



Newfoundland & Labrador
PUBLIC HEALTH LABORATORY

***E. COLI* AND COLIFORM TESTING: EVOLVING TECHNOLOGY IN A CHANGING ENVIRONMENT**

Lourens Robberts, PhD, D(ABMM), FCCM

Director

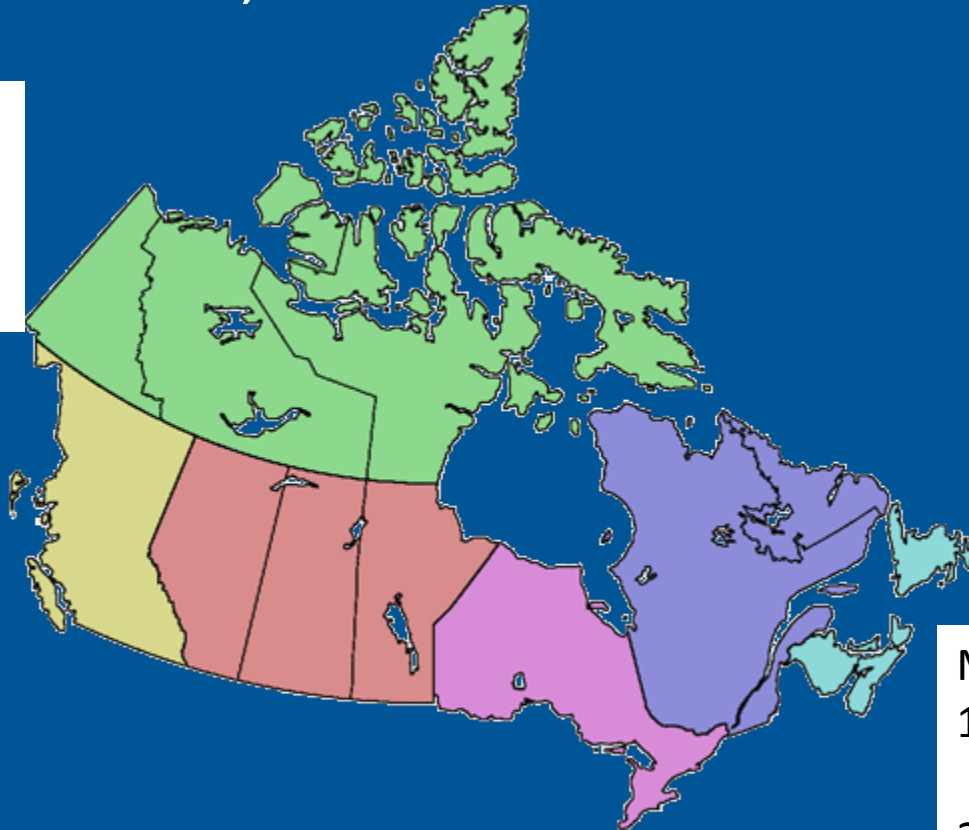
Clinical Microbiologist



Approximately 200 reported drinking water-associated disease outbreaks in Canada, 1975 - 2004

Top 3 pathogens:

1. Giardia
2. Cryptosporidium
3. Campylobacter



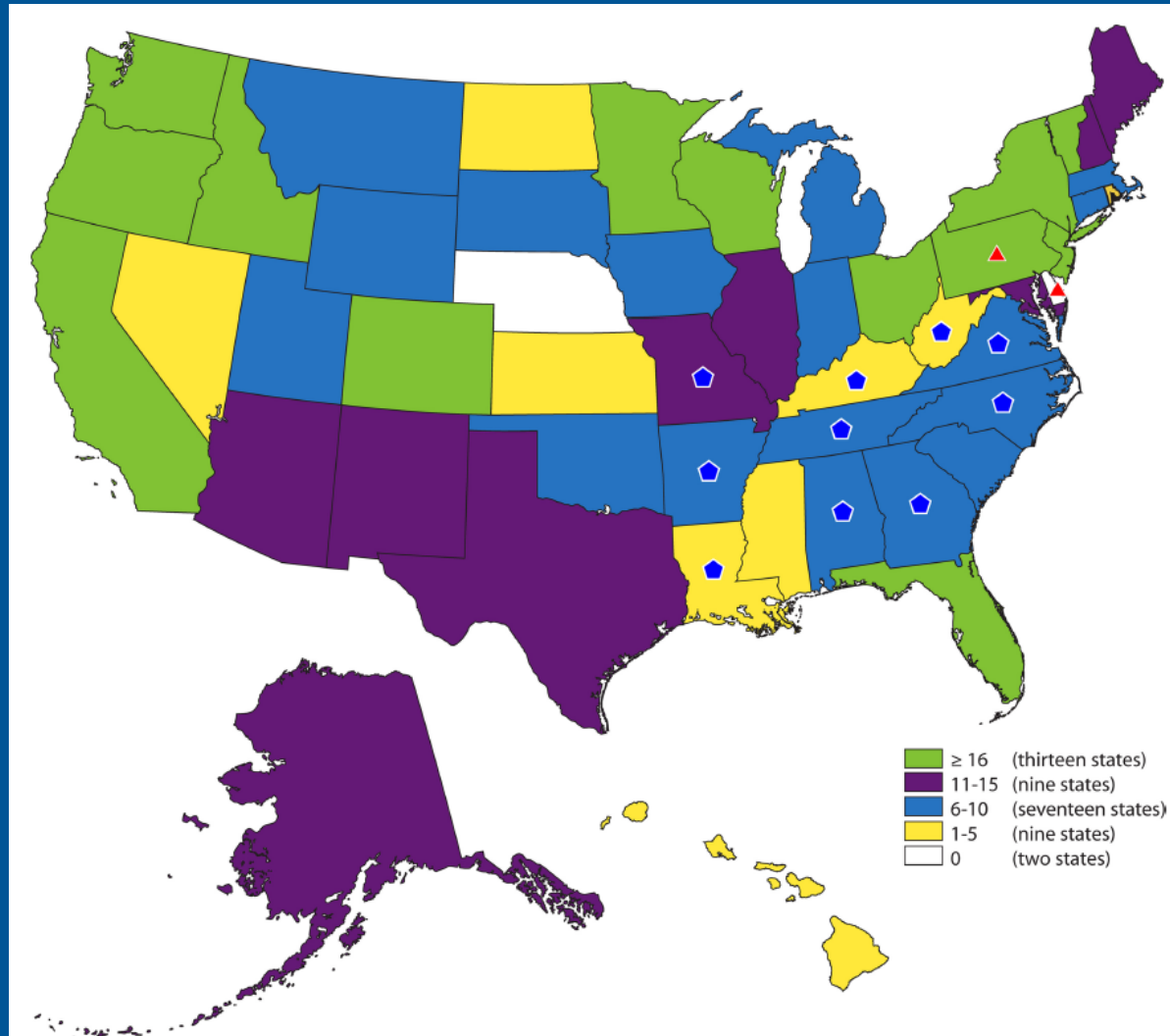
Main Causes

1. Surface water source (watershed)
2. Mechanical problems (Spring)

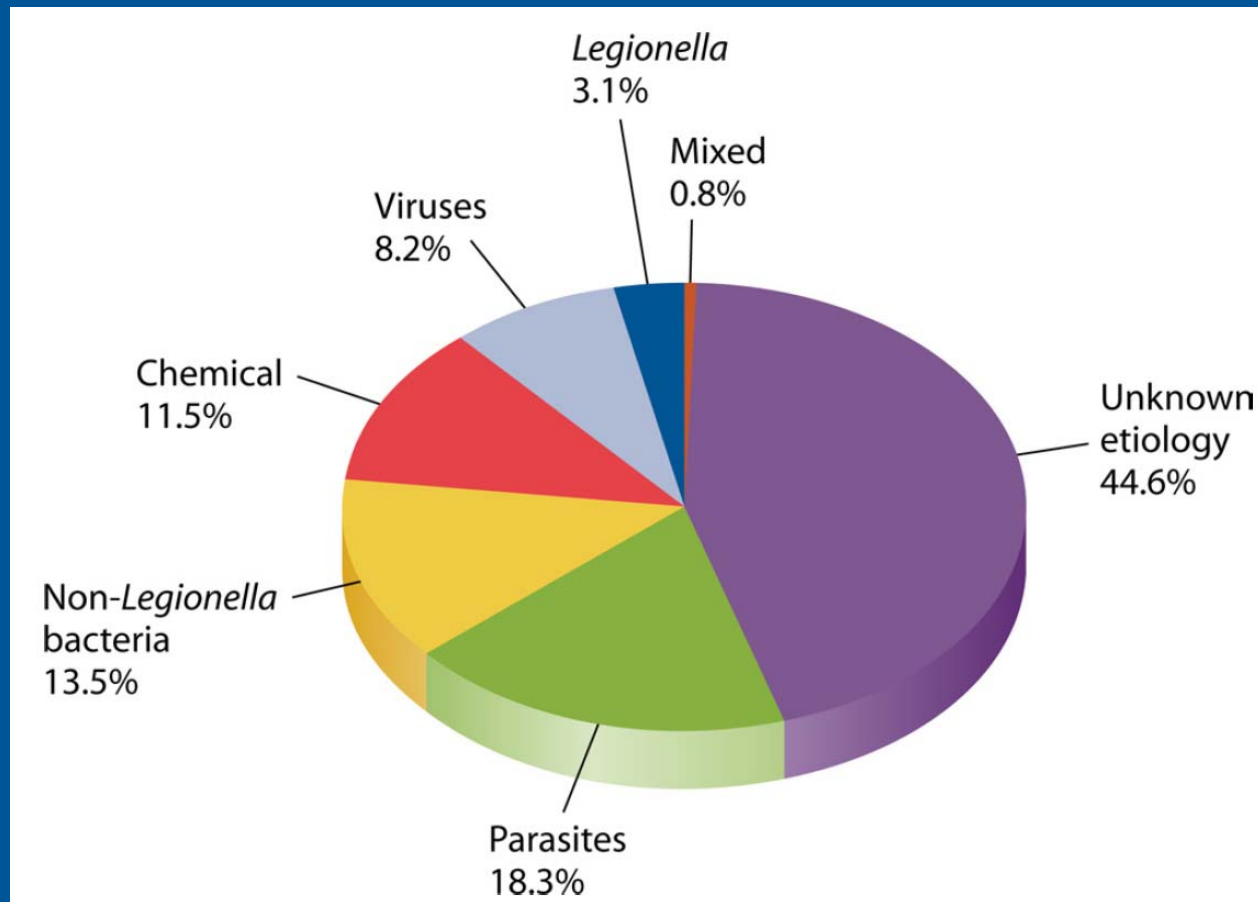
Charron, D.F., et al. J Toxicol Environ Health, 2004



Drinking water-associated disease outbreaks (n=780), USA 1971 - 2006

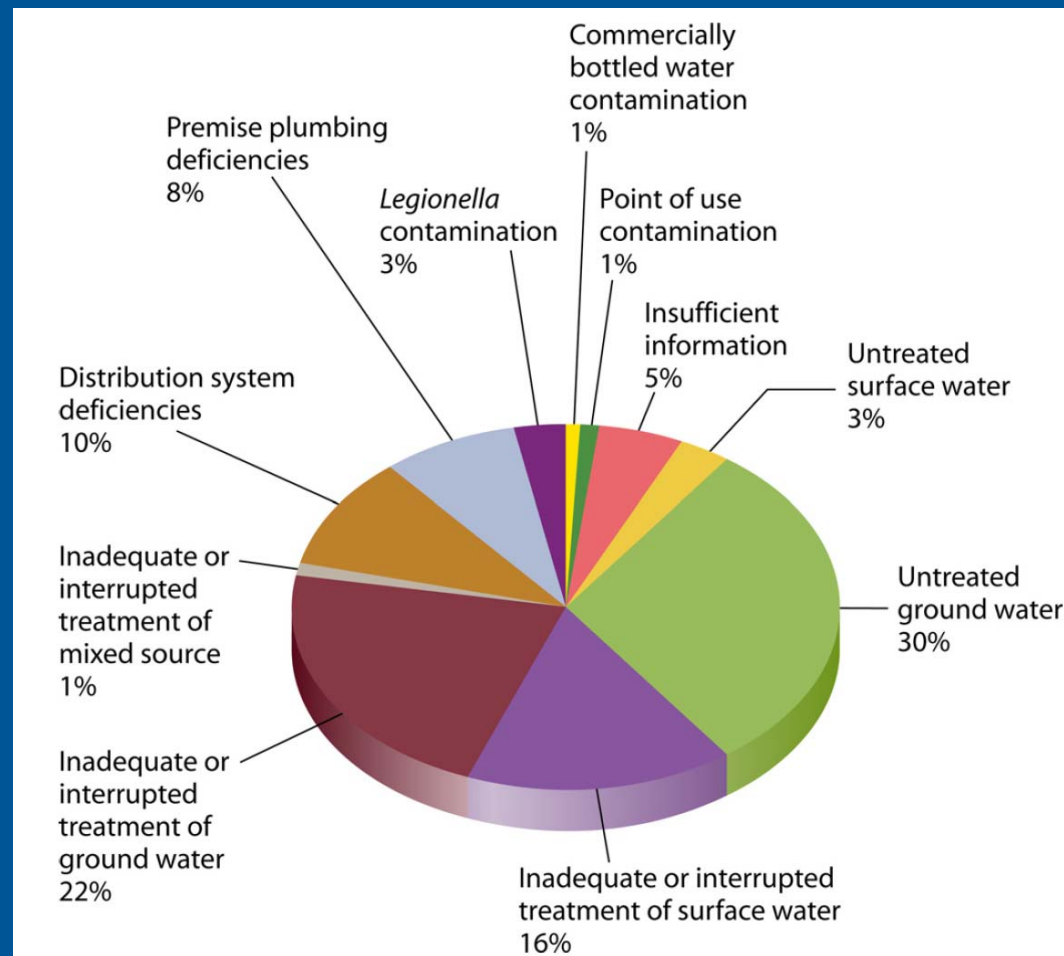


Percentage of etiological agents of drinking water-associated disease outbreaks (*n*=780), USA 1971 - 2006



Craun, G.F., et al. Clin Microbiol Rev 2010

Percentages of deficiencies (n=801) in 780 outbreaks associated with drinking water, USA 1971 - 2006

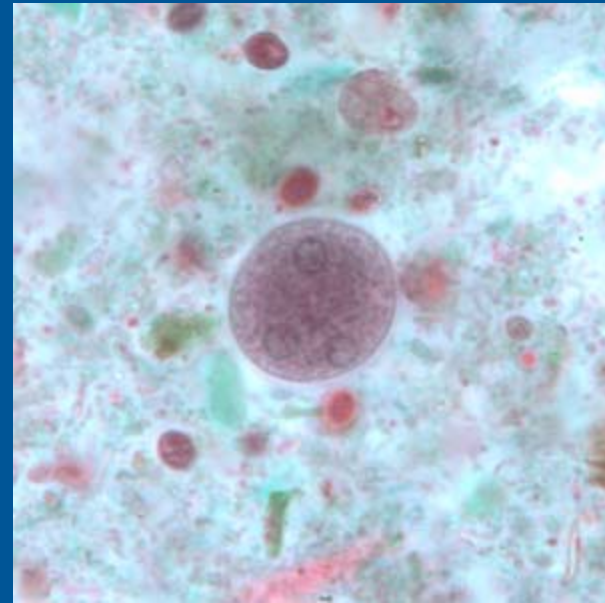


Clinical Case #1

64 year old woman presents to her doctor with diarrhea, tachycardia and dehydration. She explained that her symptoms started a week ago but yesterday morning when she woke up she felt very ill. Her husband has been feeling unwell with bloating, intermittent diarrhea and cramps for a few days now. They have not eaten anything out of the ordinary in the last month. They have not travelled in the past 6 months

Her doctor collects one stool specimen every other day for a total of 3 specimens for laboratory investigation.

Initial stool specimen showed few cysts *Entamoeba coli* and *Endolimax nana*. The Laboratory report includes a comment that these organisms are not pathogenic.

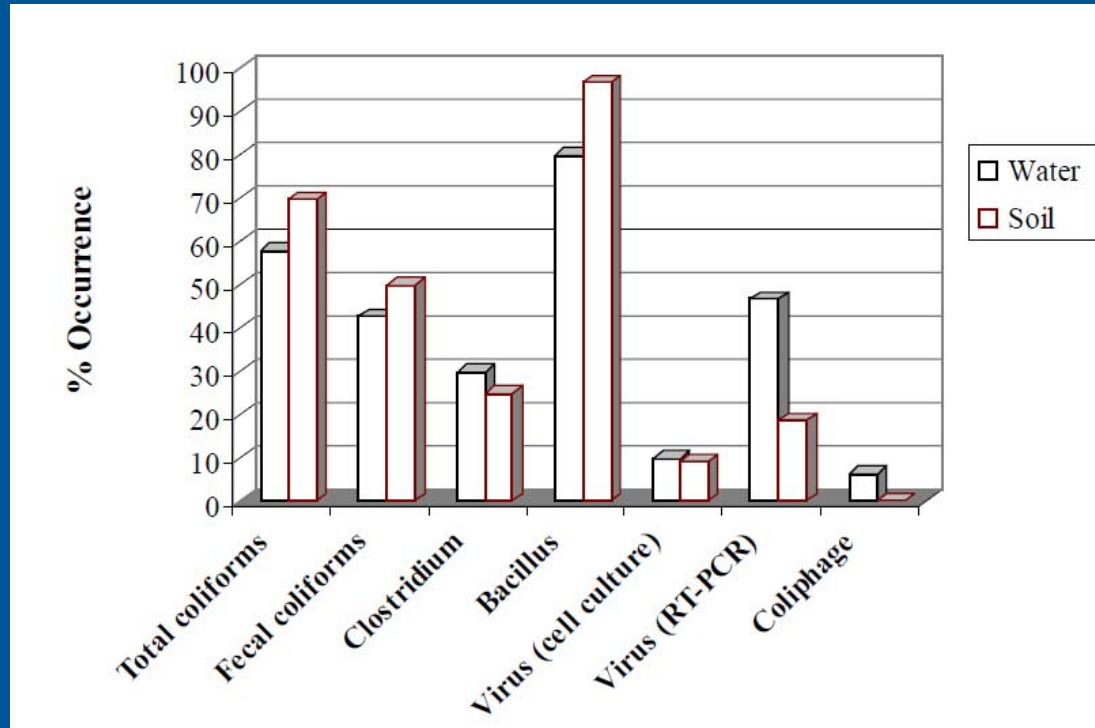


The physician recognizes that although the organisms are non-pathogenic, their presence suggest exposure to contaminated water. This may explain the vague symptoms the patient experienced a week ago but not her current bout of severe diarrhea and weakness. He orders viral studies.

Within 24 hours the results comes back as being POSITIVE for Norovirus.



Microbial Occurrence in Water and Soil Samples Around Sewer Lines



LeChevalier et al, U.S. Environmental Protection Agency

Microbial Occurrence in Water and Soil Samples Around Sewer Lines

Organism	Water CFU or PFU/100 ml	Soil CFU or PFU/100 gm
Total Coliforms	< 2 - 1.6×10^3	< 2 - 1.6×10^4
Fecal Coliforms	< 2 - 1.6×10^3	< 2 - 1.6×10^4
<i>Clostridium</i>	0 - 2.5×10^3	0 - 1×10^5
<i>Bacillus</i>	0 - 4.6×10^6	0 - 1.2×10^8
Phage	0 - 1×10^4	0

CFU, colony-forming units; PFU, plaque-forming units

LeChevalier et al, U.S. Environmental Protection Agency



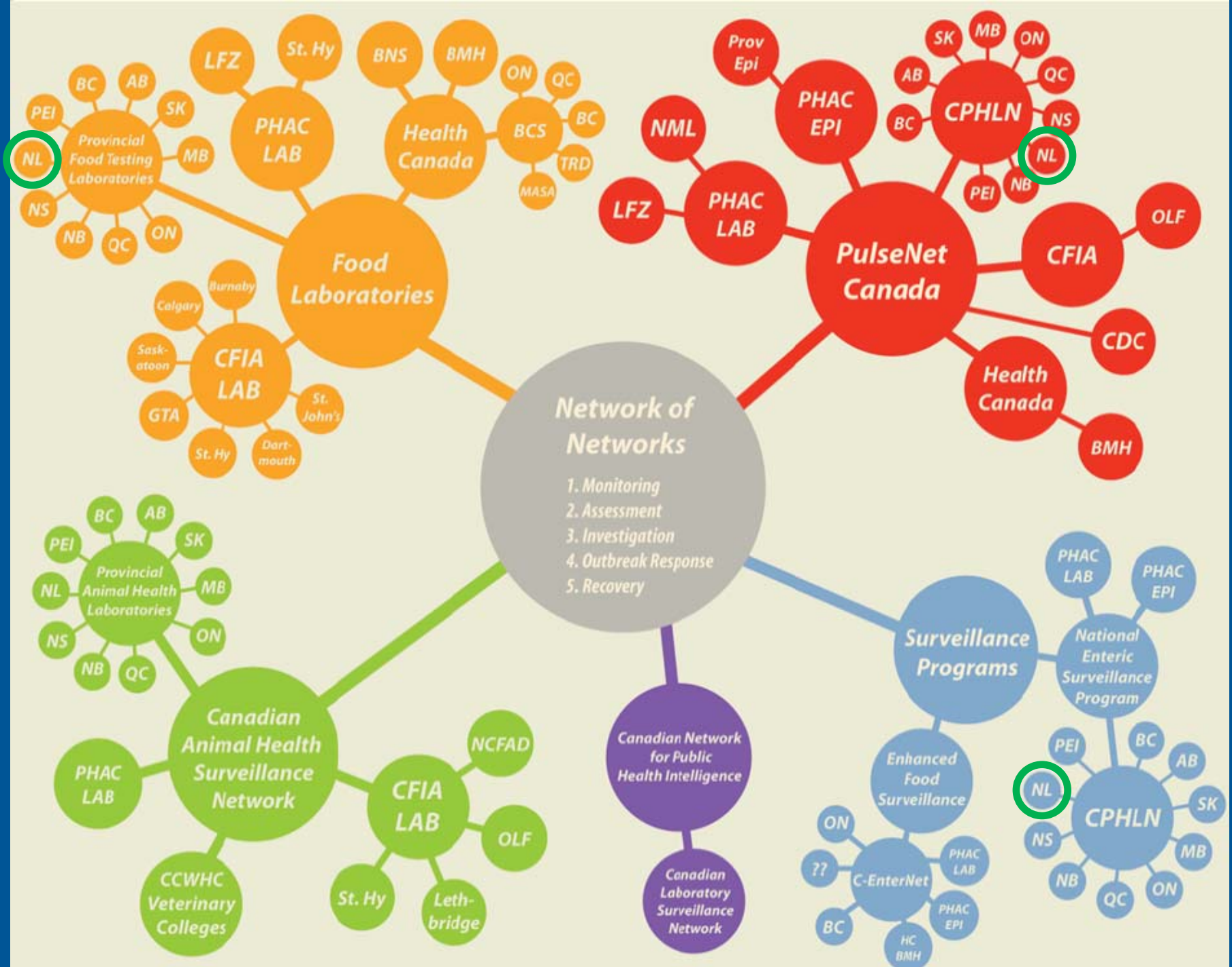
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Public Health
Agency of Canada

Agence de la santé
publique du Canada



Bacteriological Water Testing in Newfoundland and Labrador

Testing in NL is performed by the Public Health Laboratory with 6 Regional Health Authority Microbiology laboratories serving as satellite testing sites

Ensures near-community testing

Expedient transport and turn-around-time

PHL in St. John's (lead laboratory)

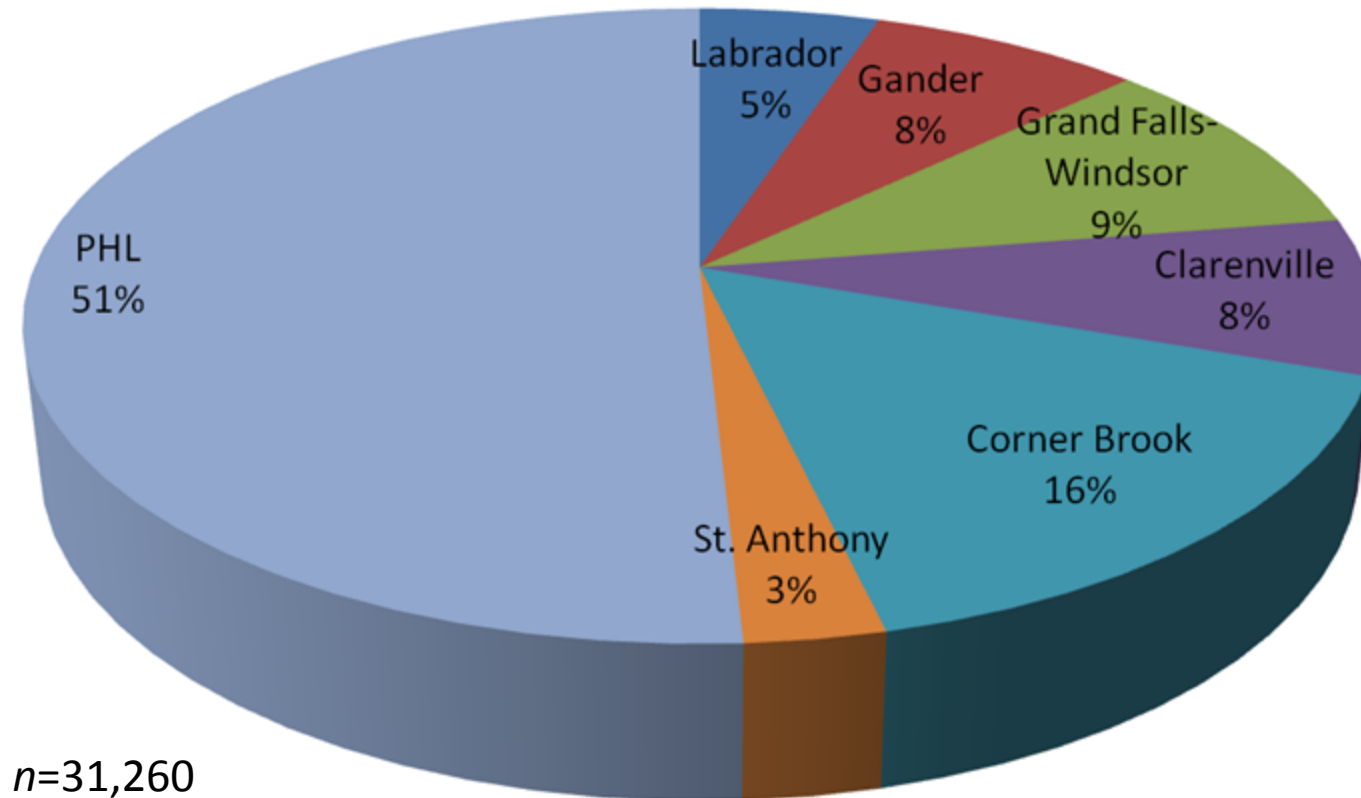
- Gander & Grand Falls
- Corner Brook
- Clarenville
- St. Anthony
- Goose Bay



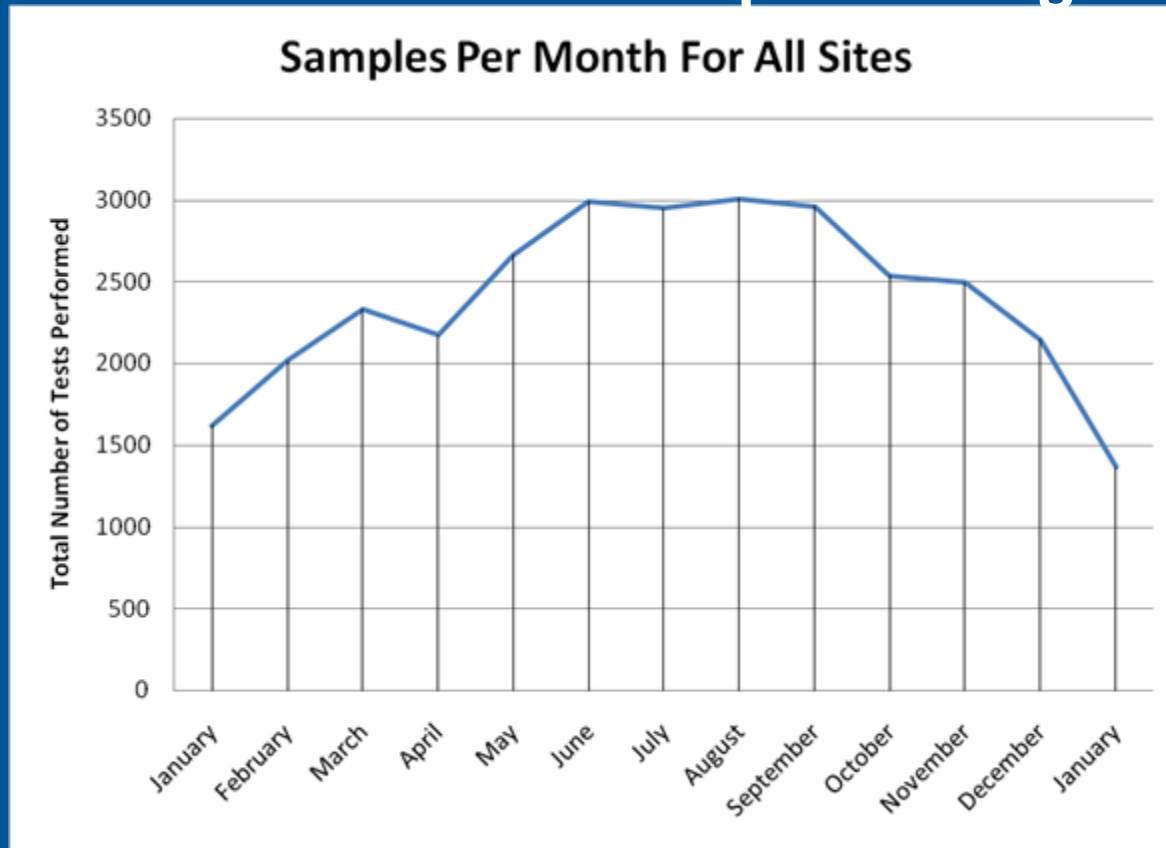
MicroLabNet

Provincial Water Sample Testing

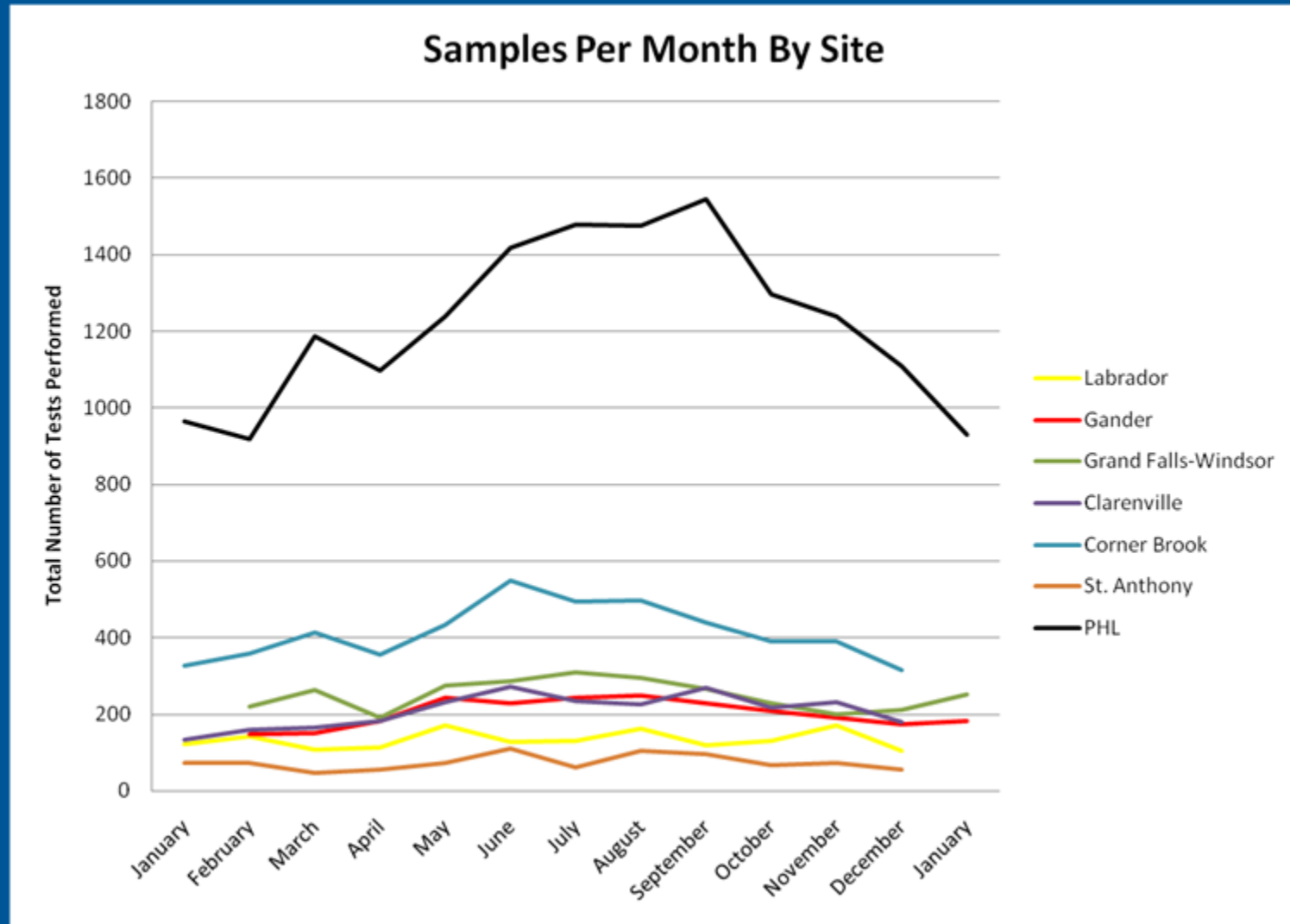
Total Testing By Site, 2011



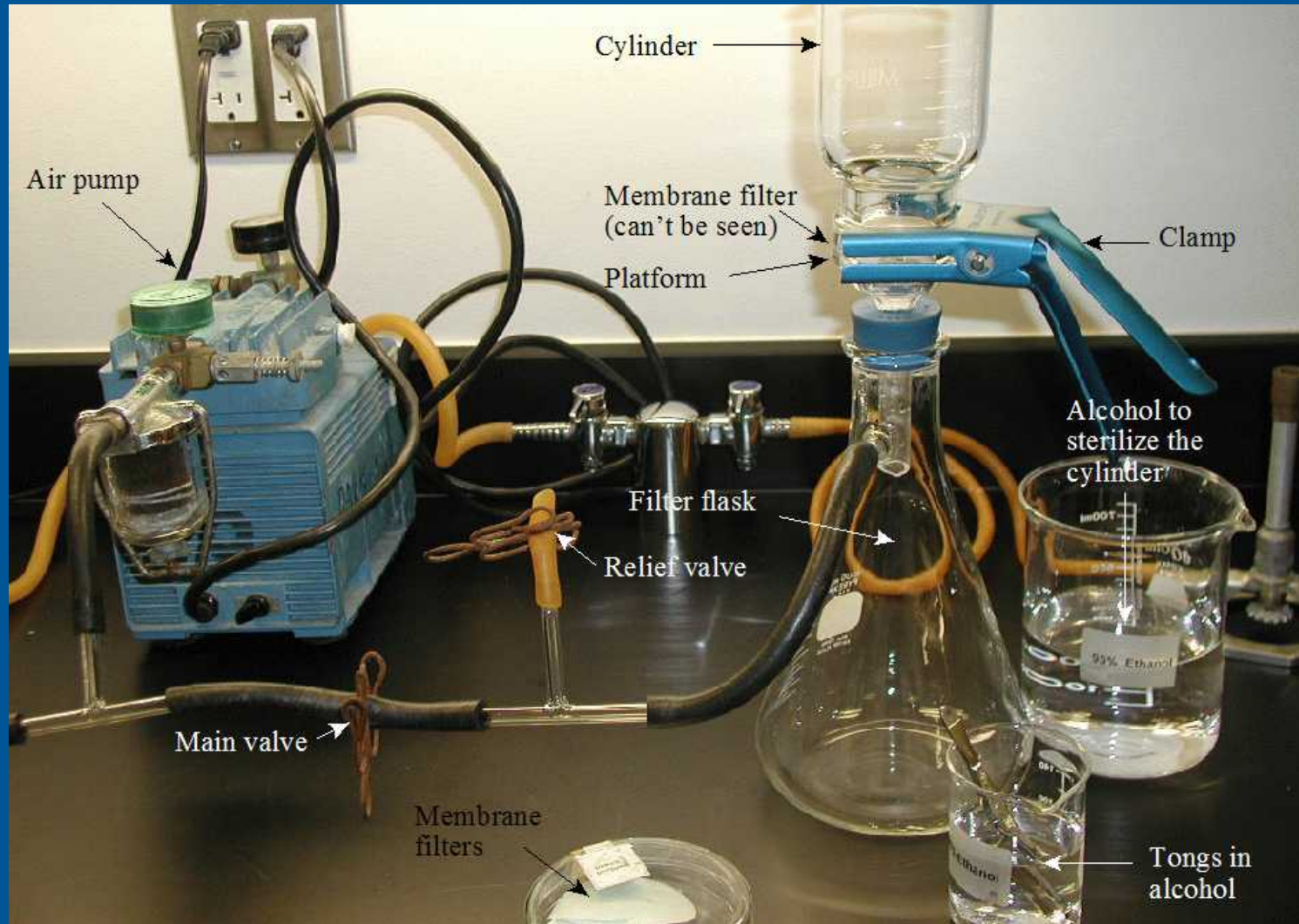
Provincial Water Sample Testing



Provincial Water Sample Testing



Current method: Membrane Filtration, cultures on DC Agar





coliform

E. coli

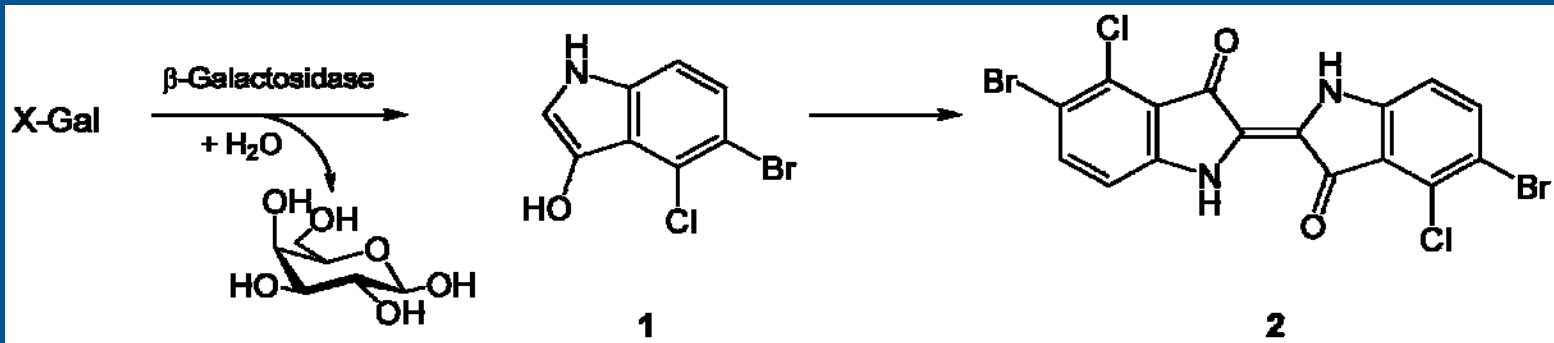
coliform

Overgrown

E. coli



DCA Medium *E. coli* detection



X-Gal: 5-bromo-4-chloro-indolyl- β -D-galactopyranoside

2: 5,5'-dibromo-4,4'-dichloro-indigo

Membrane Filtration using DC Agar

Pros:

Quantitative value (CFU/ml)

Cons:

Repetitive stress injuries – multiple manipulations, standing etc

Labor intensive

Multiple quality control (media, filters, buffers, UV sterilization)

Operator experience

Operational Capacity (one technologist, seasonal, 1545 specimens in September)

Performance Evaluation of Colitag[®] Presence/Absence Test

Method



Add reagent packet
Incubate 16 – 48h

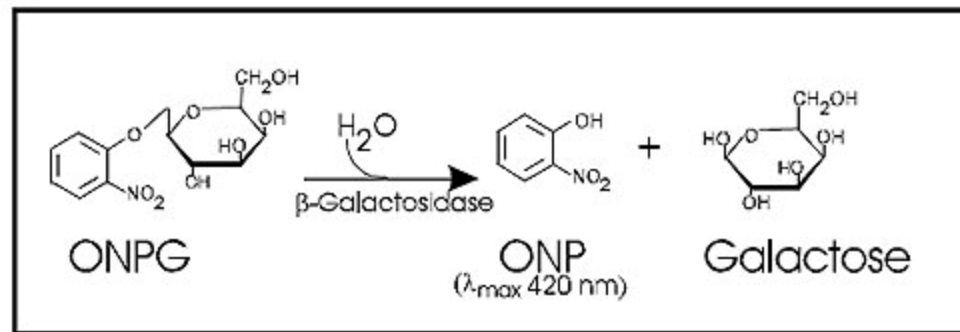


Read color
Yellow = coliforms
Clear = no coliforms/E. coli



Read fluorescence
Positive = E. coli
Clear = no E. coli

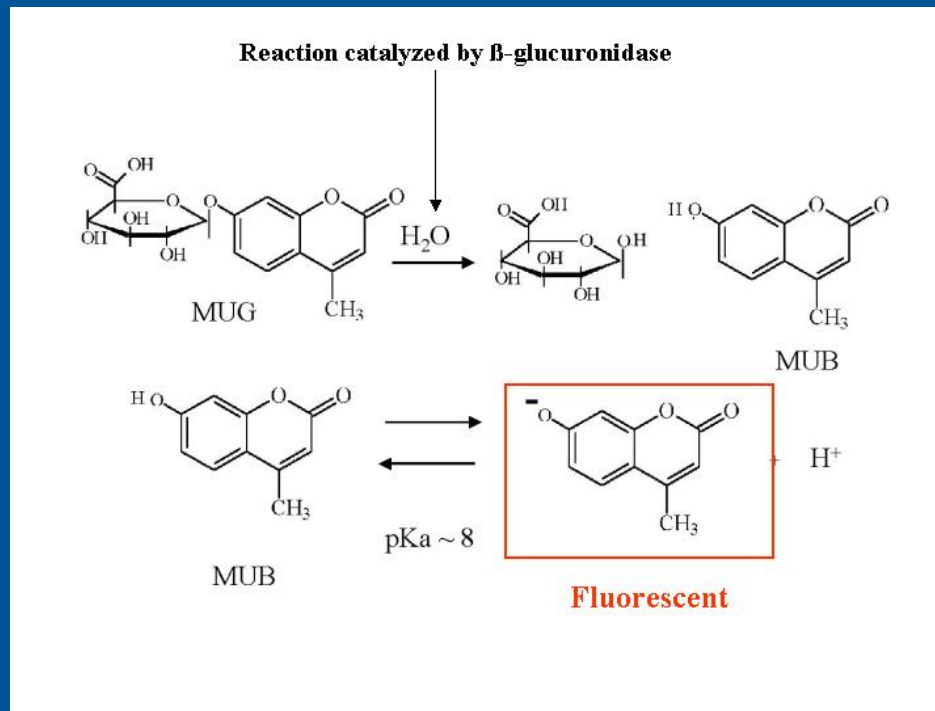
Coliform Detection



ONPG: o-nitrophenol-β-D-galactoside

ONP: o-nitrophenol

E. coli Detection



MUG: 4-methylumbelliferyl- β -D-glucuronide

MUB: 4-methylumbelliferone

Presence/Absence Test using Colitag

Pros:

Minimum hands-on

Minimal QC

Can be read after 16 hours incubation

Easy interpretation

No overgrowth

Cons:

Cost (reagent)

Performance Evaluation of Colitag[®] Presence/Absence Test

Method

Parallel testing using MF-DCA and Colitag (55 routine E. coli / coliform-positive samples)

Discrepancies investigated by identification of isolates on DCA or in Colitag media

Coliform: (MF-DCA as standard)

Colitag

Sensitivity: 78%

Specificity: 100%

Correlation: 82%

E. coli (discrepancies investigated)

Colitag

Sensitivity: 84%

Specificity: 100%

Correlation: 93%

MF-DCA

Sensitivity: 100%

Specificity: 48%

Correlation: 75%

Performance Evaluation of Colitag[®] Presence/Absence Test

Method

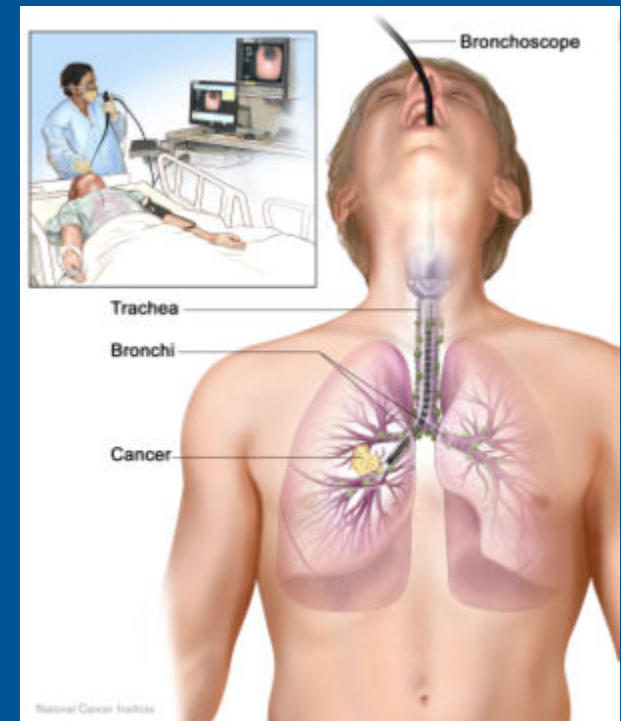
DCA	Sensitivity	100% (77-100)
	Specificity	78% (60 - 89)
Private	PPV	36.7%
	* 11.3 NPV	100%
Public	PPV	3.5%
	* 0.8 NPV	100%

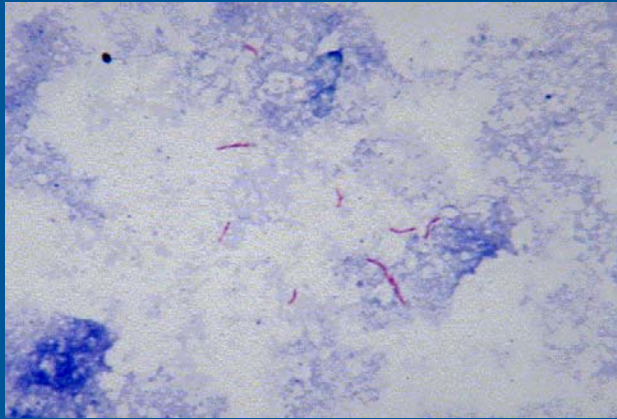
Colitag	Sensitivity	76% (50 - 92)
	Specificity	100% (88 - 100)
Private	PPV	100%
	11.2 NPV	97%
Public	PPV	100%
	0.8 NPV	99.8%

* Prevalence of E. coli in samples tested

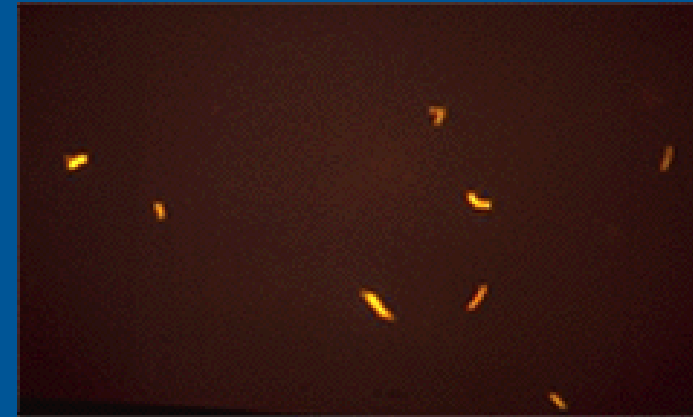
Clinical Case #2

71 year old woman with chronic obstructive pulmonary disease (COPD) shows worsening of her lung function and requires hospitalization. In the hospital a bronchoscopist performs a bronchoalveolar lavage (BAL) and sends the BAL fluid to the laboratory for culture.





Ziehl-Neelson Acid Fast Stain



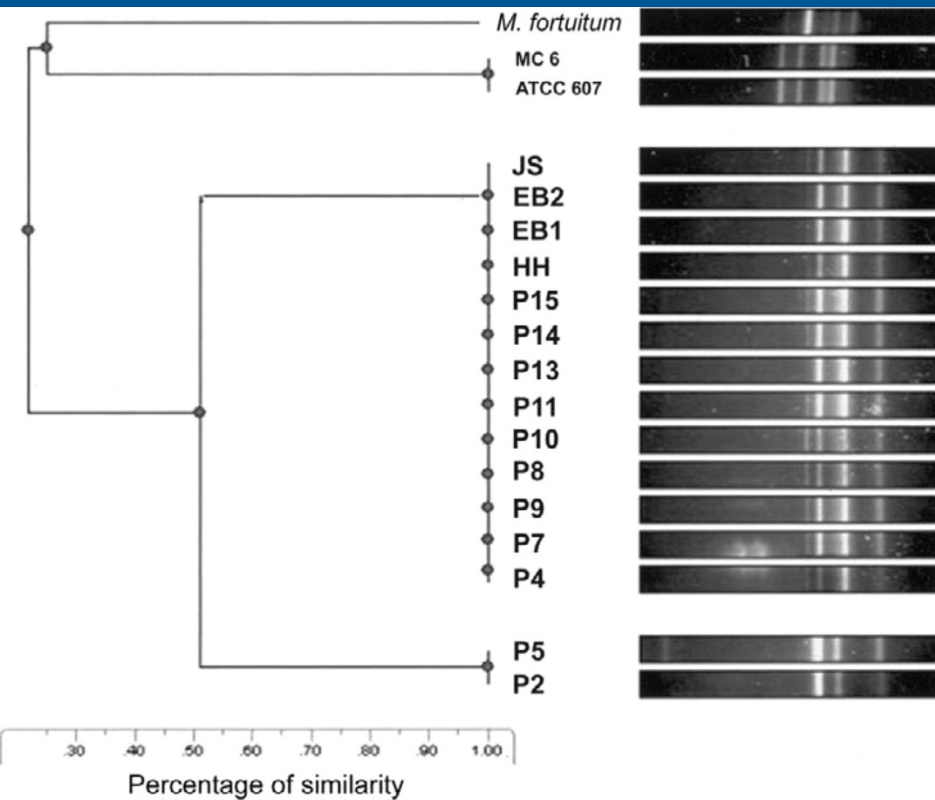
Auramine Fluorescent Stain



Mycobacterium fortuitum

Her doctor is perplexed with her patient's inability to clear the bacterium, *Mycobacterium fortuitum*, which has been troubling the patient for 3 years. The patient has not been able to clear the infection completely.

Repeated antibiotic therapy seems to clear the airway infection after 2 weeks of treatment, but the patient has been returning with repeated flare-ups of her lung disease. The doctor has been treating the patient with Clindamycin and she gets better every time. The *M. fortuitum* remains sensitive to the antibiotic.

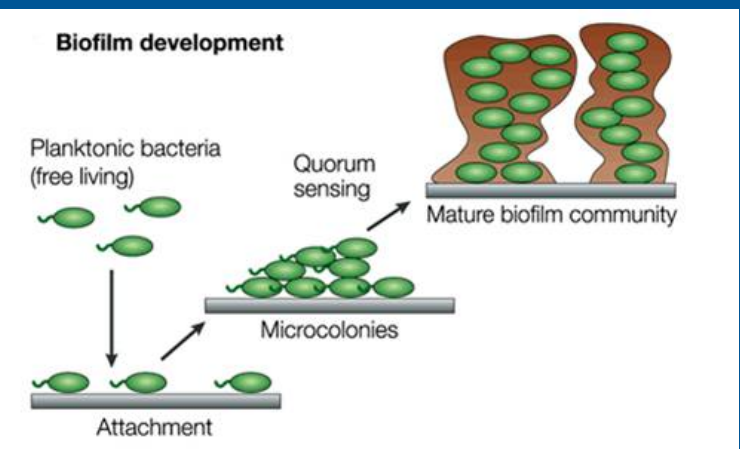
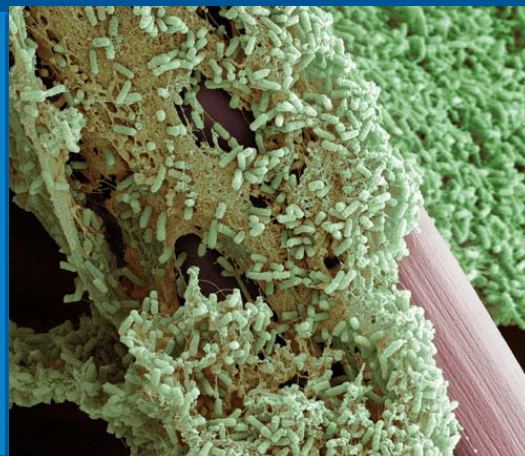


Products of Randomly Amplified Polymorphic DNA (RAPD) PCR suggests that all the patient's isolates are the same. Given that the patient was successfully treated indicates re-infection from a source in the patient's environment.



The doctor realizes that her patient's community has been on boil water advisory for a long time, and although the patient has been diligent to boil her water for consumption she did not realize the threat from bacteria being aerosolized during other activities.

Her patient is likely the victim of repeated infection from her environment (e.g. shower).



ACKNOWLEDGEMENTS

Laura Gilbert (Public Health Laboratory Informatician)

Florence Stead (PHL Supervisor)

Bernadette Noftall (Technologist II)

Tony Cumby (Technologist I)

Patricia Hawco (Technologist I)