ENV H 433: Environmental & Occupational Sampling and Analysis - Microbial Contaminants

Winter Quarter, 2021

MWF 8:30-10:20 AM

INSTRUCTOR

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TEACHING ASSISTANTS

Tyler Gerken: tgerken@uw.edu
Alexandria Vingino: avingi@uw.edu

OFFICE HOURS: By Appointment

COURSE LOCATION: Remotely via Zoom (https://washington.zoom.us/j/94561028968?pwd=K3hQOXRFa0YzUmZYSVRlejZiZRklKdz09). Meeting ID: 945 6102 8968; Password: 433. (Sign in to UW Zoom (UW NetID required). Zoom classes will be recorded. Synchronous participation is required. Please let me know if you are in a different time zone.

COURSE DESCRIPTION

This course will review the sampling and analysis of microbiological contaminants in water, air, and on surfaces. Topics covered will include legal considerations, sampling and experimental design, routes of exposure, sources of exposure, standard methods, QA/QC, and data management. This course will be of use for public health professionals, microbiologists, civil and environmental engineers, and environmental scientists.

COURSE OBJECTIVES:

At the conclusion of this class, students should be able to:

- Recognize the various microbial contaminants in environmental and occupational settings
- Distinguish between the methods for sample collection and processing of microbial contaminants in different environmental and occupational exposure situations
- Categorize the methods for detection of microbial contaminants for different environmental and occupational exposure situations
- Formulate an appropriate experimental design for assessing environmental and occupational exposures to microbial contaminants
- Describe the advantages and disadvantages of using indicator organisms in environmental and occupational exposure assessment
- Identify the various indicator organisms in different environmental and occupational exposure situations
- Explain basic chemical and bio-safety laboratory precautions
- Describe quality assurance and quality control (QA/QC) procedures used in conducting environmental microbiology research
- Analyze, report, and manage scientific data related to environmental and occupational health sciences
- Recognize the importance of the legal and regulatory framework related to environmental and occupational exposures to microbial contaminants
Critically evaluate papers in the scientific literature and identify strengths and weaknesses of the science article.

TEXTS AND REFERENCES

There is no required text for this course. Assigned readings and course materials will be available on the course webpage. The following texts are recommended references for this course:

- Multiple-Tube Fermentation Technique (9221)/Total Coliforms

- EPA Method 1604: Total Coliforms and *E. coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)
  [https://nepis.epa.gov/Exe/ZyPDF.cgi/P1002D57.PDF?Dockey=P1002D57.PDF](https://nepis.epa.gov/Exe/ZyPDF.cgi/P1002D57.PDF?Dockey=P1002D57.PDF)

- Difco™ & BBL™ Manual – online

- EPA 2012 Recreational Water Quality Criteria Fact Sheet

COURSE DESIGN:

The course will begin with lectures, which will be covered in an exam on Jan. 15, 2021. Then students will read about six laboratory experiments. Class will be held remotely via Zoom, MWF between 8:30-10:20am. Students must be in the classroom and ready to go before class starts at 8:30 AM. Each week, starting the week of January 20, students will look up research papers that use the method featured in that week's laboratory exercise. The paper must be written in English and published within the last 5 years. The paper needs to be posted to the Canvas site [first posting is Jan. 14, 2021] and each student will briefly present their papers on Monday, Wednesday, or Friday morning. Major points should be written out, not read from the paper [See Below]. The final exam will be given on March 10, 2021.

GRADING

- **First Exam (25%)**: Students will be examined for their mastery of the material presented in the introductory lectures during weeks 1 and 2. The exam will consist of approximately 20 questions and the format will be multiple choice, short answer, and true/false. The first exam will be closed book and given on Jan 15, 2021.

- **Weekly Quizzes (10%)**: In preparation for the 'experiment', reviewing the Youtube videos are required before the first day of class for each laboratory experiment. Weekly quizzes will assess your understanding of the background knowledge, theory, and experimental setup for each of the laboratory experiments conducted.

- **Weekly Problem Sets (10%)**: Students will have a weekly problem set for each laboratory experiment. Students will need to read the laboratory protocol and answer any questions about what will normally be done. The final will be based on the experimental setup, protocol, and the problem sets. Data sets will be provided to students on the first day of class for that lab. **Homework is due by 5pm, submitted via Canvas the day before the 3rd day of the lab**, and the class will go over the homework the following class period.
  - **Ex: Lab 1: MPN multiple tube fermentation timeline**:
    - Data sets will be given to students on Jan. 22nd
    - Homework problem set is due on Canvas by 5pm on Jan. 26th
    - Homework will be reviewed during class on Jan. 27th

- **Participation in class (20%)**: Answering questions in class and general participation will be noted as well as giving critical evaluations on papers in the scientific literature with identification of its strengths and weaknesses. Please note that each student will present 1 paper each week for 6 weeks. In addition, a minimum of 8 questions asked by each student about another student’s presentation is required to receive 100% for participation. **Papers need to be selected on Canvas by noon on the Thursday, the week before the presentation**. The first lab is January 20, but the first lab with papers will be
Jan 22 meaning the paper for presentation needs to be on Canvas by noon January 14, 2021. Each student needs to select a unique paper that is written in English and peer reviewed. It must use the method used the week of lab and must be on bacteria.

- **Final Exam (35%)**: Closed book in class. The final exam will be given on **March 10, 2021**, the week before finals week.

**LABORATORY POLICIES**

- Arrive on time and be ready to start right at 8:30 AM. This is critical. **Attendance will be taken.**
- **Weekly quizzes will begin at 8:30 and will take ~15 minutes.**
- Turn in assignments on time. Late assignments will be marked down.
- Come to class prepared (keep up with the readings).
- Be courteous
- **ASK QUESTIONS!**

**IMPORTANT CLASS POLICIES**

**Land Acknowledgment**

We honor the people on whose land we are guests because that is the protocol for this area. Washington state is home to 29 federally recognized and five unrecognized tribes. Including a land acknowledgment statement such as the one written below demonstrates our collective humility and respect for the original caretakers of the land:

"The University of Washington acknowledges the Coast Salish people of this land, the land which touches the shared waters of all tribes and bands within the Duwamish, Suquamish, Tulalip and Muckleshoot nations."

**Diversity Statement**

Diverse backgrounds, embodiments and experiences are essential to the critical thinking endeavor at the heart of University education. In SPH, students are expected:

1. To respect individual differences, which may include, but are not limited to, age, cultural background, disability, ethnicity, family status, gender, immigration status, national origin, race, religion, sex, sexual orientation, socioeconomic status and veteran status.
2. To engage respectfully in the discussion of diverse worldviews and ideologies embedded in course readings, presentations and artifacts, including those course materials that are at odds with personal beliefs and values.
3. To encourage students with concerns about classroom climate to talk to their instructor, adviser, a member of the departmental or SPH EDI Committee, the Assistant Dean for EDI, or the program’s director.

**Access and Accommodation**

Your experience in this class is important to us. If you have already established accommodations with Disability Resources for Students (DRS), please communicate your approved accommodations to me at your earliest convenience so we can discuss your needs in this course.

If you have not yet established services through DRS, but have a temporary health condition or permanent disability that requires accommodations (conditions include but not limited to; mental health, attention-related, learning, vision, hearing, physical or health impacts), you are welcome to contact DRS at 206-543-8924 or uwdrs@uw.edu or [http://depts.washington.edu/uwdrs/](http://depts.washington.edu/uwdrs/). DRS offers resources and coordinates reasonable accommodations for students with disabilities and/or temporary health conditions. Reasonable accommodations are established through an interactive process between you, your instructor(s) and DRS. It is the policy and practice of the University of Washington to create inclusive and accessible learning environments consistent with federal and state law. Requests for accommodations or services must be arranged in advance.

**Religious Accommodations**
Washington state law requires that UW develop a policy for accommodation of student absences or significant hardship due to reasons of faith or conscience, or for organized religious activities. The UW's policy, including more information about how to request an accommodation, is available at Religious Accommodations Policy. Accommodations must be requested within the first two weeks of this course using the Religious Accommodations Request form.

Bias concerns

The Office of the Dean has a student concern policy, a faculty concern policy and standard HR procedures for staff concerns. Our 2018 climate survey states that most people in SPH do not report bias incidents because they do not know where to go. Students are encouraged to report any incidents of bias to someone they feel comfortable with, including instructors, advisers or department staff. They can email dcinfo@uw.edu for immediate follow up. Bias concerns can be anonymously and confidentially reported at this link, Data is collected by the Assistant Dean for EDI and the Director of Program Operations for Student and Academic Services and tracked for resolution and areas are identified for further training.

Pronouns

According to the UW First Year Programs, being an ally is not just about intention, it’s also about behavior. We share our pronouns because we strive to cultivate an inclusive environment where people of all genders feel safe and respected. We cannot assume we know someone’s gender just by looking at them. So we invite everyone to share their pronouns. Faculty training and consultation on pronoun use is available for SPH faculty from the Assistant Dean for EDI.

UW Academic Integrity Statement

Students at the University of Washington (UW) are expected to maintain the highest standards of academic conduct, professional honesty, and personal integrity.

The UW School of Public Health (SPH) is committed to upholding standards of academic integrity consistent with the academic and professional communities of which it is a part. Plagiarism, cheating, and other misconduct are serious violations of the University of Washington Student Conduct Code (WAC 478-120). We expect you to know and follow the university’s policies on cheating and plagiarism, and the SPH Academic Integrity Policy. Any suspected cases of academic misconduct will be handled according to University of Washington regulations. For more information, see the University of Washington Community Standards and Student Conduct website.

Classroom Climate

The UW School of Public Health seeks to ensure all students are fully included in each course. We strive to create an environment that reflects community and mutual caring. We encourage students with concerns about classroom climate to talk to your instructor, your advisor, a member of the departmental or SPH Diversity Committee and/or the program director Victoria Gardner, Assistant Dean for Equity, Diversity, & Inclusion, vg@uw.edu is a resource for students with classroom climate concerns.

Recording of Remote Class Sessions

The Zoom class sessions will be recorded. The recording will capture the presenter’s audio, video and computer screen. Student audio and video will be recorded if they share their computer audio and video during the recorded session. The recordings will only be accessible to students enrolled in the course to review materials. These recordings will not be shared with or accessible to the public. The University and Zoom have FERPA-compliant agreements in place to protect the security and privacy of UW Zoom accounts. Students who do not wish to be recorded should change their Zoom screen name to hide any personal identifying information such as their name or UW Net ID, and not share their computer audio or video during their Zoom sessions.
## COURSE SCHEDULE

For a complete, session-by-session course schedule, please see the [Modules Page](https://canvas.uw.edu/courses/1433146/assignments/syllabus).

<table>
<thead>
<tr>
<th>Date</th>
<th>Lecture</th>
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<tbody>
<tr>
<td>Jan 4</td>
<td>Introduction/overview</td>
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<tr>
<td>Jan 6</td>
<td>Lab Safety</td>
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<td>Jan 8</td>
<td>Sampling &amp; Experimental Design</td>
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<tr>
<td>Jan 11</td>
<td>QA/QC and Regulations</td>
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<tr>
<td>Jan 14</td>
<td>Submit papers on canvas (for student presentations) (Lab 1)</td>
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<tr>
<td>Jan 15</td>
<td>First Exam – Closed Book/Notes</td>
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<tr>
<td>Jan 18</td>
<td>HOLIDAY - Martin Luther King Day</td>
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<tr>
<td>Jan 20</td>
<td>Lab 0: Sterile technique, streaking plates, general micro review; Quiz on reading</td>
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<tr>
<td>Jan 22, 25, 27</td>
<td>Lab 1: MPN multiple tube fermentation, Colilert [yes/no]. Submit papers on canvas (for student presentations) on Jan 14; Quiz on reading Jan 22; Problem set due on Jan 26.</td>
</tr>
<tr>
<td>Jan 29, Feb 1, 3</td>
<td>Lab 2: IDEXX and membrane filtration to detect enterococci/E. coli. Submit papers on canvas (for student presentations) on Jan 21. Quiz on reading Jan 29; Problem set due on Feb 2</td>
</tr>
<tr>
<td>Feb 5, 8, 10</td>
<td>Lab 3: Surface sampling for S. aureus and methicillin-resistant S. aureus (MRSA). Submit papers on canvas (for student presentations) on Jan 28; Quiz on reading Feb 5; Problem set due on Feb 9</td>
</tr>
<tr>
<td>Feb 12, 17, 19</td>
<td>Lab 4: Surface sampling for vancomycin resistant enterococci. Submit papers on canvas (for student presentations) on Feb 4; Quiz on reading Feb 12; Problem set due on Feb 18 (extended)</td>
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<tr>
<td>Feb 15</td>
<td>HOLIDAY - Presidents' Day</td>
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<tr>
<td>Feb 22, 24, 26</td>
<td>Lab 5: Salmonella in chicken. Submit papers on canvas (for student presentations) on Feb 11; Quiz on reading Feb. 22; Problem set due on Feb 25</td>
</tr>
<tr>
<td>March 1, 3, 5</td>
<td>Lab 6: Airborne Microbial contaminants. Submit papers on canvas (for student presentations) on Feb 18; Quiz on reading March 1; Problem set due on March 4</td>
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<tr>
<td>Date</td>
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<tr>
<td>March 8</td>
<td>Make up for missed student presentations; Review session with TA's</td>
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<tr>
<td>March 10</td>
<td>Final Exam - Closed Book/Notes</td>
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### Lab 0: Sterile Technique

- **Lab 0-4: Sterile Technique**
  - Sterile technique; focuses on slants
  - [Lab 1-3: Aseptic technique](https://www.youtube.com/watch?v=v0G8Hd6R-14&ab_channel=Dr.JulieWells)
  - [Lab 1-4: Streak Plate](https://www.youtube.com/watch?v=s5Fwgk_k1o&ab_channel=Dr.JulieWells)

### Lab 1: MPN Multiple Tube Fermentation Colilert

- **Lab 1-4: Multiple Tube Fermentation**
  - Background of water quality testing; multiple tube fermentation steps
  - [MULTIPLE TUBE FERMENTATION (MTF) - Water Systems Technology](https://www.youtube.com/watch?v=uPZ_YFWUzoE&list=PLmATW0pvCR3vP0TQjCblKogmbDKfyqutt&ab_channel=AWETDSNmlau)
  - [8-13: Multiple Tube Fermentation](https://www.youtube.com/watch?v=msVEDf2wGSA&ab_channel=Dr.JulieWells)

#### Day 1:
- Presumptive - Lauryl Tryptose Broth (LTB):
  - Begins at 27:30; ends: 31:53
  - Begins again: 36:07; ends 38:22

### Lab 11: Lab 11

- [8-13: Water Sample Testing](https://www.youtube.com/watch?v=msVEDf2wGSA&ab_channel=Dr.JulieWells)
Day 2: Confirmatory - Brilliant Green Lactose Bile (BGLB):
-Start: 1:18:20

Day 3: Readout
-Start: 1:34:10, end:1:37:39

How to detect total coliforms, E. coli using colilert; 4min

Colilert® Test Overview and How To Instructions (https://www.youtube.com/watch?v=23tPNP4WsAE&ab_channel=KossodoCanal)

MPN method for water quality testing

MPN Most Probable Number Water Quality Testing: Microbiology (https://www.youtube.com/watch?v=9eaoErq6INc&ab_channel=GrowGrayMatterDr.FrankO%27Neill)

Overview of MacConkey agar

MacConkey Agar (https://www.youtube.com/watch?v=f0pgH7adv7A&ab_channel=CatalystUniversity)

Resource needed:
Table 9221.IV MPN Index and 95CI.JPG

[Available under modules tab]

Lab 2: IDEXX and membrane filtration
Covers Quanti-tray 2000 (Colilert)

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<thead>
<tr>
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<tbody>
<tr>
<td>Day 1:</td>
<td><strong>Purpose:</strong> to test a water sample for the presence of coliform bacteria <em>Uses endo agar</em></td>
</tr>
<tr>
<td>- Start: 0:20, end: 27:29</td>
<td><strong>Lab 8-12 Water Sample Testing (membrane filtration) <a href="https://www.youtube.com/watch?v=msVEDf2wGSA&amp;ab_channel=Dr.JulieWells">Link</a></strong></td>
</tr>
<tr>
<td>Day 2:</td>
<td><strong>Membrane filtration method to count bacteria in water <a href="https://www.youtube.com/watch?v=Gvl_Qvsr3z4&amp;t=131s&amp;ab_channel=MicrobiologyteachingvideosatNUIGalway">Link</a></strong></td>
</tr>
<tr>
<td>- Start: 38:36, End: (talks about endo agar), End:</td>
<td><strong>Resource needed:</strong> IDEXX Quanti-Tray®/2000 MPN Table <a href="https://www.idexx.com/files/qt97mpntable.pdf">Link</a></td>
</tr>
<tr>
<td>1:18:20</td>
<td><strong>Lab 3:</strong> Surface Sampling for MRSA Air sampling using settle plates</td>
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<tr>
<td>Sampling using contact agar plates</td>
<td><img src="https://www.youtube.com/watch?v=KdndbLKHxmw&amp;ab_channel=HardyDiagnostics" alt="Image" /></td>
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<tr>
<td>Environmental Surface Sampling Using Contact Agar Plates</td>
<td><img src="https://www.youtube.com/watch?v=KdndbLKHxmw&amp;ab_channel=HardyDiagnostics" alt="Image" /></td>
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<tr>
<td>An example of using a contact RODAC plate</td>
<td><img src="https://www.youtube.com/watch?v=pZCiiWHEgvyA&amp;ab_channel=AAMUFoodMicro" alt="Image" /></td>
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<tr>
<td>Sampling with Rodac Plates</td>
<td><img src="https://www.youtube.com/watch?v=pZCiiWHEgvyA&amp;ab_channel=AAMUFoodMicro" alt="Image" /></td>
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<tr>
<td>Surface swabbing technique</td>
<td><img src="https://www.youtube.com/watch?v=7z4JRQlGOag&amp;ab_channel=ALSLimited" alt="Image" /></td>
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<tr>
<td>Good Surface Swabbing Technique</td>
<td><img src="https://www.youtube.com/watch?v=7z4JRQlGOag&amp;ab_channel=ALSLimited" alt="Image" /></td>
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<tr>
<td>Mannitol salt agar (MSA): background, how to interpret results</td>
<td><img src="https://www.youtube.com/watch?v=ON4qQafGfc&amp;ab_channel=CatalystUniversity" alt="Image" /></td>
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</tr>
<tr>
<td>Screening for hemolysis using blood agar</td>
<td><img src="https://www.youtube.com/watch?v=uVU1JXfXwVk&amp;ab_channel=CatalystUniversity" alt="Image" /></td>
</tr>
<tr>
<td>Microbiology: Hemolysis/Blood Agar</td>
<td><img src="https://www.youtube.com/watch?v=uVU1JXfXwVk&amp;ab_channel=CatalystUniversity" alt="Image" /></td>
</tr>
<tr>
<td>Background: How to differentiate, health effects,</td>
<td>Staphylococcus aureus</td>
</tr>
</tbody>
</table>
virulence factors (TST, PVL, hemolysin, enterotoxin, exfoliation), treatment, MRSA/VRSA

https://www.youtube.com/watch?v=wdo3E2w0cl8&ab_channel=Osmosis

Surface sampling background, surface sampling technique with contact plates and swabs

Environmental Surface Sampling using Contact Agar Plates

https://www.youtube.com/watch?v=KdndbLKhxmw&t=45s&ab_channel=HardyDiagnostics

Lab 4: Surface Sampling for VRE

Vancomycin and VRE: Microbiology

https://www.youtube.com/watch?v=puYOGhtXvco&ab_channel=GrowGrayMatterDr.FrankO%27Neill

Background on VRE

Vancomycin and VRE: Microbiology

https://www.youtube.com/watch?v=puYOGhtXvco&ab_channel=GrowGrayMatterDr.FrankO%27Neill

Isolating microorganisms with information on morphology, 3 streak plating

Isolating Microorganisms at 13:48

https://www.youtube.com/watch?v=hxausVA8a3E&ab_channel=ProfessorSimms

Practice with counting and calculating colonies

CFU Calculation

https://www.youtube.com/watch?v=jdnOjoOO6qY&ab_channel=Bio-Resource

Lab 5: Salmonella in Chicken

Use for HE agar and how to interpret

HE Agar

https://www.youtube.com/watch?v=KPzP4B3nYbo&ab_channel=DelacyRhodes
<table>
<thead>
<tr>
<th>TSI slant inoculation and interpretation</th>
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<tbody>
<tr>
<td>How to Inoculate and Interpret a TSI slant <a href="https://www.youtube.com/watch?v=FuOcN3wB0VM&amp;ab_channel=MCCCMicrobiology">Video</a></td>
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<th>Composition of XLD agar</th>
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<tr>
<td>XLD Differential Media <a href="https://www.youtube.com/watch?v=5hRy1_YtL1U&amp;ab_channel=DiseaseMedia">Video</a></td>
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<th>XLD agar result interpretation</th>
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<tr>
<td>XLD Agar guide <a href="https://microbenotes.com/xylose-lysine-deoxycholate-xld-agar/">Website</a></td>
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<tr>
<th>Lab 6: Airborne Microbial contaminants</th>
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<tbody>
<tr>
<td>How to set up airborne microbe lab with three different exposure periods and how to interpret results</td>
</tr>
<tr>
<td>Procedure: Lab Ch 10 Airborne Microbes mov 3 13 2020 <a href="https://www.youtube.com/watch?v=l93tpLlYrgc&amp;t=11s&amp;ab_channel=MicrobesProf">Video</a></td>
</tr>
</tbody>
</table>

| Results: Lab movie results Airborne microbes 3 14 2020 [Video](https://www.youtube.com/watch?v=fv9FGo2StHY&ab_channel=MicrobesProf) |