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Cambodia, Kingdom of Water...or is it?

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Cambodia, Kingdom of Water

.....or is it?



Overview of Presentation

- How I got to Cambodia
- The Problem – lack of clean drinking water
- Biosand Filters– How they work
- Pathogens in the Water – Who are they?

BSU's Commitment To Cambodia!



- Fall 2006, Dr. Kevin Curry approached to set up lab for the Middletown Rotary Club.
- Water for Cambodia
- Pannasastra University in Phnom Penh
- My research team



Cambodia Research Team 2013



Jen Conway

- Research student with me for 3 years
- Wants to improve the lives of others



Heidi Lima

- 2nd trip to Cambodia
- Teacher and interested in global education



Dr. Jenna Mendell

- Microbiologist, researcher, mentor and professor!



Zach Ripatrazzone

- Research student with me for 2 years
- Natural research talent and teacher



Rachel Toews

- 2nd trip to Cambodia
- Wants to help with water issues and pollution problem

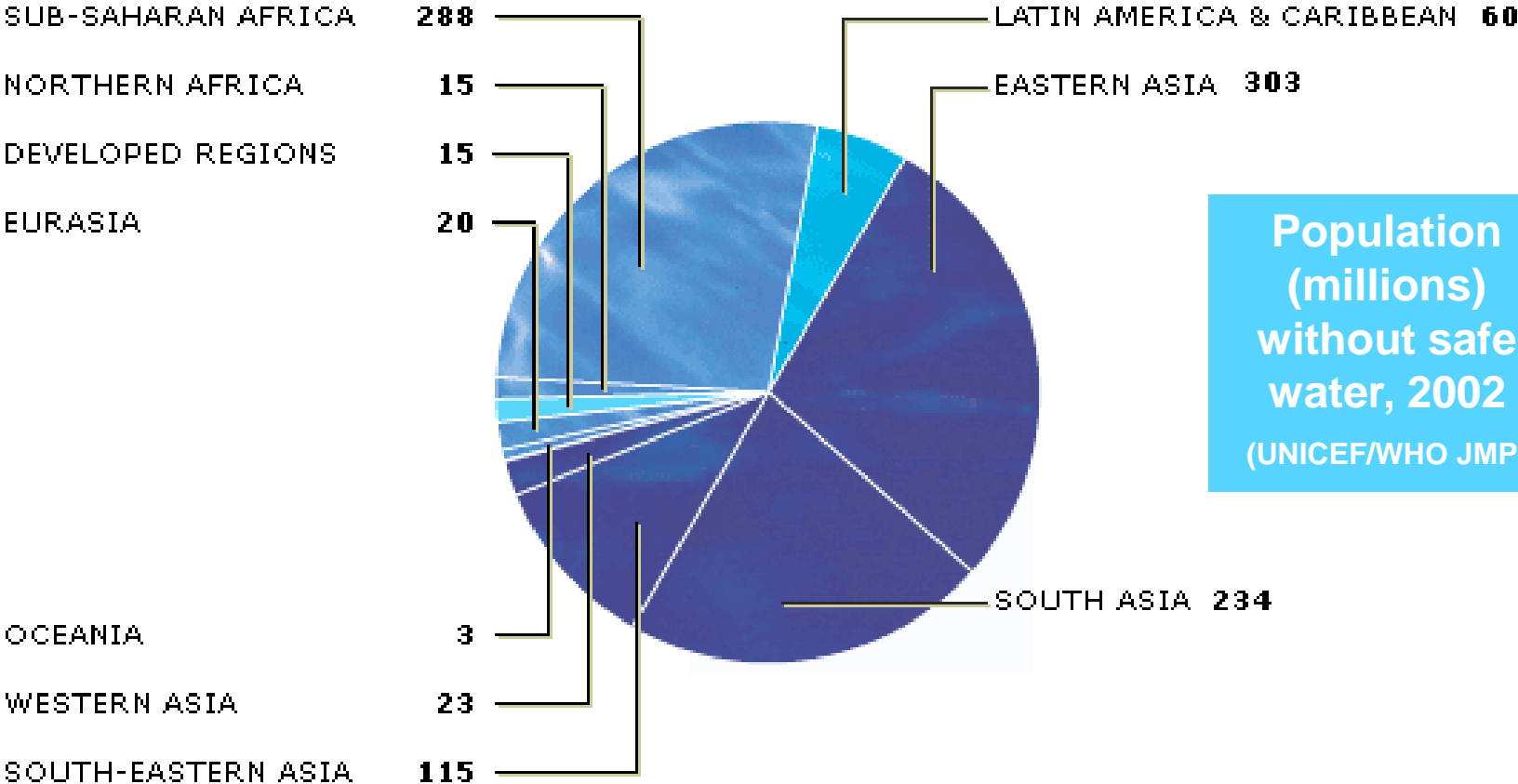
Cambodia.....Kingdom of Water

But is this water safe to drink?

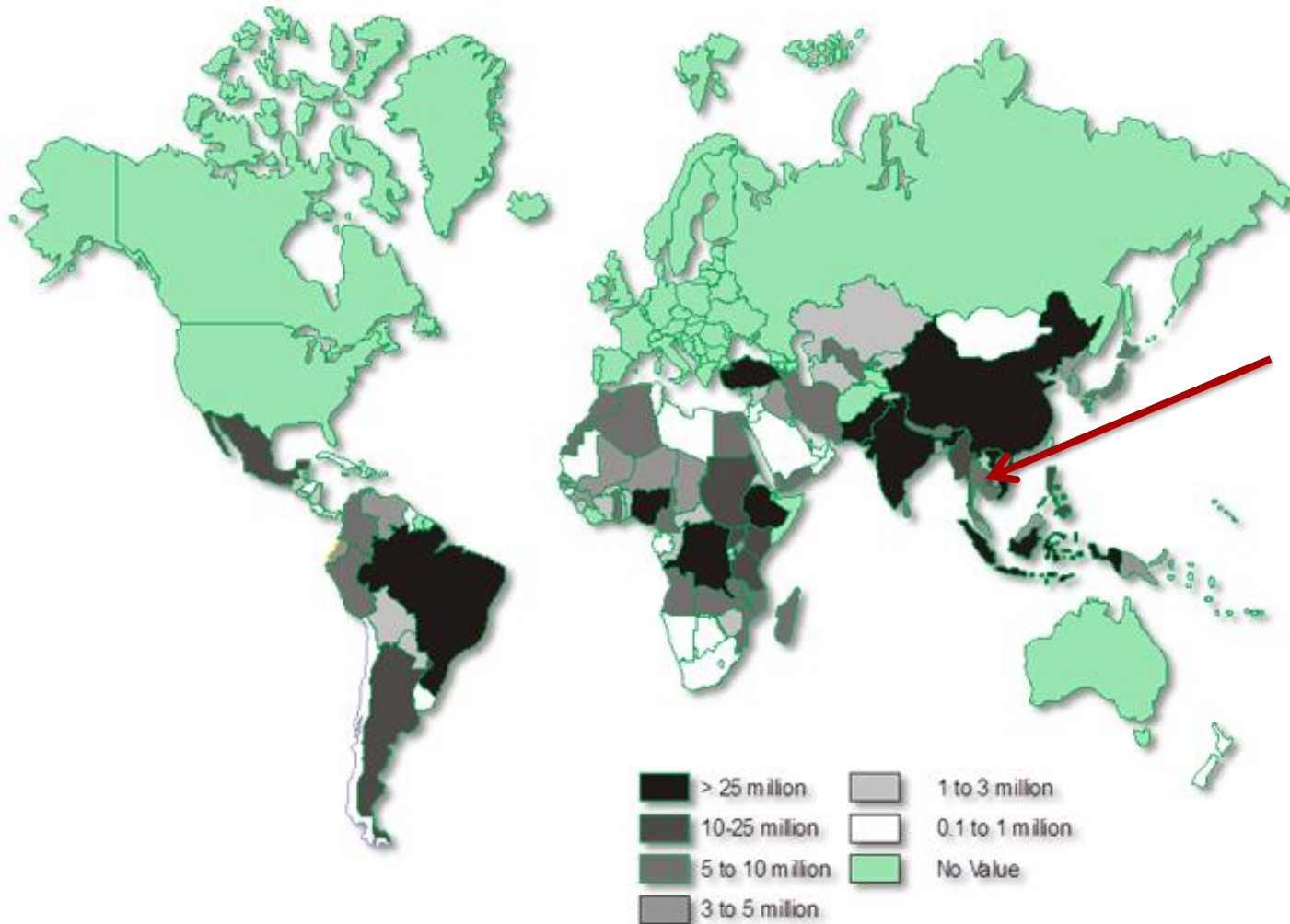


- Located in Southeast Asia, surrounded by Thailand, Vietnam and Laos.
- Population of 14,952,665.
- 31% live below the poverty line.
- Life expectancy for men is approximately 54 and 59 for women.
- Age Structure:
 - 32.2% - 0 to 14 years
 - 64.1% - 15 to 64 years
 - 3.8% - 65 plus

1.1 Billion People in the World Lack Access to Clean Drinking Water



Global Distribution of People Without Access to Clean Water



The Problem

- Over 50% of the rural Cambodian population does not have access to safe drinking water.
- Mortality due to waterborne diseases in Cambodia is high.
- 20% of the deaths of children under 5 are due to waterborne diseases.



Children of the World

- 443 million school days are lost each year due to water related diseases.
- 5,000 Children die each day as a result of diarrhea.
- On average, this equals to one child dying every 20 seconds.



The Problem Continued





The Problem with Sanitation

Water Sources



Pond



Pump



Well

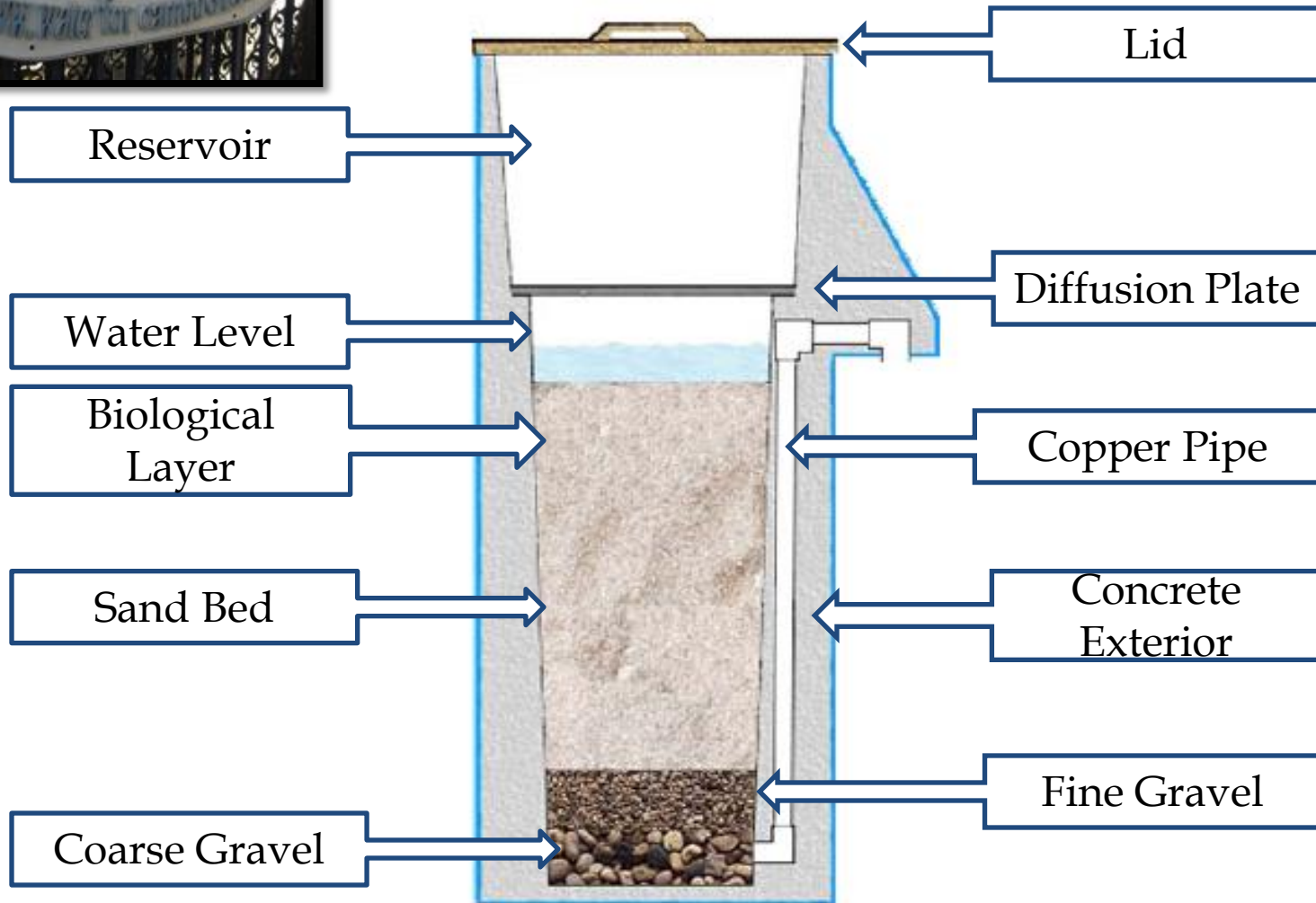
What Can Be Done?

- Water for Cambodia
 - Over 10,000 Biosand Filters installed
 - Literacy program
 - Sanitation classes





Heavy Biosand Filters (HBSF)



Installing Biosand Filters



Installation



The End Result....

- Removes up to 99% of bacteria
- Removes 99.9% of the protozoa
- Removes 70-99% of viruses

- Biosand filters will last about 8-10 years with proper care and maintenance.



The Next Problem



Light Biosand Filters (LBSF)



- Light biosand filters are currently being developed and tested.
- Light biosand filters are a good alternative to the heavy biosand filters because they are lighter in weight, work the same way and are easier to install in the villages.

The Two Projects



- Side-by-side comparison of the HBSF and LBSF to test for effective water filtration.
- Identification and assessment of microorganisms present in the Siem Reap River.

- WFC project has installed thousands of “Point of Use” water filtration system (concrete biosand filters)
- Installation in remote villages is difficult, yet there is a significant need for purified drinking water
- An alternative to the HBSF is a lighter, PVC filter (LBSF)





HBSF vs. LBSF



- A side-by-side comparison of these two filters was conducted in Siem Reap
- The HBSF filters have been shown to decrease the number of *E. coli* cells to a safe level
- *E. coli* is an indicator species of fecal contamination
- Are these LBSF as efficient at reducing *E. coli* as the HBSF?

Water for Cambodia Project in Siem Reap

- Heavy and light biosand filters already in use
- Water samples were collected from the Siem Reap River
- This water was treated by HBSF or LBSF
 - Varying volumes of the treated water was filtered onto a paper membrane and transferred onto media
 - Colonies were counted and colony forming units (CFUs) per 100 milliliters of water could be calculated

We expected a significant reduction in the number of bacteria present in water samples filtered through the LBSF, similar to that seen in water filtered by the HBSF.



If this is the case, then there is the potential for many more POU filtration systems to be installed in these remote villages, including floating villages, thus providing safe drinking water for the families that live there.

Materials & Methods



Traditional Concrete Heavy
Biosand Filter



New Light PVC
Biosand Filter

River Water Collection Siem Reap River



Feed Filters



Measure Flow Rate



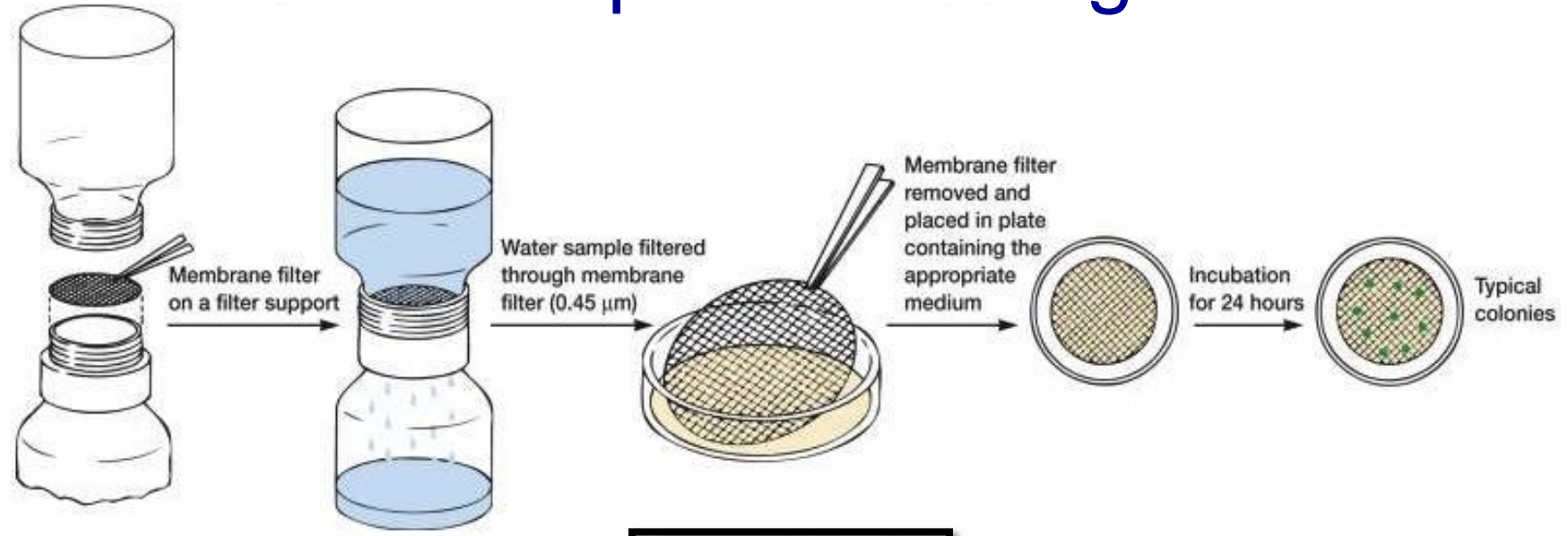
Collection of Treated Water Samples



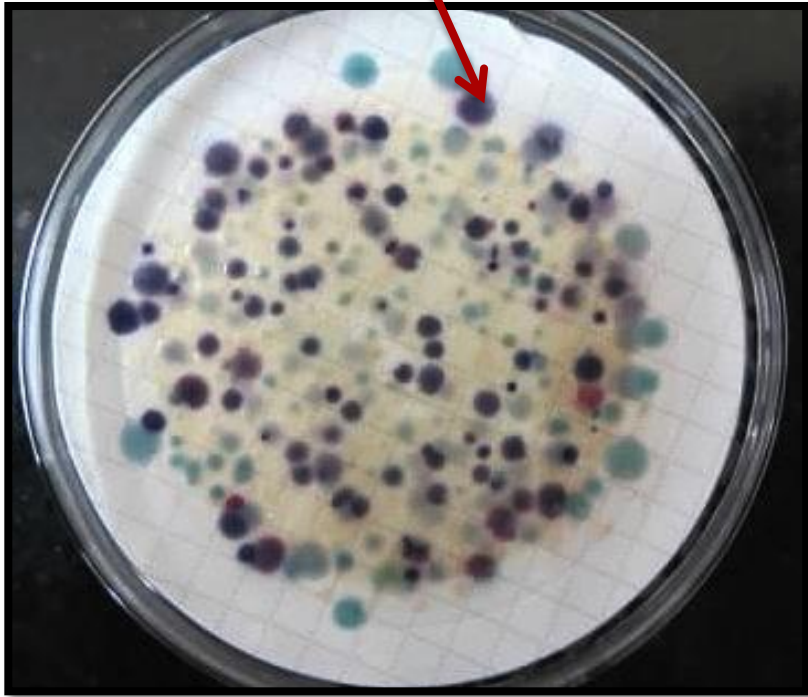
We weren't In Kansas Anymore...



Filtering Treated and Source Water Samples & Plating



Colony Counts



Results: Siem Reap River Water

Source Water	Sample 1	Sample 2	Sample 3
1 mL	53	64	69
2 mL	74	71	22*
5 mL	252	245	262

Bacterial counts for unfiltered water samples from the Siem Reap River.

- Tested untreated Siem Reap River water
- Used 1 mL, 2 mL, and 5 mL quantities
- Compared with treated water samples



Results From HBSF Treated Water

Heavy Biosand Filter	Run #1			Run #2		
Filter 1	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	negative
50 mL	TFTC (8)	TFTC (5)	TFTC (2)	TFTC (4)	TFTC (7)	23
100 mL	TFTC (7)	TFTC (4)	TFTC (8)	34	43	38
Filter 2	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	negative
50 mL	0	0	0	TFTC (3)	TFTC (1)	TFTC (4)
100 mL	0	0	0	TFTC (2)	TFTC (5)	TFTC (8)
Filter 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	negative
50 mL	TFTC (1)	TFTC (1)	0	TFTC (7)	TFTC (6)	TFTC (10)
100 mL	TFTC (4)	0	TFTC (5)	TFTC (7)	TFTC (4)	TFTC (1)

- Heavy Biosand Filter bacterial counts
- 17 of 18 bacterial counts for 50 mL samples were zero or TFTC
- 15 of 18 bacteria counts for 100 mL samples were zero or TFTC



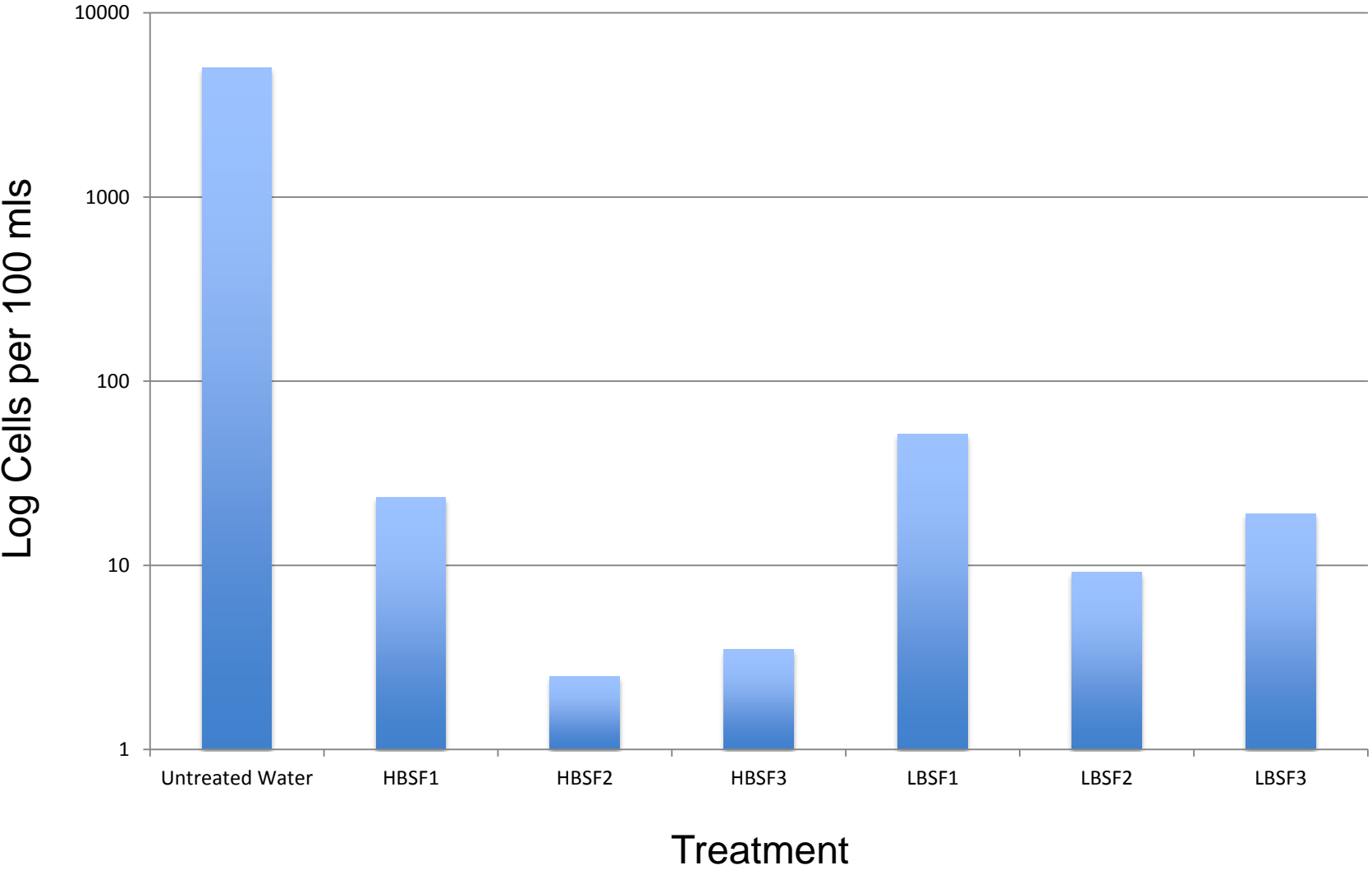
Results From LBSF Treated Water

- Light Biosand Filter bacteria counts
- 13 of 18 bacteria counts for 50 mL samples were TFTC
- 11 of 18 bacteria counts for 100 mL samples were TFTC

Light Biosand Filter	Run #1			Run #2		
Filter 1	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	negative
50 mL	TFTC (14)	TFTC (6)	TFTC (18)	64	62	51
100 mL	TFTC (19)	27	TFTC (19)	91	80	74
Filter 2	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	negative
50 mL	TFTC (1)	TFTC (2)	TFTC (2)	TFTC (8)	TFTC (7)	TFTC (7)
100 mL	TFTC (2)	TFTC (8)	TFTC (3)	TFTC (11)	TFTC (15)	TFTC (16)
Filter 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Blank	negative	negative	negative	negative	negative	positive (3)
50 mL	TFTC (4)	TFTC (6)	TFTC (7)	TFTC (16)	24	21
100 mL	TFTC (8)	TFTC (8)	TFTC (10)	24	33	31



What All of This Data Means.....



Identifying Pathogenic Bacteria from the Siem Reap River

- Currently only testing for *E. coli* which serves as an indicator species for fecal contamination.
- Many more organisms belonging to the families *Enterobacteriaceae* and *Vibrionaceae*, which have also been shown to cause gastrointestinal disease.
- Little is known about microbial community, including other pathogens found in the water source.
- Goal is to identify these organisms.

Inoculating Broth Cultures

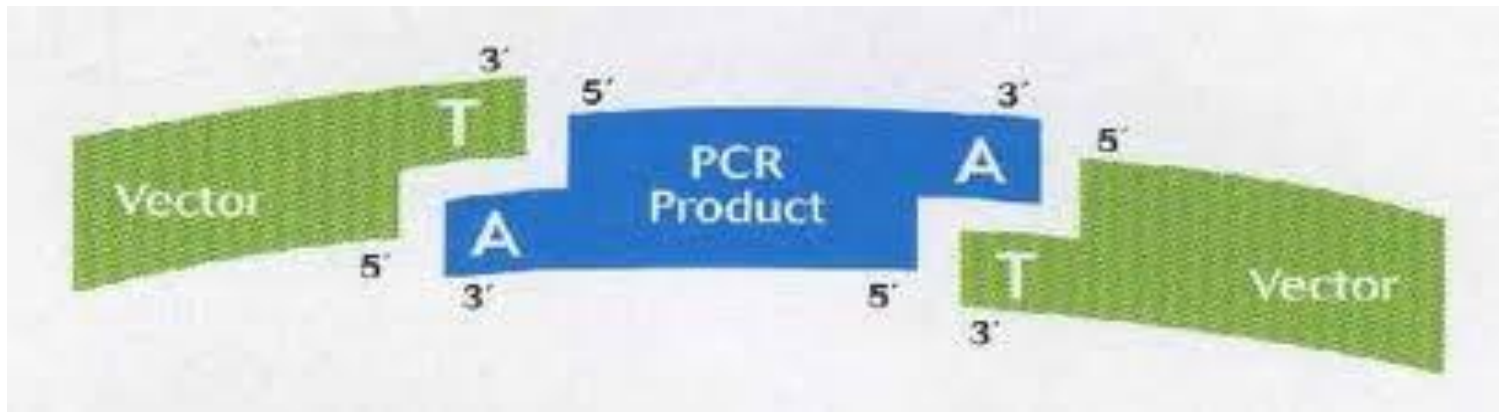


Building a Clone Library

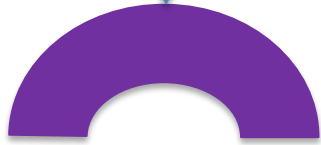
1. Extract total community DNA from your sample (soil, water, gastrointestinal contents, etc)
2. PCR amplify 16S rRNA gene in triplicate
3. Run PCR on gel to confirm specific amplification
4. Ligate PCR products into plasmids
5. Transform *E. coli* with plasmids
6. Grow *E. coli* to high cell density
7. Extract Plasmids and Sequence

Cloning Reaction - Ligation

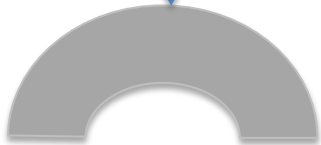
- Ligation between linearized plasmid and PCR product
 - overhanging 3' "T" of vector lines up with overhanging "A" at 3' end of PCR product
- Each plasmid vector only takes up one 16S rRNA gene from the PCR reaction



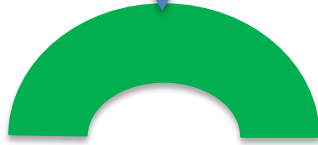
16S rRNA gene
from Organism 1



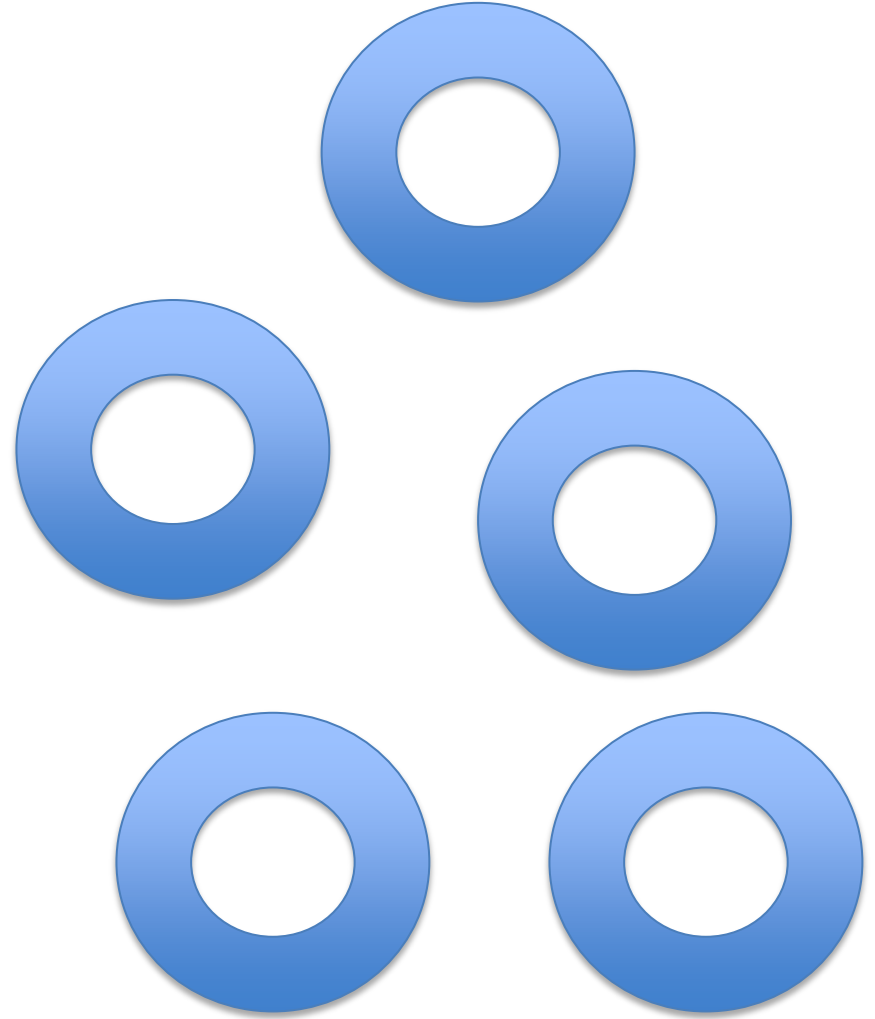
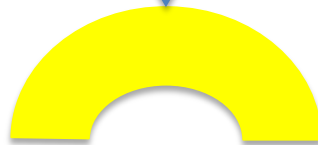
16S rRNA gene
from Organism 2



16S rRNA gene
from Organism 3



16S rRNA gene
from Organism 4



Cloning Reaction - Transformation

- After we have the 16S rRNA genes ligated into the plasmids, we need to grow these plasmids to high copy number
- Use *E. coli* to do this
- Each *E. coli* cell only takes up one plasmid
- Spread *E. coli* onto selective media, and each colony is comprised of cells that contain a plasmid with one “type” of 16S rRNA gene

Cloning Reaction - Transformation



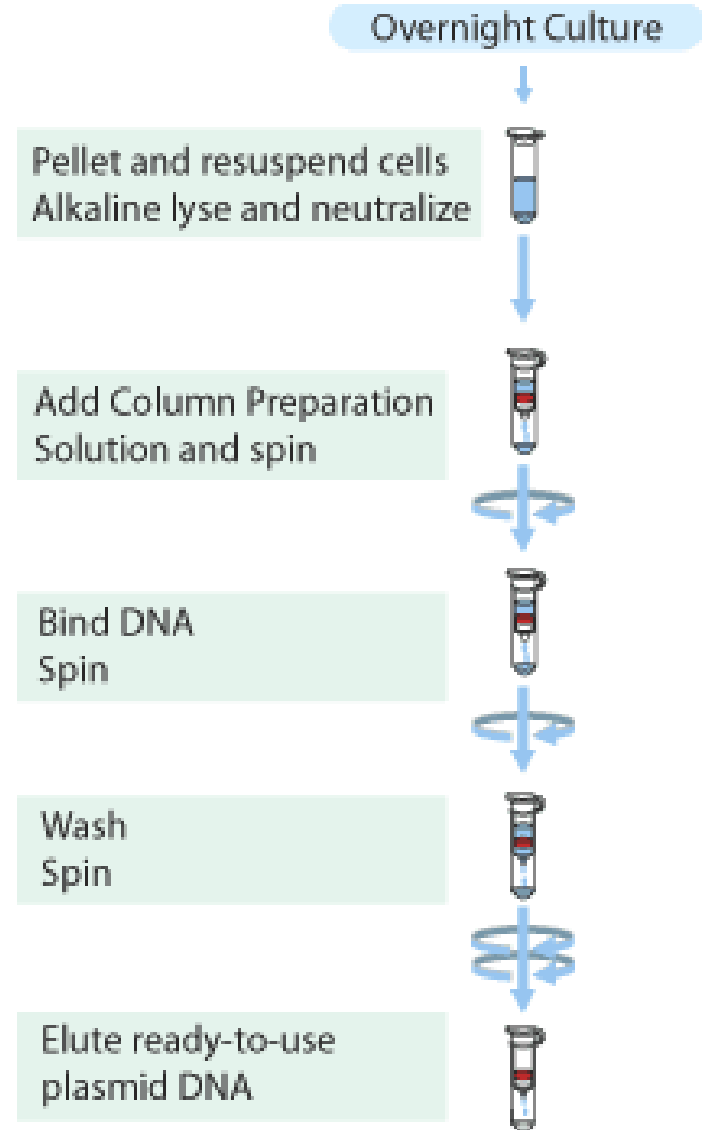
Screening Colonies for Insert

- White colonies have taken up plasmid + insert
- Blue colonies have taken up plasmid - insert
- White colonies screened for insert using plasmid specific primers (confirmation)

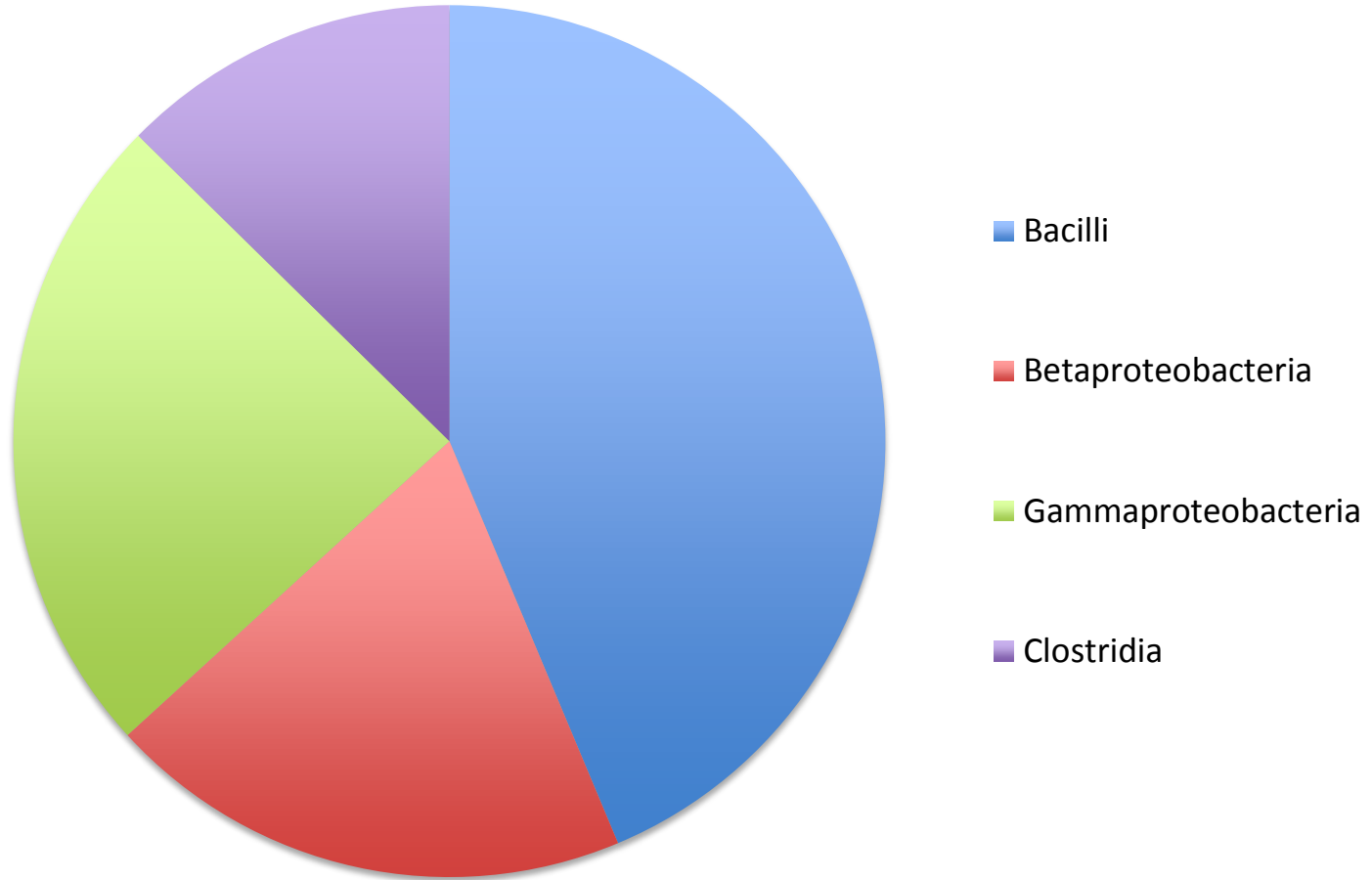


Now what?

- Select white colonies and and grow ON in broth with ampicillin
- Spin cells down and isolate plasmids
- Qiagen QIAprep Spin Miniprep Kit
- Spec to obtain appropriate concentration
- Send samples for sequencing
- Analyze sequences



Classes of Bacteria in the Siem Reap River



Conclusions

- HBSF have been shown to reduce *E. coli* but they can be difficult to install in remote villages
- LBSF have the potential to be a beneficial alternative
- There is still a lack of information with these LBSF
- LBSF are reducing *E. coli* that we see in the water, however further testing needs to be done to test if these filters are as effective at filtering out bacteria as HBSF

Conclusions

- Previous studies have shown that the predominant organisms found in fecal-contaminated water include *Vibrio* species, *E. coli*, *Streptococci*, *Staphylococci*, *Salmonella* species, and *Bacillus*.
- Of the organisms examined in our clone library, many are water borne pathogens..
- By understating the distribution of the pathogenic microbial contaminants within the water source, we can better identify the sources of contamination.
- In the future, this information can be applied to assess the overall risk this water source presents to human health as well as develop appropriate treatment protocols.

Awe Coon Cheran

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QUESTIONS?



Ot Benya Haa!



