### Microbiome Introductory Unit

<table>
<thead>
<tr>
<th>Required Time</th>
<th>Objectives:</th>
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</table>
|               | • Students will explain microbiomes  
|               | • Students will distinguish between a reputable and non-reputable source  
|               | • Each student will formulate a question related to microbiomes and hypothesize an answer  
|               | • Each Student will plan an experiment to test his/her hypothesis  
|               | • Students will conclude if their hypotheses was correct, based on data obtained in the experiment  
|               | • Students will complete a lab report summarizing their results |

<table>
<thead>
<tr>
<th>Class Period #1: Introduction</th>
<th>Class Period #2: Student Research</th>
<th>Class Period #3:</th>
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</table>
| Bell Question: “Why does poop smell?”  
| Introduce the responsibility of microbes for various smells in/on the human body – armpit odor, foot odor, feces, breath  
| “What is a microbe?” – bacteria, fungi, viruses  
| Microbes are mostly good, a few bad ones  
| Pathogens cause disease  
| “What is a microbiome?”  
| Collection of microbes living in an area/environment  
| Where do microbiomes exist on the human body?  
| Microbiome is an important part of our health  
| Read part of The Guardian article, “Is your Gut Making You Sick?” by Ann Robinson (8-1-16)  
| Different bugs in/on different parts of the human body  
| Clostridium difficile (C. diff.) infections  
| Microbiome and autoimmune disease  
| Antibiotics effect on the microbiome  
| What does a microbiome look like? | Bell Question: “Are antibacterial soaps more effective than normal soap?”  
| Discuss what makes a reference reputable and what is fact vs opinion?  
| Class is split into groups, dependent on number of instructors in the room. Each group reads aloud an op-ed and the FDA release about antibiotic soap and discusses which is an acceptable reference  
| Students use their devices to research the skin microbiome  
| Students organize information to be shared with the class  
| Spend the final 10 minutes discussing what the students learned | Bell Question: “Write three questions you have about skin microbiomes.”  
| Students will plan an experiment investigating a question related to microbiomes |
| Experiment Design | o Each student will have access to 4 LB agar-fill petri dishes, 8 cotton swabs and a permanent marker  
o Students must identify a question they can answer with the available materials. Examples:  
  ▪ Does the microbiome differ from my left hand to my right hand?  
  ▪ If I touch the doorknob, does my microbiome change to be more similar to the doorknob?  
  ▪ Does my friend have a similar microbiome on his/her right hand as my right hand?  
o Students develop a hypothesis to answer their question, based on their research  
o Students begin to formulate an experimental protocol |
|---------------------|--------------------------------------------------|
| 10 minutes of Living Environment Class | | • Students review and finalize their protocols  
• Students use sterile cotton swabs to take samples and plate them  
• Plates are incubated at 37°C Celsius for one (1) week |
| Class Period #4: Observations | | • Bell Question: “Do you think owning a pet affects your microbiome?”  
• Students observe their plates and take clear notes as to what they see and smell  
• Students create a lab report to share their results and conclusion |
| Literature | | • “Is your gut making you sick?” by Ann Robinson in The Guardian, 8-1-16  
• [http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM378615.pdf](http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM378615.pdf)  
| Materials | | • Student laptops  
• LB agar plates  
• Sterile cotton swabs  
• “Question Notebooks”  
• Lab Report Template  
• Print-outs of literature  
• Permanent Markers  
• 37°C Celsius Incubator |

**Living Environment Standards Met:**

<p>| 1.1a | Scientific explanations are built by combining evidence that can be observed with what people already know about the world. |
| 1.2a | Inquiry involves asking questions and locating, interpreting, and processing |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1.2b</td>
<td>Inquiry involves making judgments about the reliability of the source and relevance of information.</td>
</tr>
<tr>
<td>1.3b</td>
<td>All scientific explanations are tentative and subject to change or improvement. Each new bit of evidence can create more questions than it answers. This leads to increasingly better understanding of how things work in the living world.</td>
</tr>
<tr>
<td>2.1</td>
<td>Devise ways of making observations to test proposed explanations.</td>
</tr>
<tr>
<td>2.2</td>
<td>Refine research ideas through library investigations, including electronic information retrieval and reviews of the literature, and through peer feedback obtained from review and discussion.</td>
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<tr>
<td>2.3</td>
<td>Develop and present proposals including formal hypotheses to test explanations; i.e., predict what should be observed under specific conditions if the explanation is true.</td>
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<tr>
<td>3.1</td>
<td>Use various methods of representing and organizing observations (e.g., diagrams, tables, charts, graphs, equations, matrices) and insightfully interpret the organized data.</td>
</tr>
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<td>3.3</td>
<td>Assess correspondence between the predicted result contained in the hypothesis and actual result, and reach a conclusion as to whether the explanation on which the prediction was based is supported.</td>
</tr>
<tr>
<td>3.4a</td>
<td>Hypotheses are valuable, even if they turn out not to be true, because they may lead to further investigation.</td>
</tr>
<tr>
<td>5.2a</td>
<td>Homeostasis in an organism is constantly threatened. Failure to respond effectively can result in disease or death.</td>
</tr>
<tr>
<td>5.2b</td>
<td>Viruses, bacteria, fungi, and other parasites may infect plants and animals and interfere with normal life functions.</td>
</tr>
<tr>
<td>6.1d</td>
<td>The number of organisms any habitat can support (carrying capacity) is limited by the available energy, water, oxygen, and minerals, and by the ability of ecosystems to recycle the residue of dead organisms through the activities of bacteria and fungi.</td>
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### Microbiome Unit Extension (Best fit for an afterschool program)

<table>
<thead>
<tr>
<th>Required Time</th>
<th>• Six (6) x 40 minute periods</th>
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**Objectives:**
- Students will compose a research paper, summarizing the results of their microbiome experiment
- Students will critique a classmate’s paper via peer-review
- Students will distinguish between microbes that were found in different locations/conditions
- Students will identify some of the common microbes found
- Students will learn about polymerase chain reaction (PCR) to identify microbes

| Class Period #1: Parts of a Research Paper | • Review papers from peer-reviewed journals and students will identify the components of a research paper  
  • Students will be reminded of proper citation methods  
  • Students begin an outline of a research paper, using information from their microbiome experiments |
|-------------------------------------------|------------------------------------------------------------------------------------------|
| Class Period #2: Introduction             | • Students begin writing their introduction sections, using background information they gathered before planning their experiments  
  • Students will have access to their devices for further research |
| Class Period #3: Results and conclusion   | • Students will organize their results into a narrative and write a conclusion based on the results  
  • Students will include new questions and experiments to answer those questions |
| Class Period #4: Abstract and final draft | • Students will write an abstract, based on their introduction, results and conclusion  
  • Students will compile their entire research paper and ensure it is complete |
| Class Period #5: Peer Review              | • Students will exchange papers for peer-review. Each student’s paper will be reviewed by 3 classmates  
  • Students will write a critique for each of the three papers they review |
| Class Period #6: Future Experiments       | • Students will read “The Boston subway could help prevent disease outbreaks in the future”  
  • Students will begin to research how to identify the different species of the hand microbiome. This can be via PCR, selective media, and other microbiology methods  
  • Students will start to create an experiment to identify some of the microbes that grew on their plates. This is a lead in to the next units |
<table>
<thead>
<tr>
<th>Literature</th>
<th>• “The Boston Subway Could Help Prevent Disease Outbreaks in the Future” Business Insider, 6-28-2016</th>
</tr>
</thead>
</table>
| Materials | • Literature Printouts  
• “Question Notebooks” |

**Living Environment Standards Met:**

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<th>Standard</th>
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</tr>
<tr>
<td>1.1e</td>
<td>Ecosystems, like many other complex systems, tend to show cyclic changes around a state of approximate equilibrium.</td>
</tr>
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<td>1.2a</td>
<td>Inquiry involves asking questions and locating, interpreting, and processing information from a variety of sources.</td>
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# Microbiome Lab Report Rubric

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<th></th>
<th>Excellent 4 points</th>
<th>Good 3 points</th>
<th>Fair 2 points</th>
<th>Poor 1 point</th>
<th>Unsatisfactory 0 points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Question and Hypothesis</strong></td>
<td>Question and hypothesis demonstrate concepts beyond what was learned in the classroom. Shows critical thinking.</td>
<td>Question and hypothesis are present and variables are defined.</td>
<td>Question and hypothesis are present, but variables are not clearly defined.</td>
<td>Only question or hypothesis is written, not both.</td>
<td>No question or hypothesis present.</td>
</tr>
<tr>
<td><strong>Background information</strong></td>
<td>Background information is extensive, written in a cohesive way and properly cited.</td>
<td>Background information is substantial and organized well. No/improper citations.</td>
<td>Background information is written. No citations present.</td>
<td>Minimal background information is provided.</td>
<td>No background information present.</td>
</tr>
<tr>
<td><strong>Experimental Design</strong></td>
<td>Clear and concise so that someone else may follow the procedure and repeat the experiment exactly.</td>
<td>Experimental design is mostly clear and but makes some assumptions of knowledge.</td>
<td>Experimental design has a basic protocol</td>
<td>Experimental design lacks clear instructions.</td>
<td>No Experimental Design present.</td>
</tr>
<tr>
<td><strong>Data and Observations</strong></td>
<td>Observations and data are written clearly enough that another person could interpret and draw the same conclusion</td>
<td>Observations and data are mostly clear.</td>
<td>Observations and data are not complete.</td>
<td>Observations and data lack clarity.</td>
<td>No data or observations present.</td>
</tr>
<tr>
<td><strong>Conclusion</strong></td>
<td>Conclusion is clearly written and refers to the data and background information.</td>
<td>Conclusion is written, but does not clearly refer directly to any data or background information.</td>
<td>Conclusion shows some attempt to interpret the data.</td>
<td>Conclusion restates the observations with no interpretation of data.</td>
<td>No conclusion present.</td>
</tr>
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</table>
Microbiome: How bugs may be crucial to your health

Warning: This story could gross you out.

By Karen Weintraub
12 April 2012

It is about the microscopic bugs that live all over your body – on your skin, in your mouth, in your nose, and in particular your digestive tract. These bugs are so numerous that they outnumber your own cells by a factor of 10. You are vastly more microbe than human.

Before you get so disgusted you stop reading, consider this: many of these bugs are as essential to your life as your own cells. These microbes have been around since before humans existed, and our bodies have evolved to adapt to their presence just as they have adapted to ours.
They are also — to quote one expert — the "last frontier" of medical research, a crucial aspect of our health that scientists rarely considered until recently. It is also one of the most daunting challenges facing biologists today.

"We know next to nothing about this whole universe that we host," according to Bruce Birren, co-director of the Genome Sequencing and Analysis Program at the Broad Institute, a research collaborative between Harvard and MIT scientists. "It's as if we're coming to a planet for the first time and asking: what do we find?"

What experts like Birren are discovering is the powerful role these tiny bugs might be playing in our lives. The 1,000-or-so species of microbes that live in our guts control digestion, and possibly so much more. They are strongly linked with the rise in allergies and asthma, and with digestive problems like Crohn's disease and colitis. They also influence the immune system, and there is a growing body of evidence suggesting that gut microbes could have an influence on cancer risk. They could also dictate whether we are packing on extra pounds or liable to get diabetes.

And that is not all. Recent studies have shown that germ-free mice are much more vulnerable to stress. Two strains of mice known for their distinct personalities – one warm and friendly, the other aggressive and standoffish – swapped traits when given each other's gut microbes. Other studies have altered rats' response to heart attacks by changing the gut microbes they were fed before the attack. And a group of mice fed a high-protein diet had different gut microbes and better memory skills than mice fed a typical diet.

"We can't really understand human health without understanding how we interact with all these microbes," says Birren.

Code breakers

This year sees the culmination of two major projects seeking to better understand the full repertoire of bugs that colonize us – what scientists call the microbiome. In 2007, the United States government launched a five-year, $157 million Human Microbiome Project, aimed at sequencing the genomes of microbial populations living in the mouths, guts, armpits and other orifices of 300 healthy Americans.

The following year the European Commission launched a $29 million project called Metagenomics of the Human Intestinal Tract, or MetaHIT, which focuses on gut bacteria only. Initial findings from both efforts were presented at the International Human Microbiome Congress in Paris last month. "Both have made absolutely tremendous progress," says Dusko Ehrlich, coordinator of MetaHIT and research director at the National Institute for Agricultural Research in France.

While some researchers are wary of overhyped any results, Ehrlich says he and other scientists are confident in the potential of the human microbiome research – even more so than they were a decade ago when all the talk was about the human genome. "It's hard to be a prophet," Ehrlich says, "but we see so much more potential in the human-other-genome than in our own genome."

He cites two reasons for his optimism: genetic diversity and treatability. One person's genome differs only 0.1% from another's; while their gut genomes may differ by 50%. "Since there's so much variability, there is a much greater chance we'll be able to associate differences with disease," he says, adding that it should be easier to treat gut microbes than to make genetic changes. And he is also excited about their potential ability to predict disease. Perhaps someday, changes in someone's gut bugs could be indicators of impending illness – allowing shifts in diet or medications to restore the microbial balance before it leads to a serious health problem.

Number crunch

But while the data pour in, so begin the debates. One of the main discussions at the recent meeting concerned a study published last year, which suggested that people fall into three categories, or enterotypes as they are known, depending on the dominant group of gut bacteria living there. The idea of having a bug version of blood type that could predict a person's risk of disease is a compelling one, but follow-up results presented in Paris suggest that the boundaries between types might be fuzzier than first imagined.

Ehrlich says the only way around that problem is to study many thousands of people. "Numbers count," he says, adding that researchers in Europe, China, the United States and elsewhere need to collaborate to make sure their sample sizes are large enough to reflect meaningful differences in the microbiome. This also means researchers around the world need to develop protocols so they are all studying things the same way and their results are comparable.

Tracking the human microbiome also involves manipulating more data than scientists have ever dealt with before. Until recently, scientists had only been able to culture bacteria that could live in a petri dish; once they figured out how to separate the microbial from the human DNA, they began discovering dozens of new species. But they are collecting billions of bases, or gigabases, of DNA sequence data from complex populations — and figuring out which bits of DNA go together is only one part of the puzzle. How do you infer what those organisms are actually doing? How do they work together as an ecological system? How does this relate to human health — either normal or disease states — and how do you know whether the microbes have caused the condition, or whether they are just responding to the changing environment?

Curtis Huttenhower, assistant professor of Computational Biology and Bioinformatics at the Harvard School of Public Health, says the balance of microbes is more likely to matter than the individual strains. A few bad actors like salmonella will make you sick even in small concentrations, but for the most part, the good bacteria keep the bad in check, says Huttenhower, who studies inflammatory bowel disease. And many of us carry the bacteria Clostridium difficile around inside us all the time, with no ill effect. It is only after a shift in the microbe population — say, after a heavy-duty course of antibiotics that disrupts the balance — that the destructive power of C. difficile can be seen, causing symptoms ranging from diarrhoea to life-threatening inflammation of the colon.

Faecal transplants

Ruth, a college professor in suburban New York, knows this all too well. The 55-year-old, who asked that she be identified only by her first name, took a course of antibiotics in late 2006 for a bladder infection. A few months later, she needed antibiotics again, this time for a dental procedure — and that is when her problems began. She had terrible diarrhoea, and began losing weight and strength. Most troubling for a woman whose students still gave her credit for being “hot” on an online rating system: her hair started falling out in chunks. By May 2007 she was diagnosed with a C. difficile infection and started taking antibiotics to treat it. More than a full year later, she was still taking antibiotics — in much higher doses — and still getting sick every time she stopped.

She was feeling increasingly desperate when, doing some internet research, she came across the idea of a fecal transplant. In a faecal transplant, the faeces from a healthy person is inserted anally into the colon of another. The idea is that the bugs from the healthy person will restore the microbial balance of the sick one.

Though the concept of a faecal transplant may seem disgusting at first, being infected with C. difficile was far worse, she says. “The indignity of it is profound. You really feel dirty and contagious. You’re just walking around feeling like a freak.” Getting a faecal transplant felt like no big deal after dealing with C. difficile for so long.

Faecal donors, usually a family member or significant other, are screened for infectious diseases such as HIV and syphilis, and for lifestyle patterns that might endanger the recipient, like high-risk sexual activity.

Ruth says she felt different almost immediately after the transplant, done during a colonoscopy, and felt “almost normal” within three weeks. She was able to go off antibiotics at last, and the hopelessness she had felt for months slowly disappeared. Today, her hair has grown back, and she has recovered fully.

Her doctor, Lawrence Brandt, professor and emeritus chief of gastroenterology at Albert Einstein College of Medicine in New York, has been doing faecal transplants since 1990. He says he is struck by how successful, inexpensive, and apparently safe the procedure is — with no major adverse reactions reported. His research, some of it still unpublished, suggests faecal transplants have been 91% effective in several hundred cases worldwide. There has not yet been a gold standard, double-blind, placebo-controlled study of transplants, but there is a growing consensus that faecal transplants are a good idea for people with persistent C. difficile infections.

Someday, he predicts, the procedure will be tested and used against many more ailments, too. And eventually, drug companies will figure out how to bottle the right bacteria, and faecal transplants will not be necessary, he says. “Today, we use stool, because we haven’t yet worked out the precise formulaic combinations of organisms that are deficient in each of the diseases we are talking about.”

Population changes
Faecal transplants, of course, are not the only way to change gut microbes. As Ruth experienced, antibiotics – particularly repeated courses close together – can alter the balance, as can serious illness and shifts in diet. A gene might leave you more or less vulnerable to a bacterial hit, says Huttenhower. “If you’re predisposed and your microbial community by chance enters a high-risk state, those factors could combine to trigger disease.”

For the most part, the populations you have in early childhood will be with you the rest of your life. Even after microbe populations are disrupted by antibiotics, they tend to return to a baseline, says Graham Rook, emeritus professor of medical microbiology at University College London.

Different events, particularly early in life, can affect that baseline. A study published last year found that babies delivered by C-section had different gut microbes than those delivered vaginally – presumably because they were exposed to different bugs on their path out. MetaHIT’s Ehrlich points to other research suggesting that a baby’s microbe population changes continuously, until around two years of age, so it is not clear whether this early difference – or any microbial change in early childhood – has any long-term health implications.

Researchers believe that the microbes of people who live together can begin to resemble each other. “You’re going to share more with each other than you would compared to someone living in Chicago, but still retain a lot of a history of who you are and where you’ve been,” says Gary Huffnagle, an immunologist and professor of internal medicine at the University of Michigan. “The longer you cohabit, the theory is, the more you’ll begin to look like each other.”

Then there are the countless probiotic yoghurts and drinks marketed under the claim that consuming their “friendly” microorganisms can be good for your health. A study published last year suggests their effects might be subtle only. Comparing DNA stool samples from one identical twin who ate probiotic yoghurt with one who did not showed little difference in the make-up of their gut bacteria, but studies in mice showed probiotic yoghurt did affect the activity of genes that allow gut bacteria to break down carbohydrates.

Scientists do not know yet whether some people might respond better to probiotics and dietary changes than others. The “big promise of the future”, says Ehrlich, is that simple dietary changes could manipulate the balance of our gut bugs before diseases develop. When that time will come is impossible to predict, he says, but, at least the Human Microbiome Project and MetaHiT scientists are beginning to know which questions to ask.

Until that time comes, the 69-year-old researcher has perhaps an unconventional suggestion for younger people. If he were 18 again, he says he would deep-freeze a stool sample at a biobank, in case he ever needed a faecal transplant. See, I told you this story would gross you out.

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Biometrics
The Boston subway could help prevent disease outbreaks in the future

MOLLY SEQUIN
JUN. 28, 2016, 1:00 PM

A study published today in the American Society for Microbiology's journal mSystems could change the way we predict and prevent public health outbreaks.

Scientists from the Harvard T.H. Chan School of Public Health descended into the Boston subway system to study the germs (microbes) that are found there. With an unimaginable number of microbes moving between people and subway surfaces every single day, the scientists wanted to see how much of a threat the microbes posed to the public.

So, the team took samples from different surfaces throughout the Boston subway stations and on the trains. They swabbed seats, poles, hanging grips, walls, and the touchscreens of ticketing machines.

This was no small task. "We initially planned the study in the summer of 2012, sampled in spring and fall of 2013, and data generation took place over the course of two years after that," Curtis Huttenhower, the study's main author, told Business Insider. "Analysis and interpretation took quite a bit of time, so we could make sure the data were high quality."

The researchers found that the surface type, and the way people interact with these surfaces, were the biggest factors in determining which microbes they found. For example, skin microbes were found more on poles and hanging grips than on seat backs.

Scientists were curious about how harmful germs in public transportation systems actually are. Surprisingly, the research team found only very few worrisome pathogens on the surfaces with which people interact frequently. In fact, there were fewer virulent microbes in the subway than there are in the human intestines. In addition, the microbe communities did not change significantly across geographic areas or from station to station.

This is good news: Travelers riding the Boston subway face less harmful germs every ride than one might have expected. And importantly, now the researchers have a baseline for future comparisons. This means that if there is a public health outbreak, scientists might detect it early on by comparing their study results with new swabs.

Huttenhower says that the team is planning to continue this study in the future to see if the subway microbes are surviving or if they are actively growing on surfaces. He also hopes to take samples during different times of the
year to look for seasonal changes. For example, how would hot weather or flu season affect the microbial profile of the Boston subway?

As the database builds, scientists will be better able to predict and prevent outbreaks that lurk in the future.