swabs were also collected and put under the same incubation conditions as the plates with the phlegm samples. The swabs were taken along the edge of the faucets and also just on the inside of the faucets and along the faucet aerators. Upon inspection of the plates, three different types of colonies were identified. There are some dark yellow colonies that are round and convex in shape. There are also colonies that have irregular edges and an opaque white color. The third type of colony identified has white-grey color and is circular with a convex shape. 7. MacConkey agar with and without glucose, with lactose as a carbon source and inhibitory for gram positive organisms. Various enteric bacteria were found growing in abundance. *Streptococcus pneumoniae* was abundant with phlegm samples. Isolates of the following were also found: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Salmomella typhi* was found in the kitchen. There were colonies of *Shigella boydii*, *Pseudomonas aeruginosa*, *Serratia marcescences*, *Proteus vulgaris* and *Yersinia enterocolitica*. . Lactose utilization is was detected by the indicator dye neutral red in the med, turning red below pH 7 and is colorless above pH 7. 8. Urine samples were obtained from 40 patients of the original 42 affected. Samples were also obtained from the 2 children who have fallen ill. These samples were tested using the Bifax NOW(R) Legionella Urinary Antigen Test Kit. Urine samples were concentrated for higher sensitivity. 28 of the patients (68%) tested positive after 15 minutes, and weak bands were observed. It should be noted that cross-reactivity between serotypes is known to occur, and weakly positive bands can indicate false positives. It is also reported that Pneumonia caused by *Str. pneumoniae* gave very weak positive bands after a reaction time of 15 min.

2/9/2009 21:05

Because the data collected so far are providing mixed information, some of which supports our hypothesis of Legionella, and some of which indicates another

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organism, such as *Streptococcus pneumoniae* (both the gram-positive organisms found in the cultures & the mixed antigen test), we are requesting that the investigators perform a PCR test, using the conditions described below, on both the phlegm and water samples previously collected. Based on a Primer BLAST search of the NCBI nr database, this primer set targets a large number of *Legionella* variants, but does not amplify non-*Legionella* organisms, so bands appearing at approximately 1000 bp on a gel may be presumed *Legionella*. Target gene: hsp60 Forward primer: TGTTGAAGGTCACAAGGCAG Reverse primer: CACCGGCAACAATACCTTCT Recommended PCR conditions: initial 10 minutes @ 95°C 25 cycles: 1 minute @ 95°C 1 minute @ 56°C (based on melting temperatures for the primers of 59.87°C & 59.99°C 1 minute @ 72°C (based on product length of 944 bps) final 10 minutes @ 72°C

Max Teplitski

2/10/2009 20:08

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Investigators: please specify what should be added to the PCR reactions -- phlegm? bacterial cells off selective agar? purified DNA? Please identify positive, negative controls. If kits are involved in any of these steps, please identify suppliers. BTW, *Legionella* is a gram negative bacterium

2/11/2009 13:23

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Please use a MoBio UltraClean Microbial DNA Isolation Kit (<http://www.mobio.com/products/productdetail.php?pid=14>) to extract DNA from representative colonies of all the types found on the BCYE agar plates. In addition, please extract DNA from the previously collected phlegm and water samples using the MoBio UltraClean BloodSpin DNA Isolation Kit (phlegm, <http://www.mobio.com/products/productdetail.php?pid=16>) and MoBio UltraClean Water DNA Isolation Kit (water, <http://www.mobio.com/products/productdetail.php?pid=68>). As controls, please obtain pure cultures of any *Legionella* strain (positive control), *Streptococcus pneumoniae* (negative control, but may be the gram-positive organism that was noted earlier) and any *E. coli* strain (negative control). Extract DNA from these cultures using the same UltraClean Microbial DNA Isolation Kit as mentioned above. Aliquots of all these DNA extracts (colonies, phlegm, water, controls) should be run using the same master mix with the PCR conditions requested previously. All PCR products should be run on a 1.2% agarose gel with TAE buffer, along with a molecular ladder that includes a 1000 bp band. A correctly-sized product will appear at approximately 1000 bps (944 bps).

2/11/2009 18:02

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The MoBio UltraClean Microbial DNA Isolation kit was used to extract DNA from both types of representative colonies from the BCYE agar plates, and PCR was performed under the specified

conditions. Two of the three types of colonies from which DNA was extracted showed a distinct band at 944 bps. Phlegm samples were obtained from only 18 of the 42 surviving patients. DNA was extracted from phlegm from samples using the MoBio UltraClean BloodSpin DNA Isolation Kit, and PCR was performed under the specified conditions. Faint bands at approximately 944 bps were observed in 16 of the gels. DNA was extracted from the water samples collected from the faucets using the MoBio UltraClean Water DNA Isolation Kit, and PCR was performed under the specified conditions. The PCR detection failed - this may have been because the amount of DNA extracted from these samples was below detectable levels. Neither negative control showed a product at 1000 bps, while the positive control showed a distinct, dark band at 944 bps.

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