Household Drinking Water is a Source of Clinically Relevant Cystic Fibrosis Opportunistic Bacterial Pathogens

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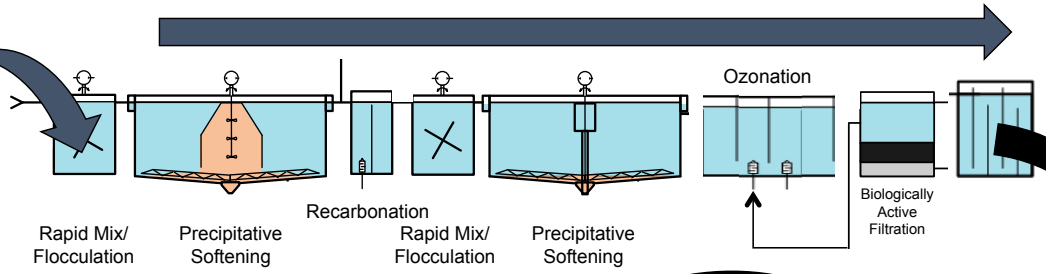
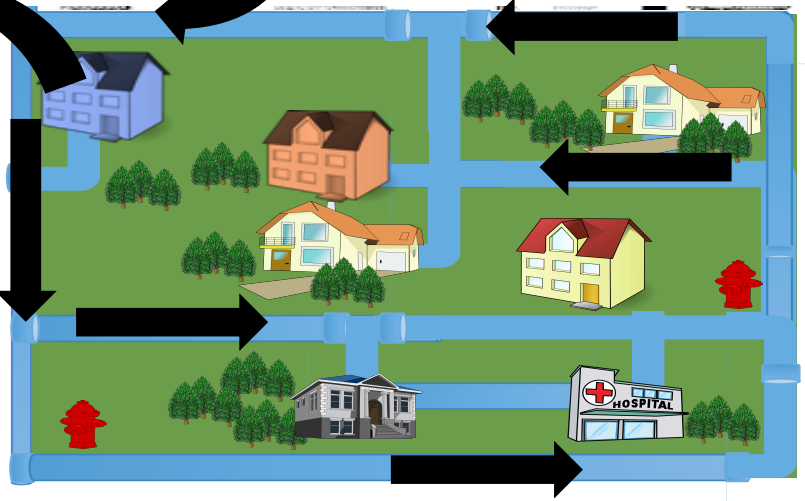
Drinking water is a source of opportunistic bacteria

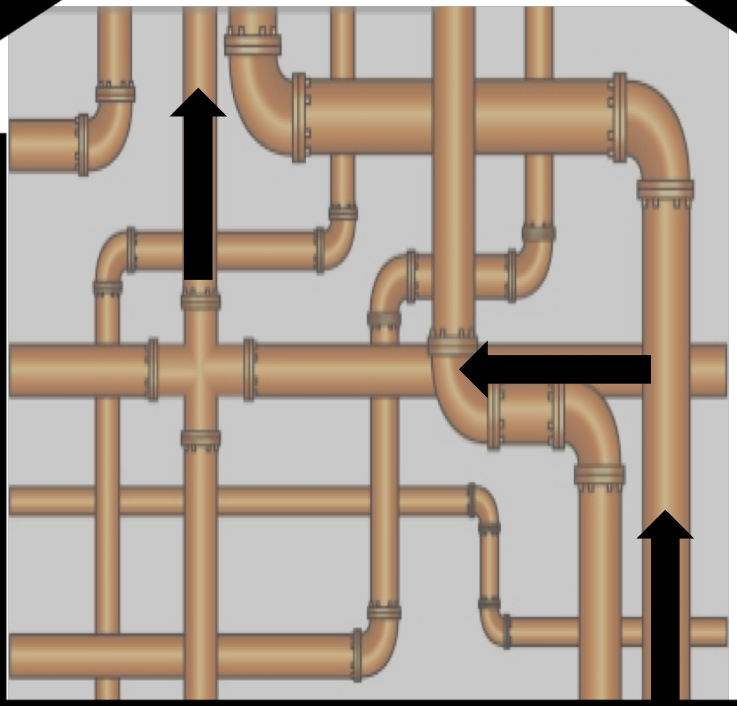
Drinking Water Treatment Plant



Distribution System



Premise Plumbing



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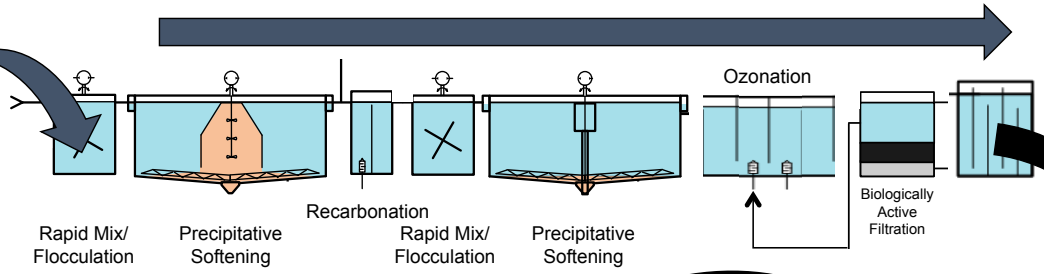

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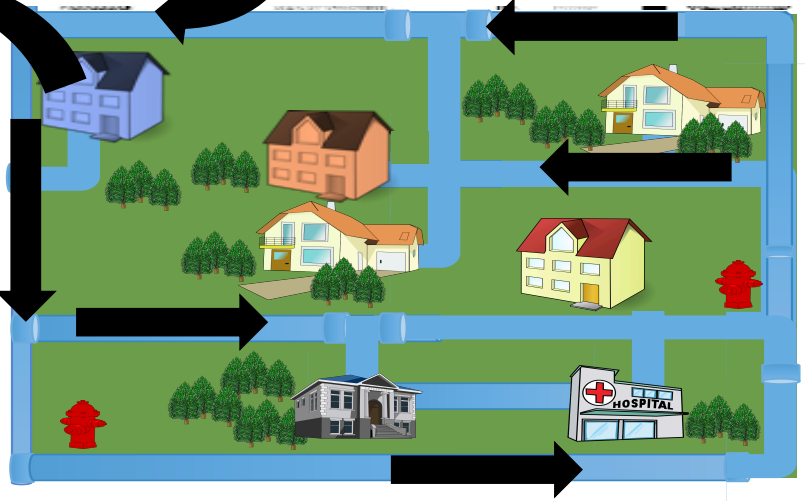

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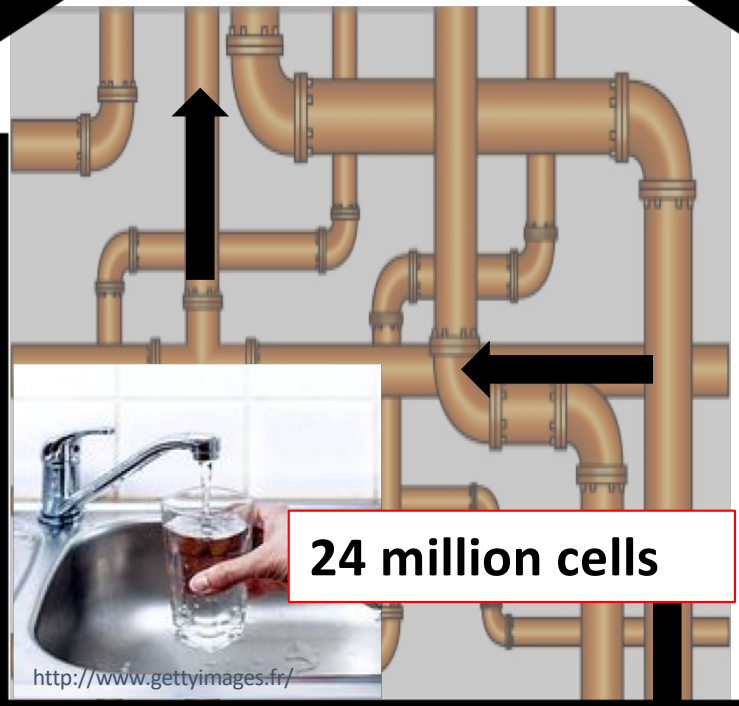

24 million cells

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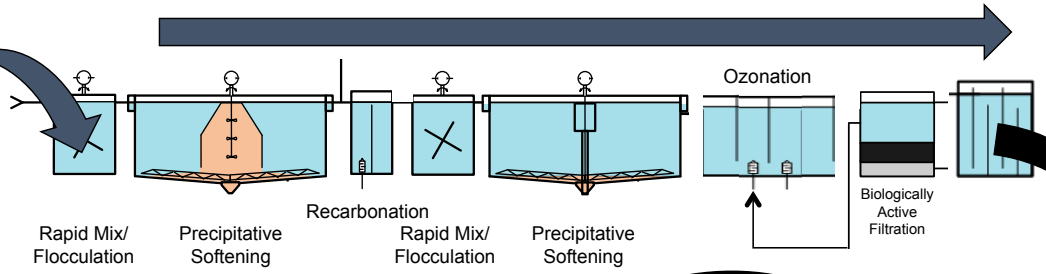

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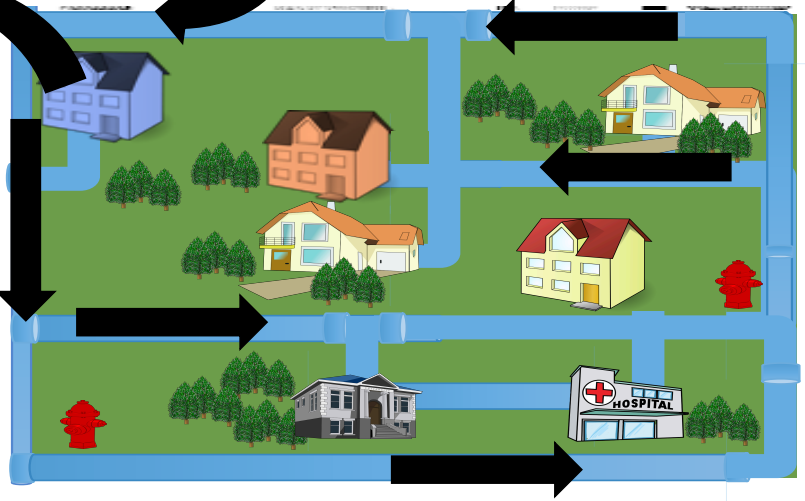

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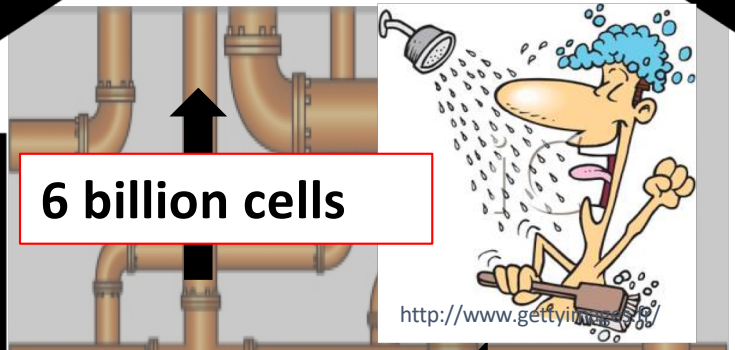

6 billion cells

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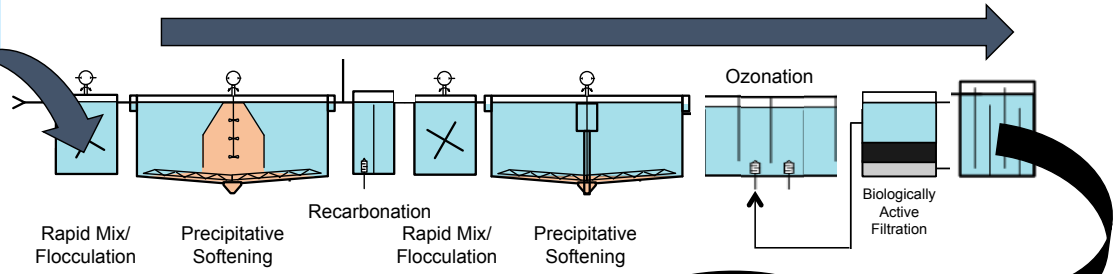

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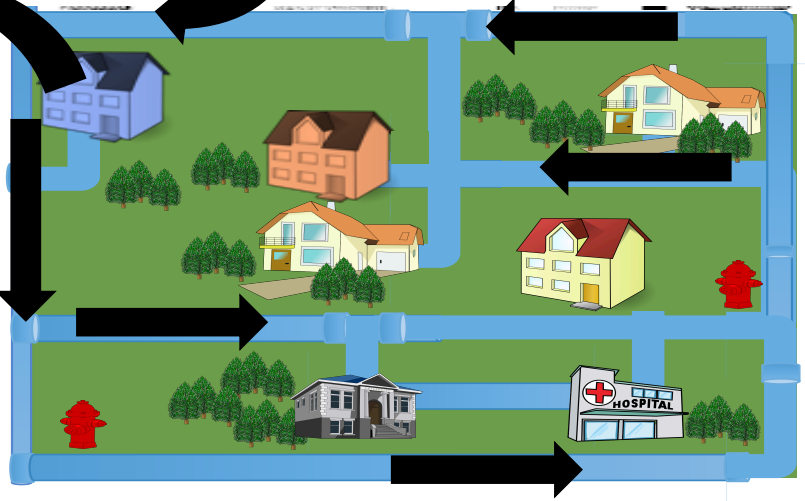

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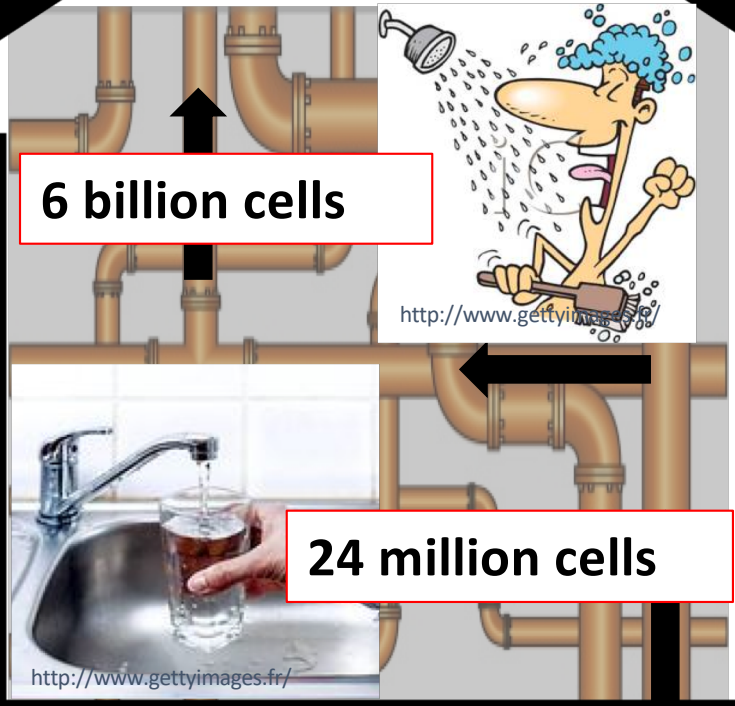

Opportunistic Pathogens
Pose a threat to the immunocompromised

- Very young
- Sufferer of chronic disease
- Very old

Cystic fibrosis predisposes individuals to opportunistic bacterial infections

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3. Nontuberculous mycobacteria
4. *Pseudomonas aeruginosa*
5. *Stenotrophomonas maltophilia*

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- Unclear where opportunists come from - environment

Opportunistic pathogens are found throughout the drinking water transect in non Cystic Fibrosis homes

- **Linear effect models indicate that distribution system & home specific factors influence opportunists' abundance**

Achromobacter	PP material[b] + Iron [c]+ *S. maltophilia*[a] + Orthophosphate[e] + Water age[c] Explains 73%
Burkholderia	Iron[b] ± Water age[a] + *P. aeruginosa*[c] – Nitrite[d] Explains 90%
Mycobacterium	Iron[b] – Water age[d] ± Pressure zone[c] ± Water type[a] + Total 16S[e] Explains 95%
P. aeruginosa	CT[d] + Iron[c] + *Burkholderia*[a] ± Water age[b] - pH[e] Explains 74%
S. maltophilia	Iron[b] – Home age[d] + *Achromobacter*[a] + *Burkholderia*[c] + Copper[e] Explains 64%

CT = Disinfectant concentration (C) x residence time (T)

Haig, SJ. *et al.,* under review

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  - unable to detect opportunistic pathogens:
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    - in the viable but non-culturable state

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- **Recently developed a high-throughput molecular approach allowing species & strain level identification of *Mycobacterium* in drinking water**

Haig et al., (2018) mBio 9(1)

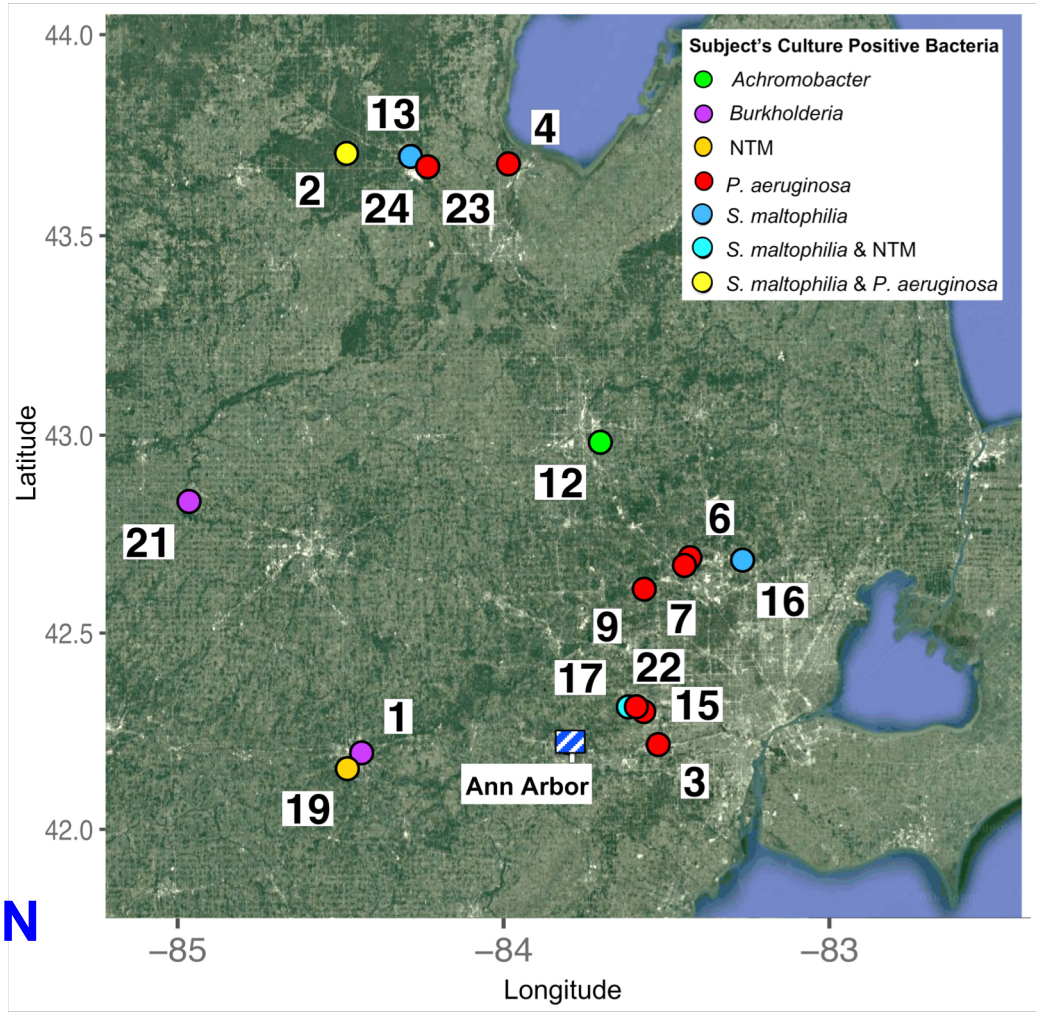
RESEARCH QUESTION

Is there a link between strains of opportunistic pathogens in drinking water and strains causing infection in persons with cystic fibrosis?

Approach to assess the link between strains causing infection in cystic fibrosis & strains in drinking water

PARTICIPANTS

- <18 years old
- Living in Michigan
- Infection for the first time
  - *Achromobacter*
  - *Burkholderia*
  - Nontuberculous mycobacteria
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*



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- Sample subject's home drinking water

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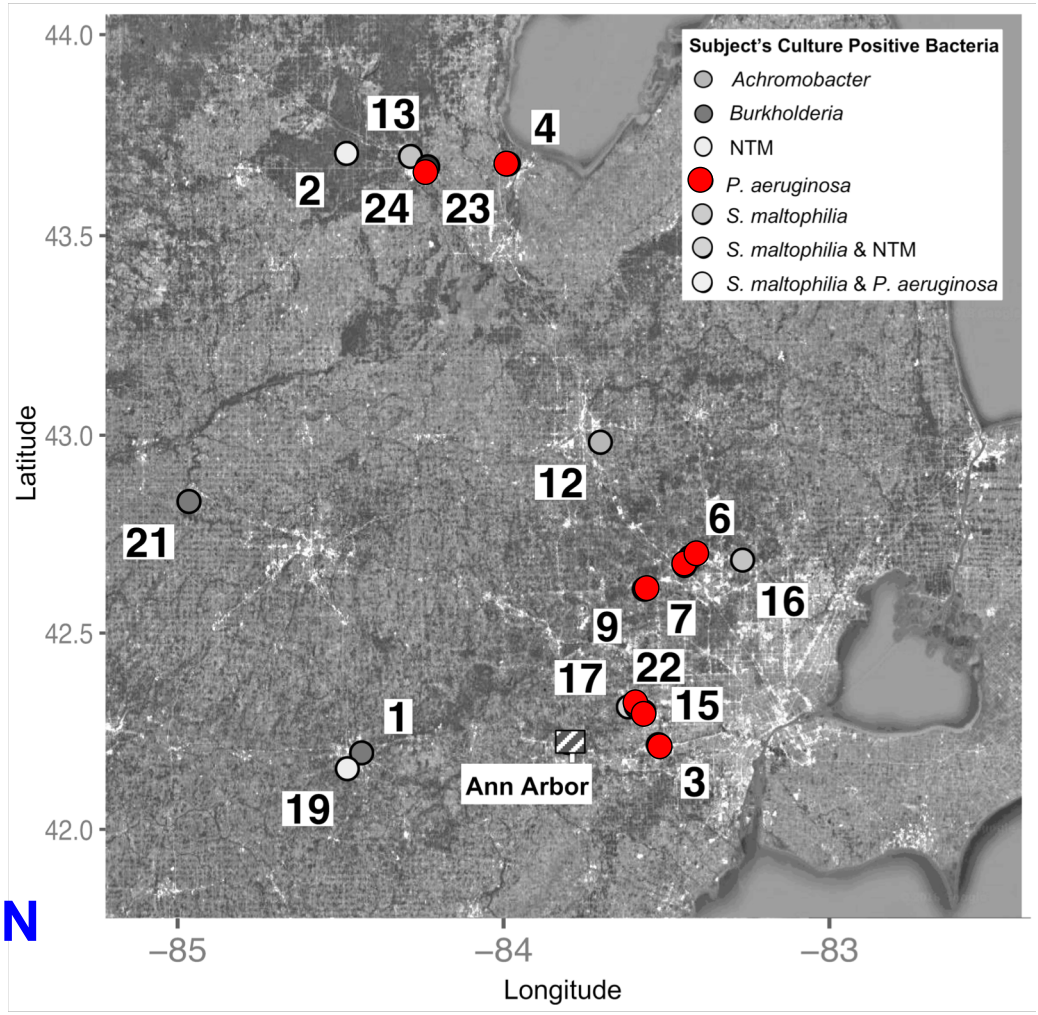

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In home sampling after ≥6 hours of water stagnation

KITCHEN

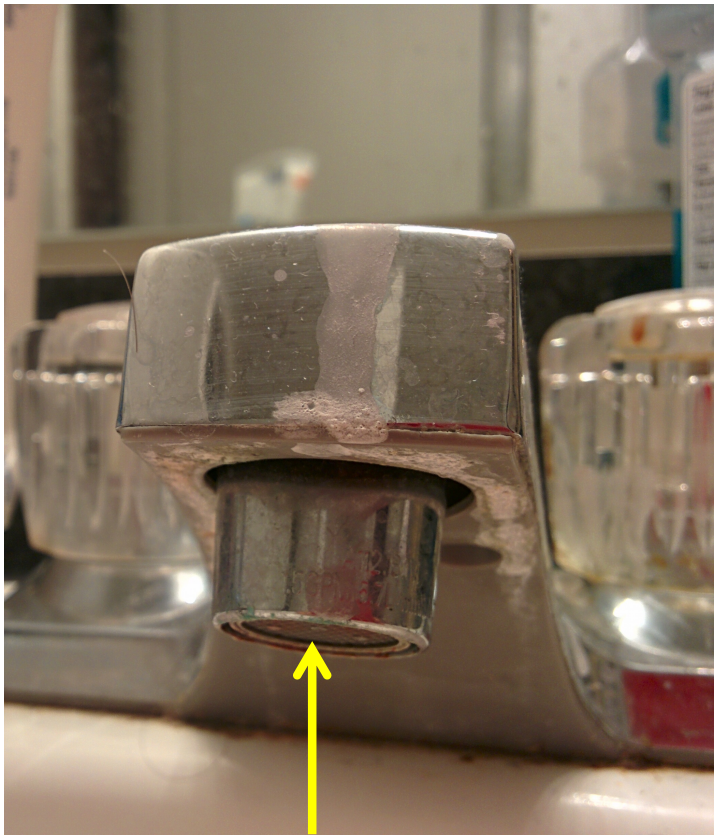
Remove faucet head

Swab aerator

First 1L premise plumbing cold water

In home sampling – additional sampling locations determined by "Water Use Survey"

- **Survey helps identify additional locations:**
  - **faucet patient interacts with the most – typically bathroom**
  - **Shower or bath**

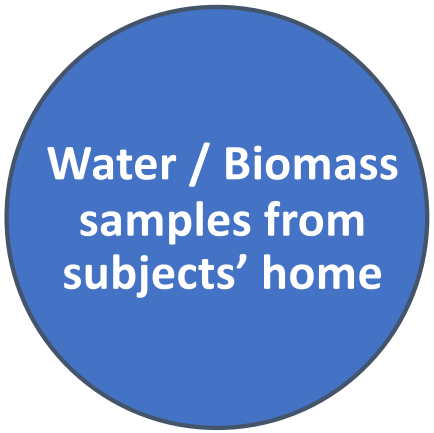


Swab aerator

1 L cold water

Shower or bath
4L Hot water

Samples are processed using culture based



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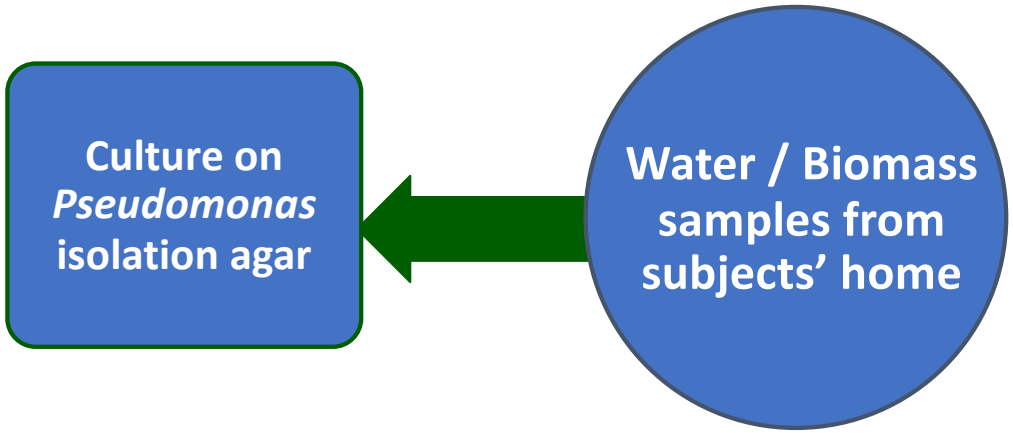

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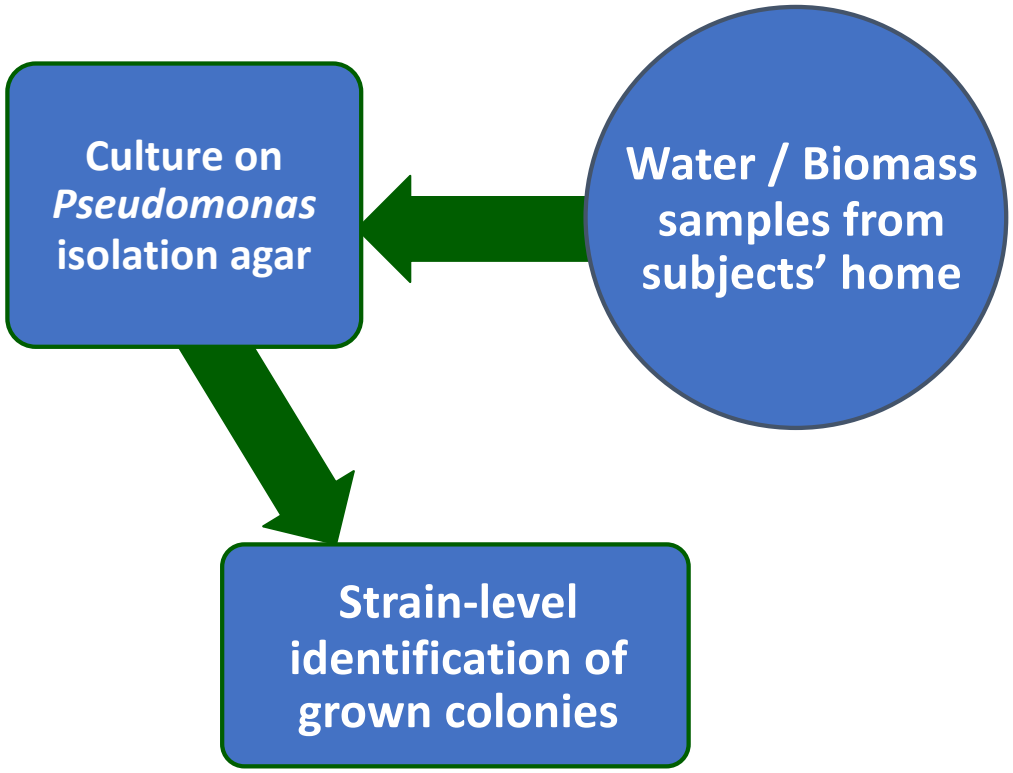

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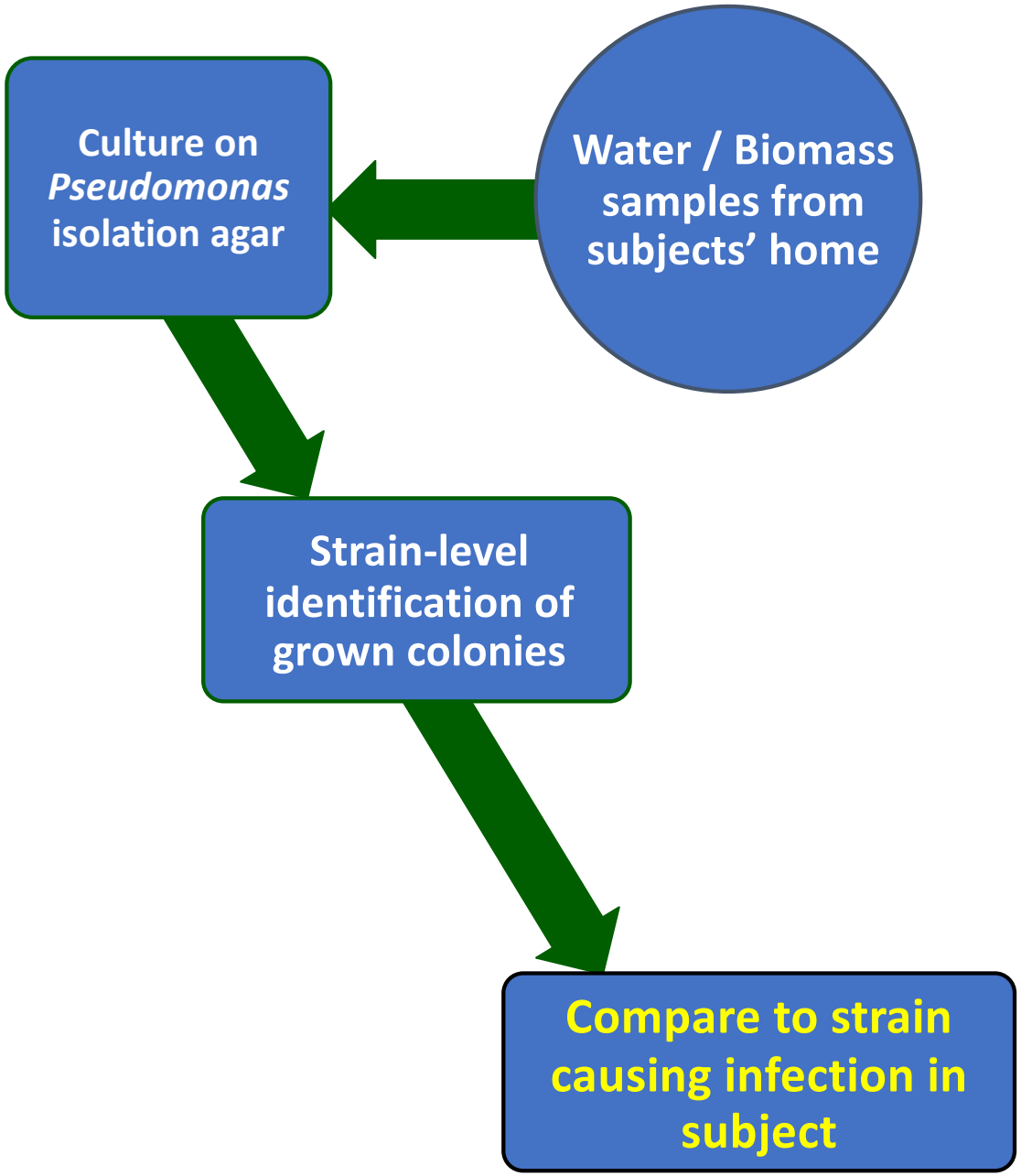

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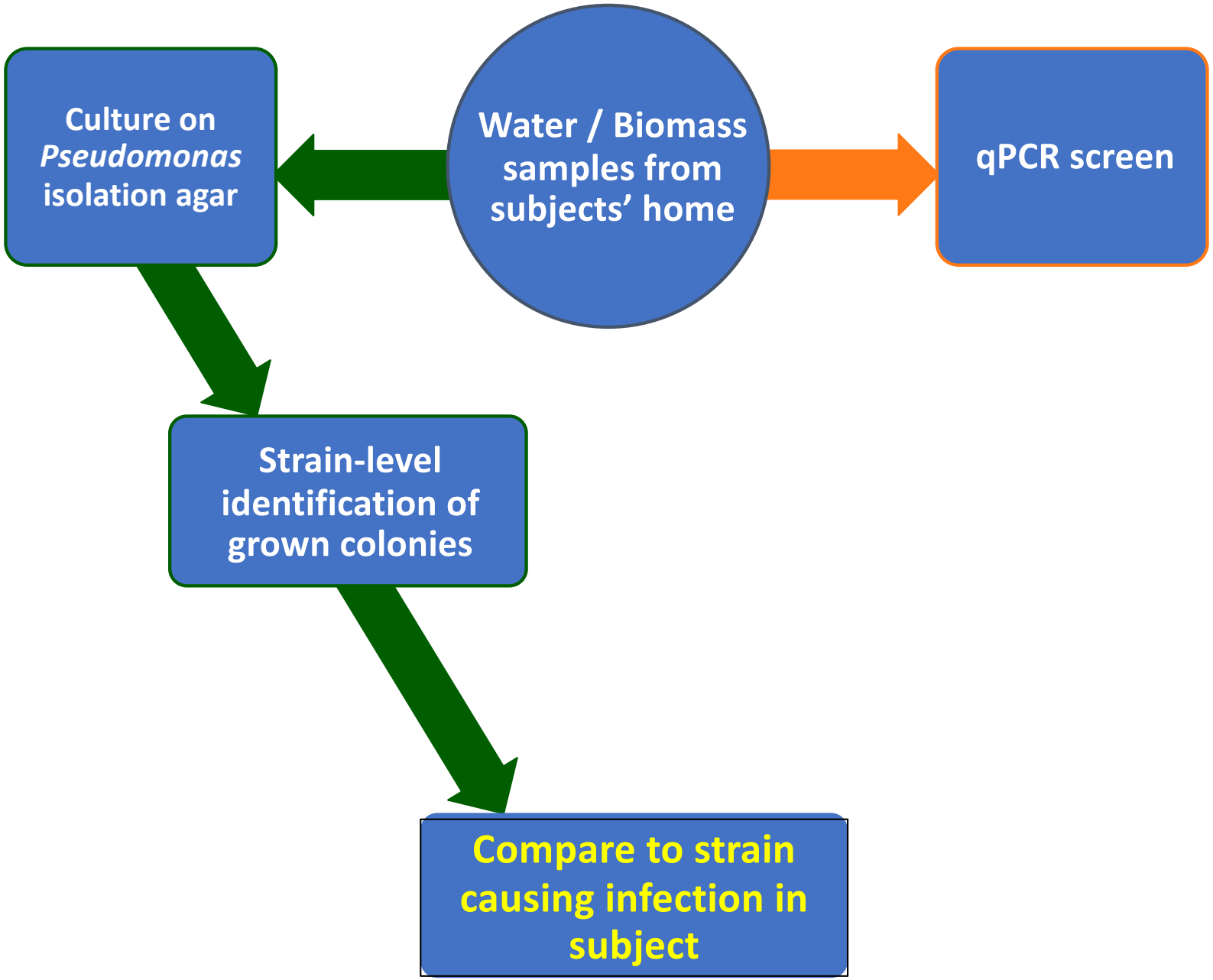

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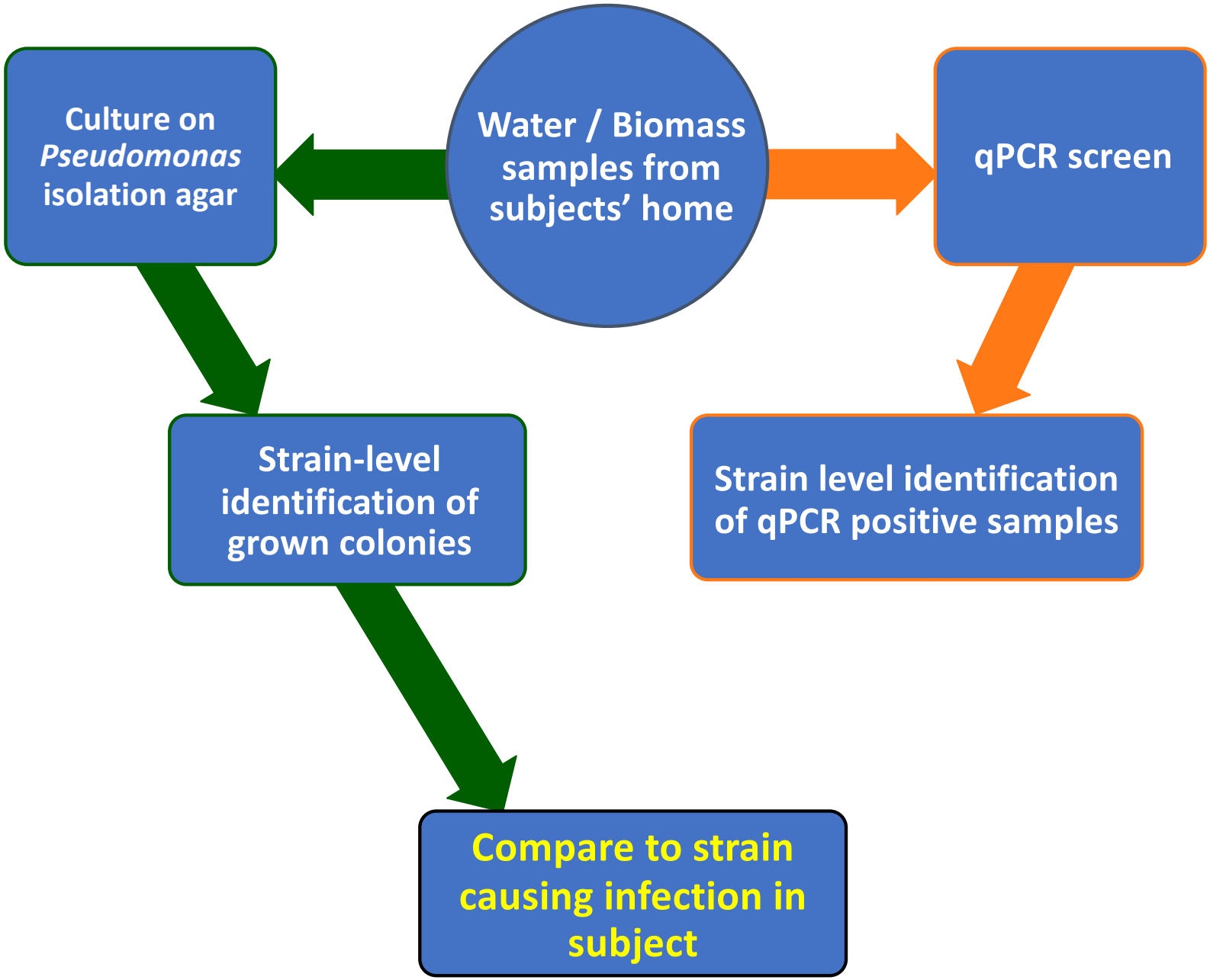

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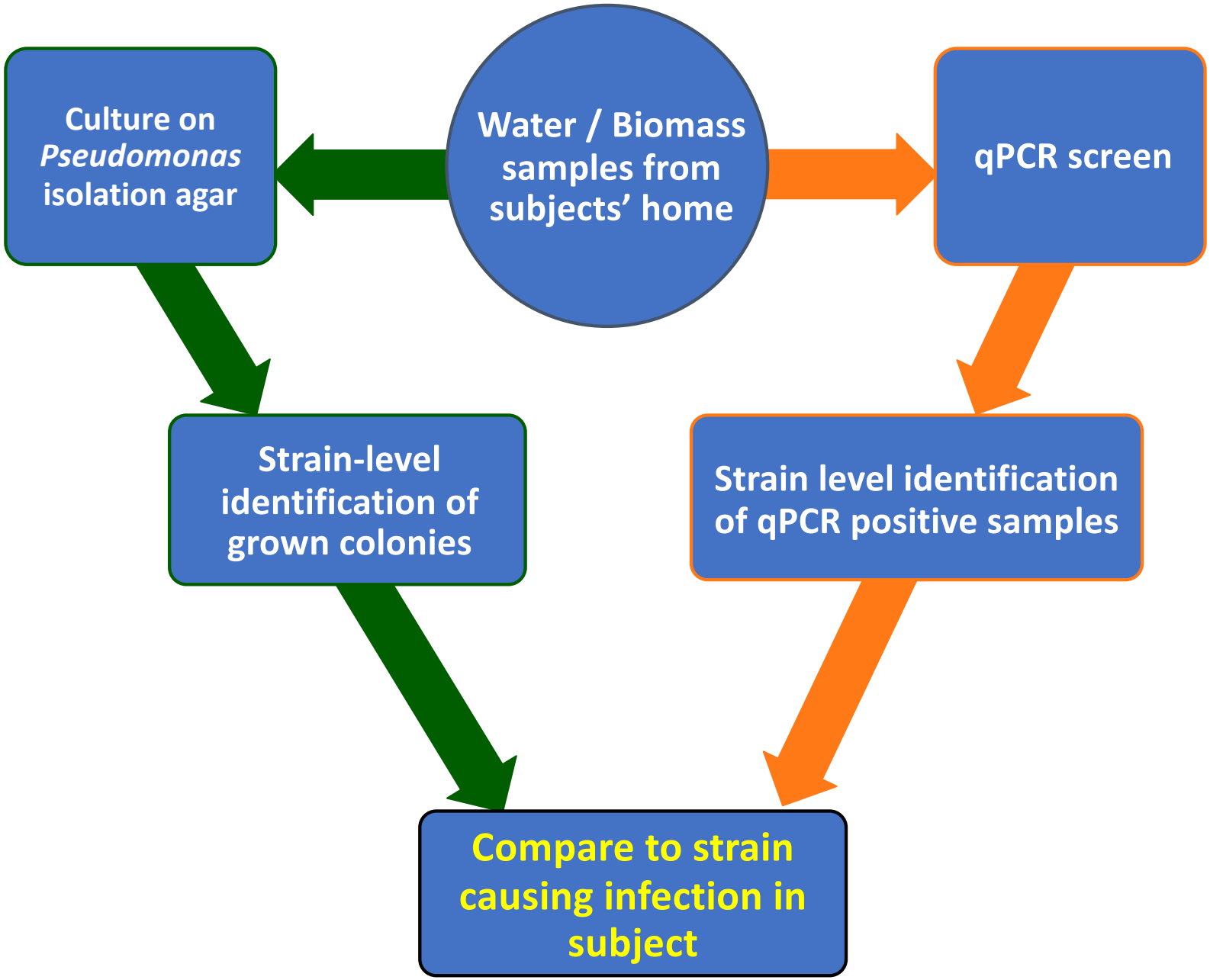

Drinking water is a source of closely related strains which are causing infection in cystic fibrosis patients

Opportunistic pathogen infecting subject	Percentage of matches between drinking water and clinical strain		Target	
	Exact	Closely related	Size (bp)	Gene
Pseudomonas aeruginosa	13%	70%	855	MARP
Achromobacter spp.	50%	0%	954	*nrdA*
Burkholderia spp.	67%	0%	1043	*recA*
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- **20% of subject's clinical isolates were found in their drinking water**
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Haig et al., (2018) Pediatric Pulmonology 53

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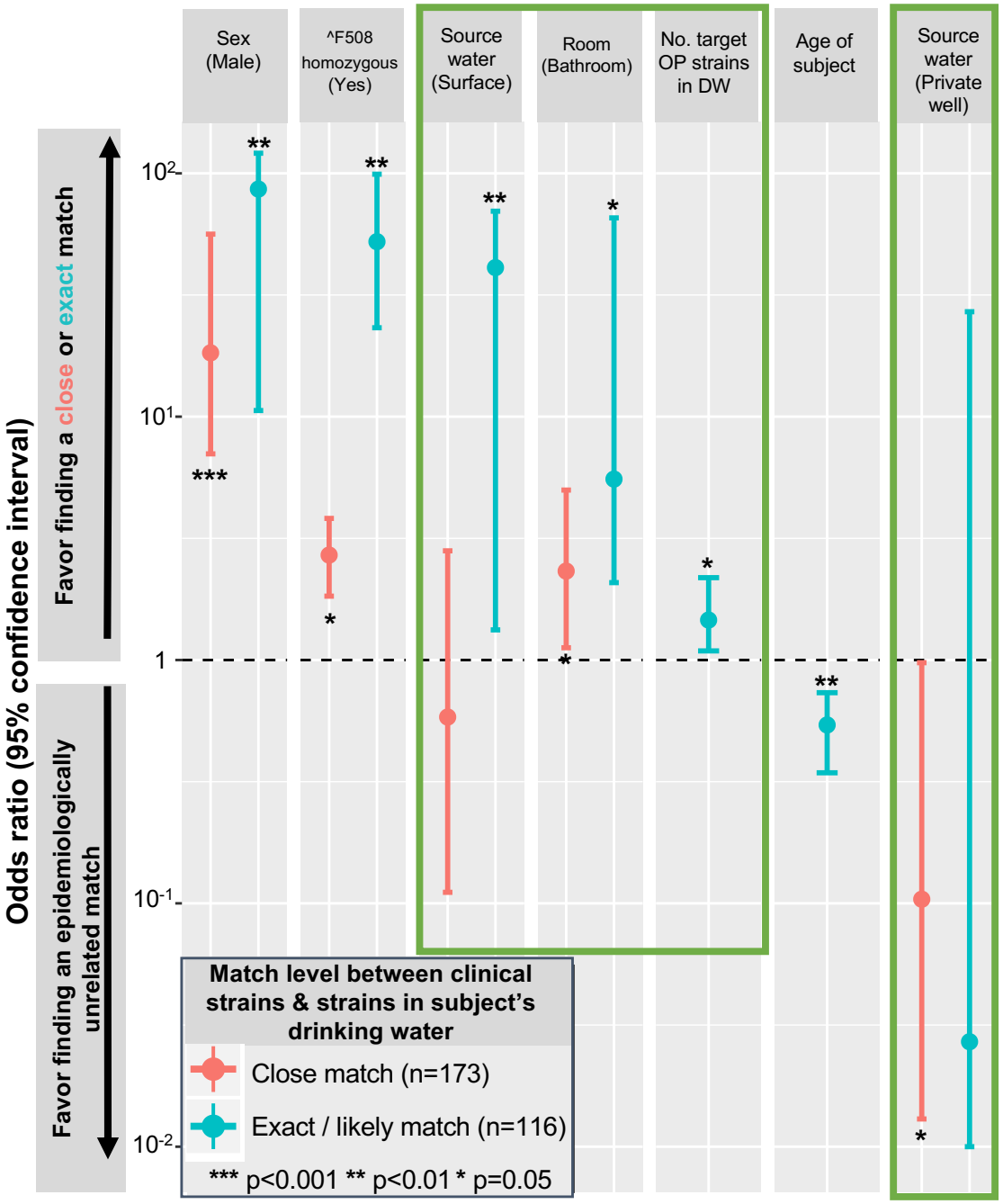
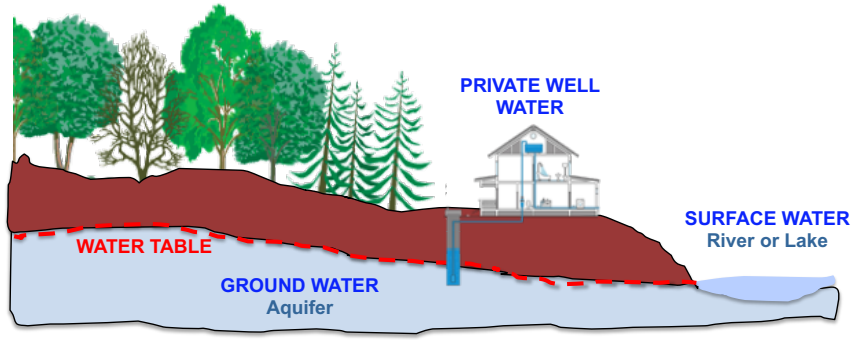
- 90% of subjects' water had at least one strain <3 SNPs different to their subject's clinical isolate
- Potential reasons for few exact match:
  a) Sampling at one point in time
  b) Bias introduced by clinical laboratory
  c) Hypermutation

Haig et al., (2018) Pediatric Pulmonology 53

Subject specific & drinking water related factors impact odds of finding a subject's clinical strain in their water





Haig, SJ. *et al.,* in preparation

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- **74% of exact matches were found in bathrooms**

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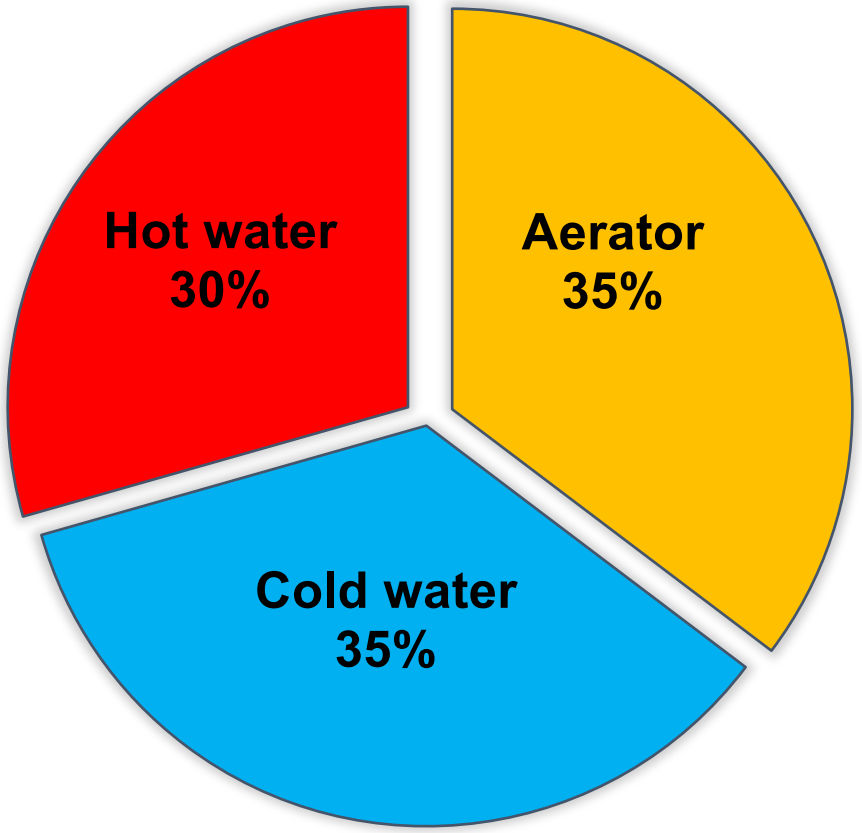

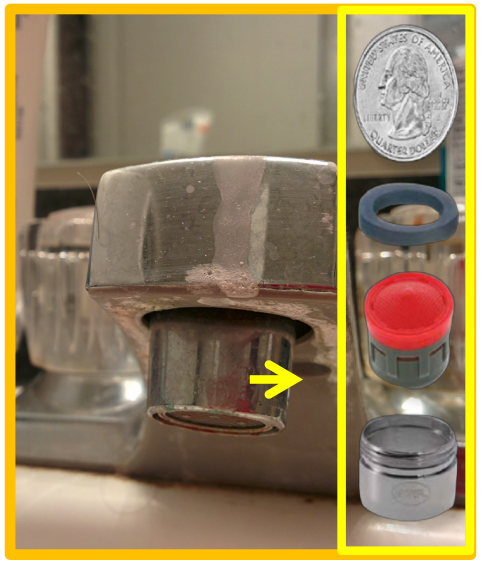

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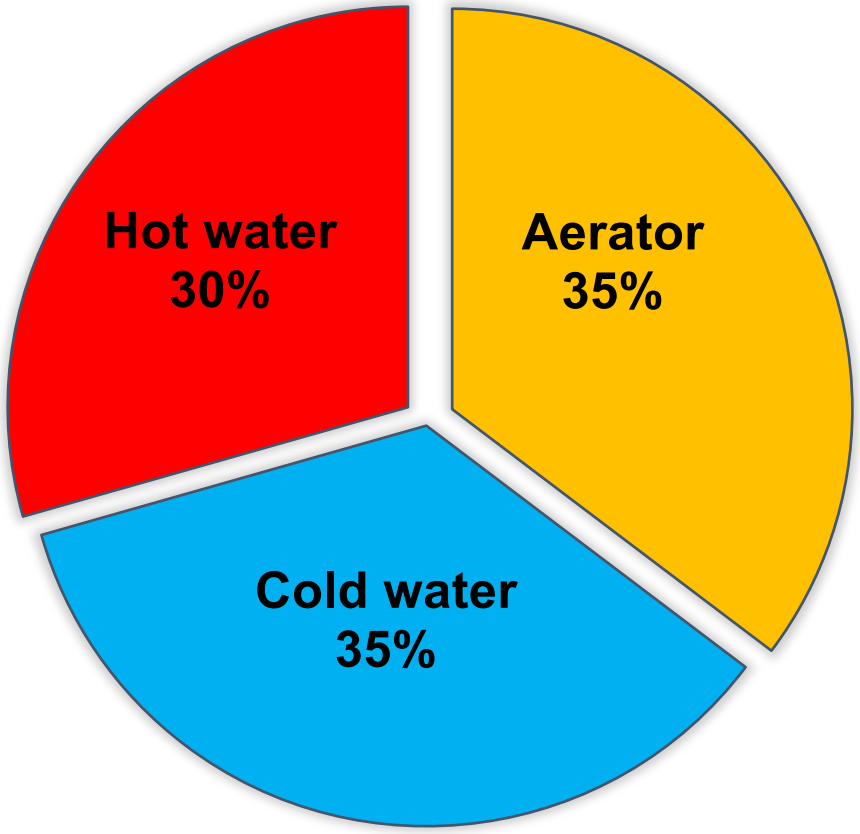
Concentration of match

~ 1300 / 8 min shower



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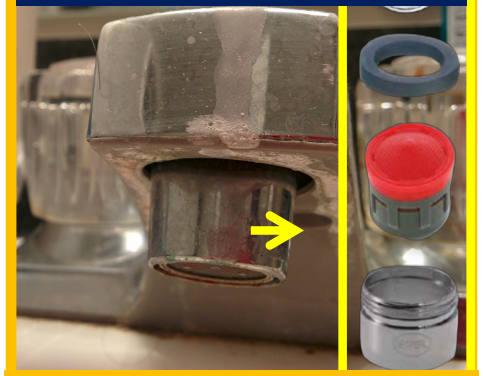

~ 1 x 10^8 / g of biomass

Concentration of match

~ 470 / L

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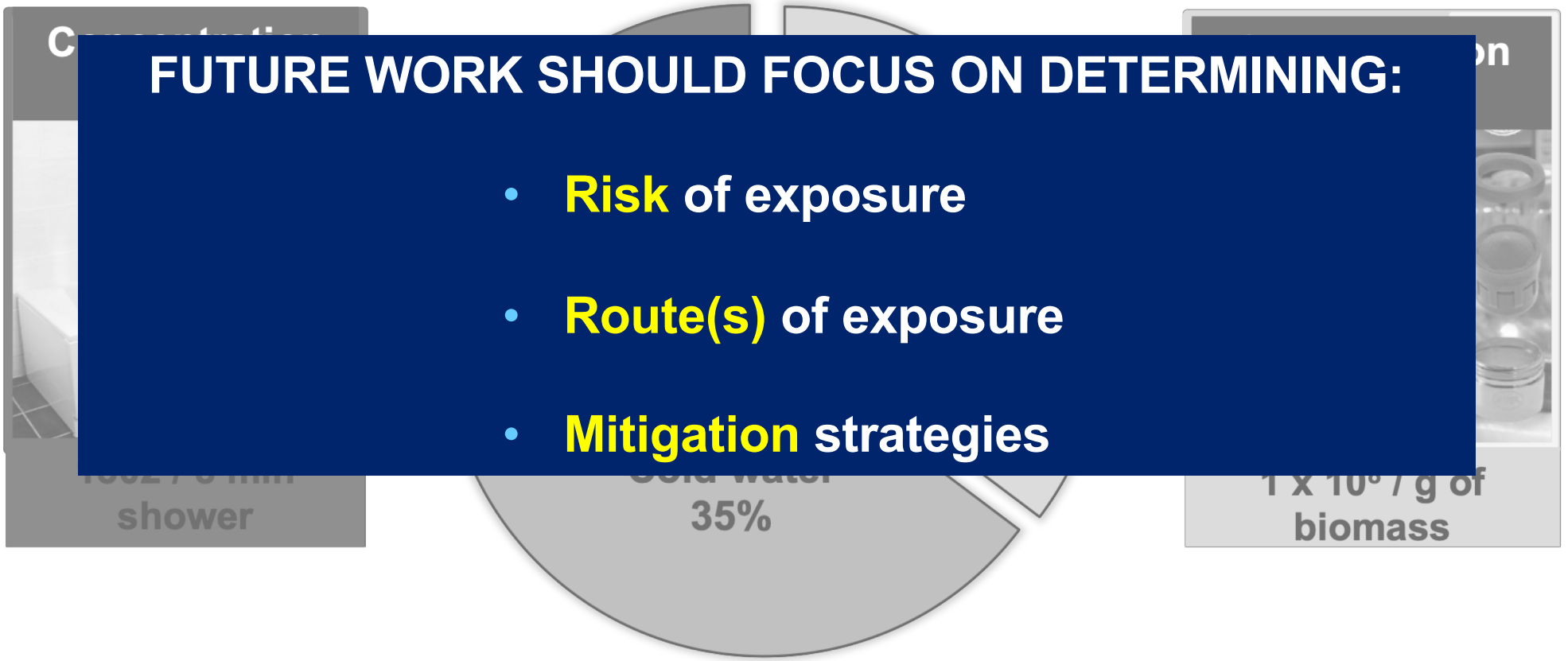
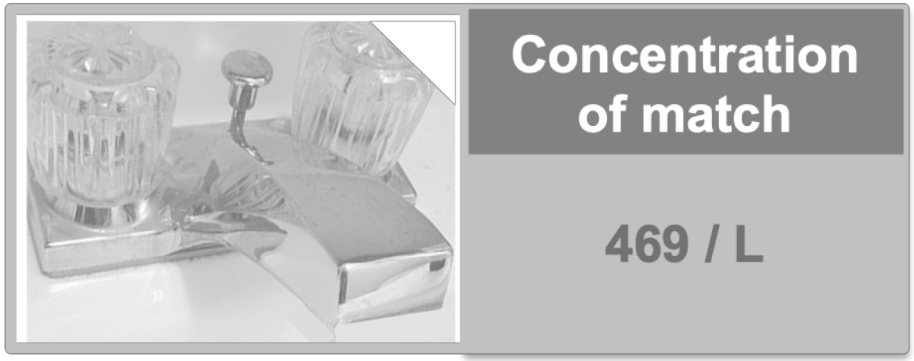


FUTURE WORK SHOULD FOCUS ON DETERMINING:

- **Risk** of exposure
- **Route(s)** of exposure
- **Mitigation** strategies



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FINDING

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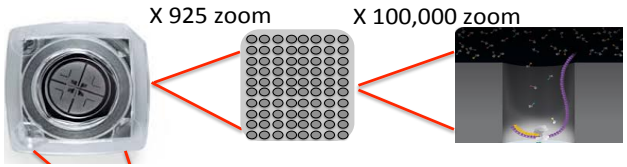
Conclusions & Next steps

- **Developed approach that provides fast & accurate strain-level screening of tap water**



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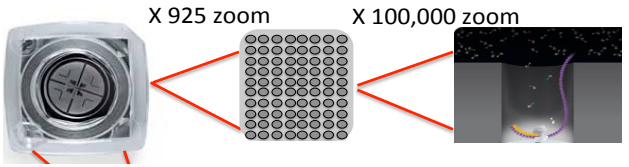

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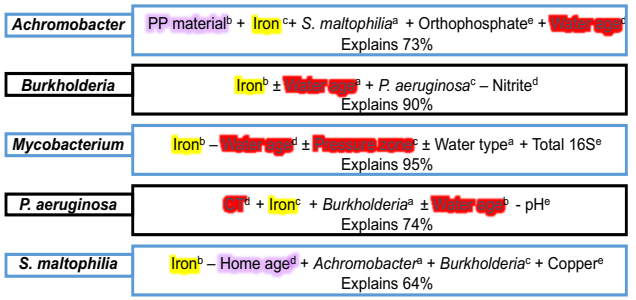
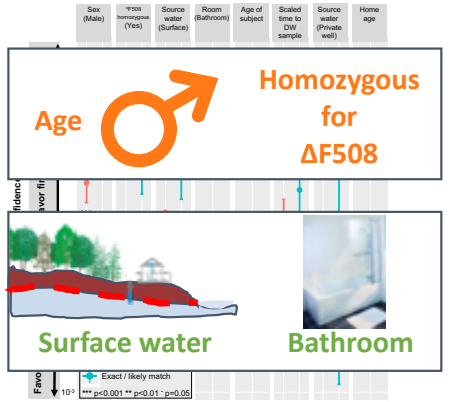
- **Important Factors Influencing:**
  - <u>abundance</u>: Iron concentration, PP material, CT, Water age and Pressure zone



  - <u>odds of finding a clinical match</u>: genetic & drinking water environment factors



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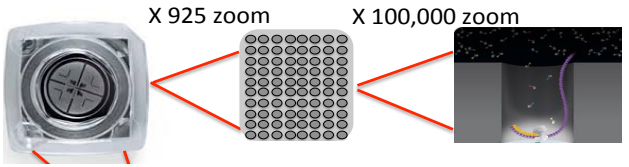

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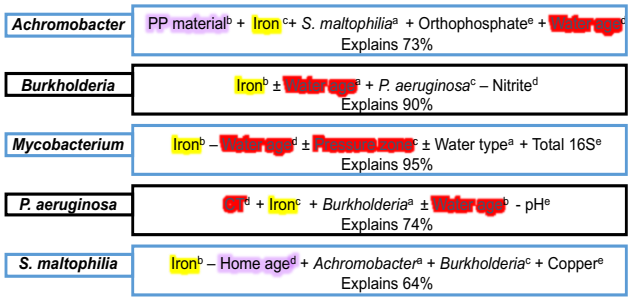

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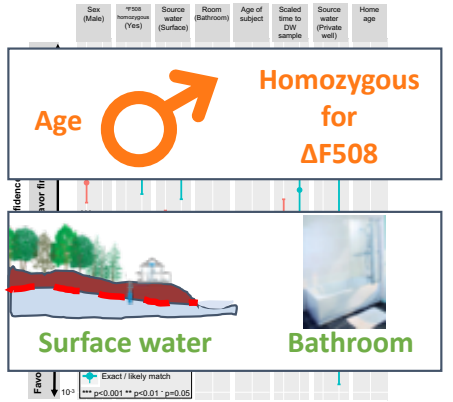
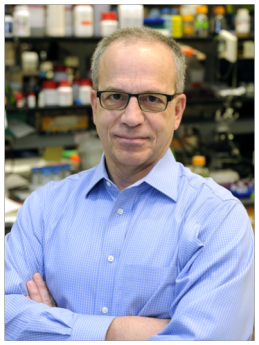


Next steps –quantify the risk of different routes of exposure and assess mitigation approaches

Acknowledgements

All the subjects who participated in our study

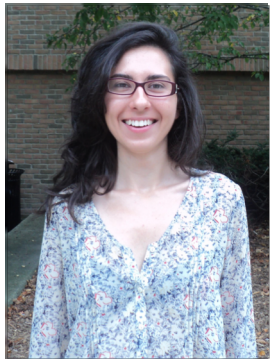
John LiPuma



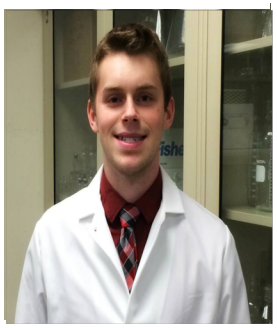
LiPuma Lab

Shannon Cahalan
Lindsay Caverly
Linda Kalikin
Theodore Spilker

Nadine Kotlarz



James Yonts



University Sequencing Core

Bob Lyons
Katherine Borysko

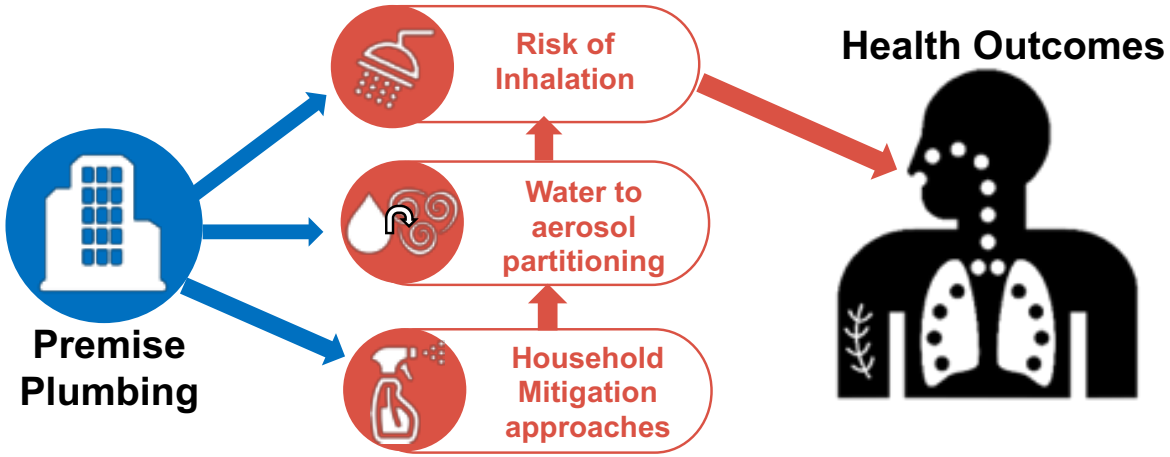
Neutral model

Christine Bassis
Arvind Venkataraman

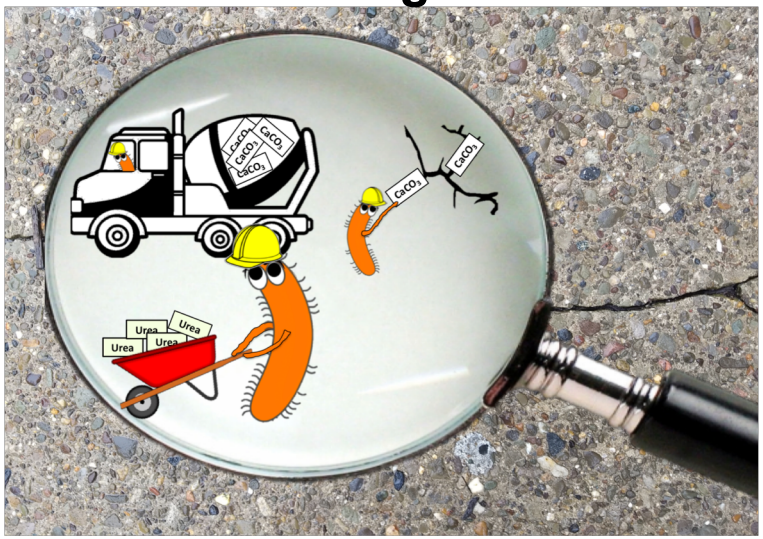
Current Research Projects in the Haig Group

Haig Shower Lab – Under construction

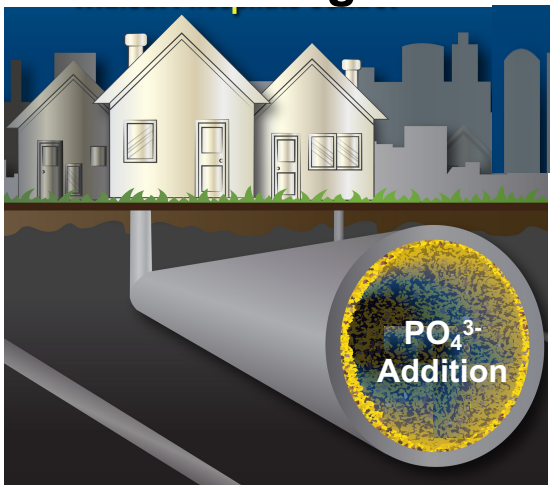
INvestigating **H**ome water & **A**erosols' **L**inks
to opportunistic pathogen **E**xposure (INHALE)



Self-healing concrete

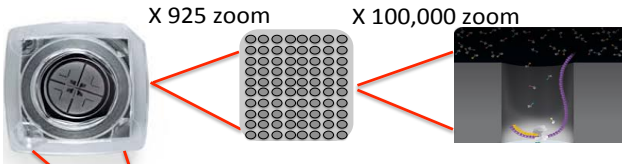


Corrosion control impact on opportunistic pathogens in Pittsburgh drinking water



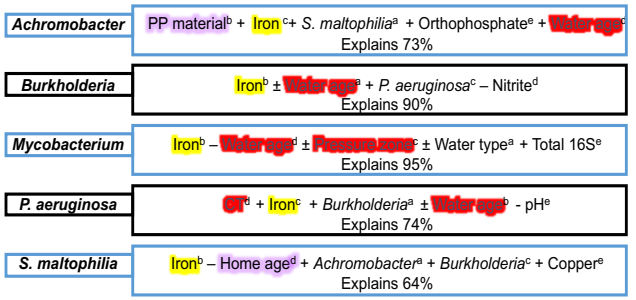
Conclusions & Next steps

- Developed approach that provides fast & accurate strain-level screening of tap water

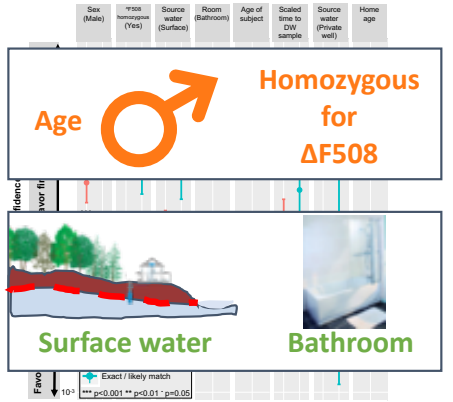


- Drinking water can be a source of bacterial strains that cause infections in people with CF

- **Important Factors Influencing:**
  - <u>abundance</u>: Iron concentration, PP material, CT, Water age and Pressure zone



  - <u>odds of finding a clinical match</u>: genetic & drinking water environment factors



Next steps –quantify the risk of different routes of exposure and assess mitigation approaches