Isolation and Identification of Legionella at the MD DHMH Laboratories Administration

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Division of Virology and Immunology

State of Maryland Department of Health and Mental Hygiene Laboratories Administration

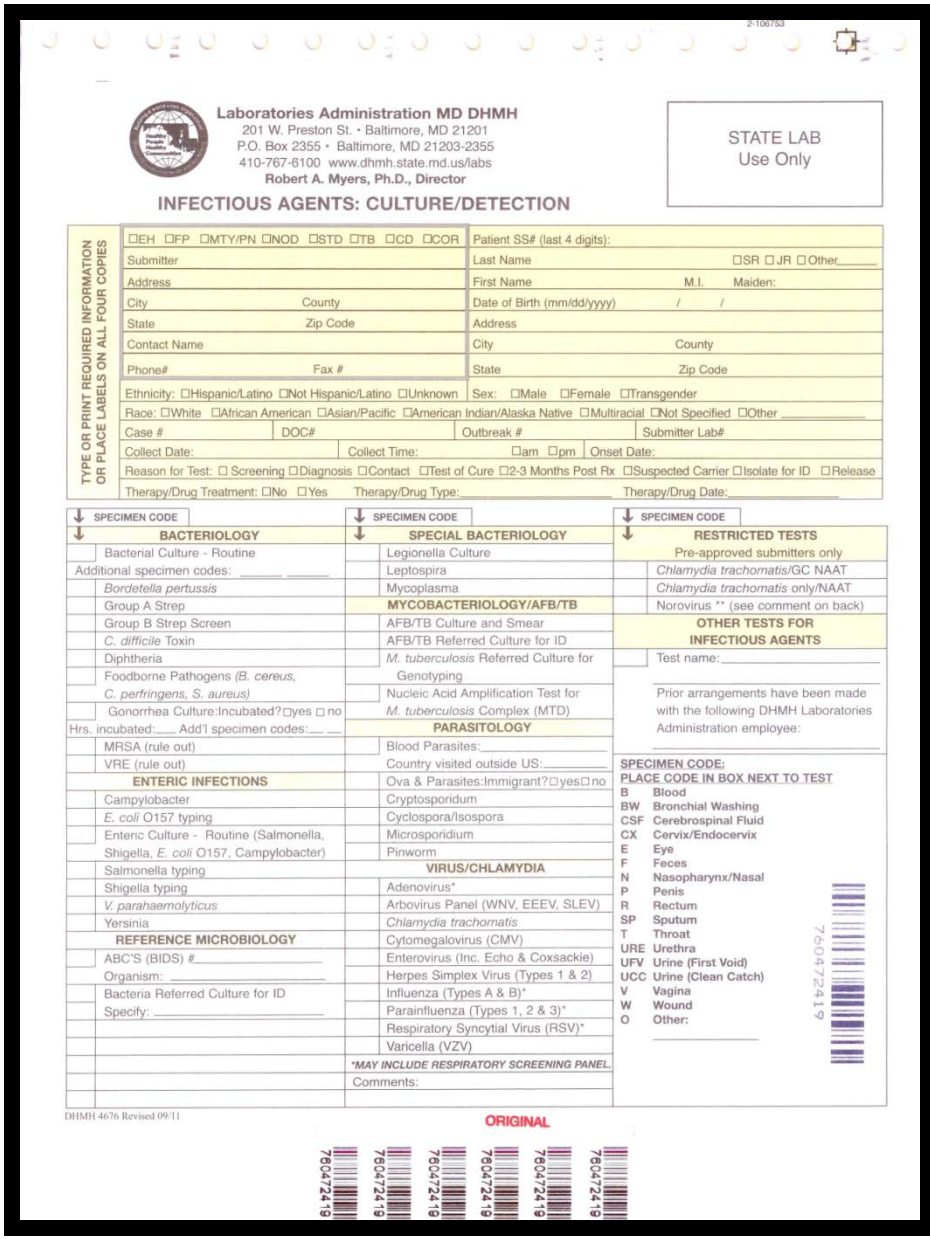
MARYLAND

Clinical Tests for Legionella Performed at the MD DHMH Laboratories

- **Culture/ Direct Fluorescent Antibody (i.e., sputum, lung tissue)**
- **Serology IFA**
- **Urine Antigen EIA/ Rapid**

Additional informational found at MD DHMH Guide to Public Health Lab Services: http://dhmh.maryland.gov/laboratories/docs/guide.pdf

Clinical Test Request Submission Form for Legionella



Laboratories Administration MD DHMH
201 W. Preston St. • Baltimore, MD 21201
P.O. Box 2355 • Baltimore, MD 21203-2355
410-767-6100 www.dhmh.state.md.us/labs
Robert A. Myers, Ph.D., Director

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INFECTIOUS AGENTS: CULTURE/DETECTION

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL FOUR COPIES

☐EH ☐FP ☐MTY/PN ☐NOD ☐STD ☐TB ☐CD ☐COR | Patient SS# (last 4 digits):
Submitter | Last Name ☐SR ☐JR ☐Other
Address | First Name M.I. Maiden:
City County | Date of Birth (mm/dd/yyyy) / /
State Zip Code | Address
Contact Name | City County
Phone# Fax # | State Zip Code
Ethnicity: ☐Hispanic/Latino ☐Not Hispanic/Latino ☐Unknown | Sex: ☐Male ☐Female ☐Transgender
Race: ☐White ☐African American ☐Asian/Pacific ☐American Indian/Alaska Native ☐Multiracial ☐Not Specified ☐Other
Case # | DOC# | Outbreak # | Submitter Lab#
Collect Date: | Collect Time: ☐am ☐pm | Onset Date:
Reason for Test: ☐ Screening ☐Diagnosis ☐Contact ☐Test of Cure ☐2-3 Months Post Rx ☐Suspected Carrier ☐Isolate for ID ☐Release
Therapy/Drug Treatment: ☐No ☐Yes | Therapy/Drug Type: | Therapy/Drug Date:

SPECIMEN CODE

BACTERIOLOGY
- Bacterial Culture - Routine
- Additional specimen codes: _____ _____
- *Bordetella pertussis*
- Group A Strep
- Group B Strep Screen
- *C. difficile* Toxin
- Diphtheria
- Foodborne Pathogens (*B. cereus, C. perfringens, S. aureus)*
- Gonorrhea Culture:Incubated?☐yes ☐ no
- Hrs. incubated:___ Add'l specimen codes:___ ___
- MRSA (rule out)
- VRE (rule out)

ENTERIC INFECTIONS
- Campylobacter
- *E. coli* O157 typing
- Enteric Culture - Routine (Salmonella, Shigella, *E. coli* O157, Campylobacter)
- Salmonella typing
- Shigella typing
- *V. parahaemolyticus*
- Yersinia

REFERENCE MICROBIOLOGY
- ABC'S (BIDS) #_____
- Organism: _____
- Bacteria Referred Culture for ID
- Specify: _____

SPECIMEN CODE

SPECIAL BACTERIOLOGY
- Legionella Culture
- Leptospira
- Mycoplasma

MYCOBACTERIOLOGY/AFB/TB
- AFB/TB Culture and Smear
- AFB/TB Referred Culture for ID
- *M. tuberculosis* Referred Culture for Genotyping
- Nucleic Acid Amplification Test for *M. tuberculosis* Complex (MTD)

PARASITOLOGY
- Blood Parasites:_____
- Country visited outside US:_____
- Ova & Parasites:Immigrant?☐yes☐no
- Cryptosporidum
- Cyclospora/Isospora
- Microsporidium
- Pinworm

VIRUS/CHLAMYDIA
- Adenovirus*
- Arbovirus Panel (WNV, EEEV, SLEV)
- *Chlamydia trachomatis*
- Cytomegalovirus (CMV)
- Enterovirus (Inc. Echo & Coxsackie)
- Herpes Simplex Virus (Types 1 & 2)
- Influenza (Types A & B)*
- Parainfluenza (Types 1, 2 & 3)*
- Respiratory Syncytial Virus (RSV)*
- Varicella (VZV)

MAY INCLUDE RESPIRATORY SCREENING PANEL.

Comments:

SPECIMEN CODE

RESTRICTED TESTS
Pre-approved submitters only
- *Chlamydia trachomatis*/GC NAAT
- *Chlamydia trachomatis* only/NAAT
- Norovirus ** (see comment on back)

OTHER TESTS FOR INFECTIOUS AGENTS
- Test name:_____

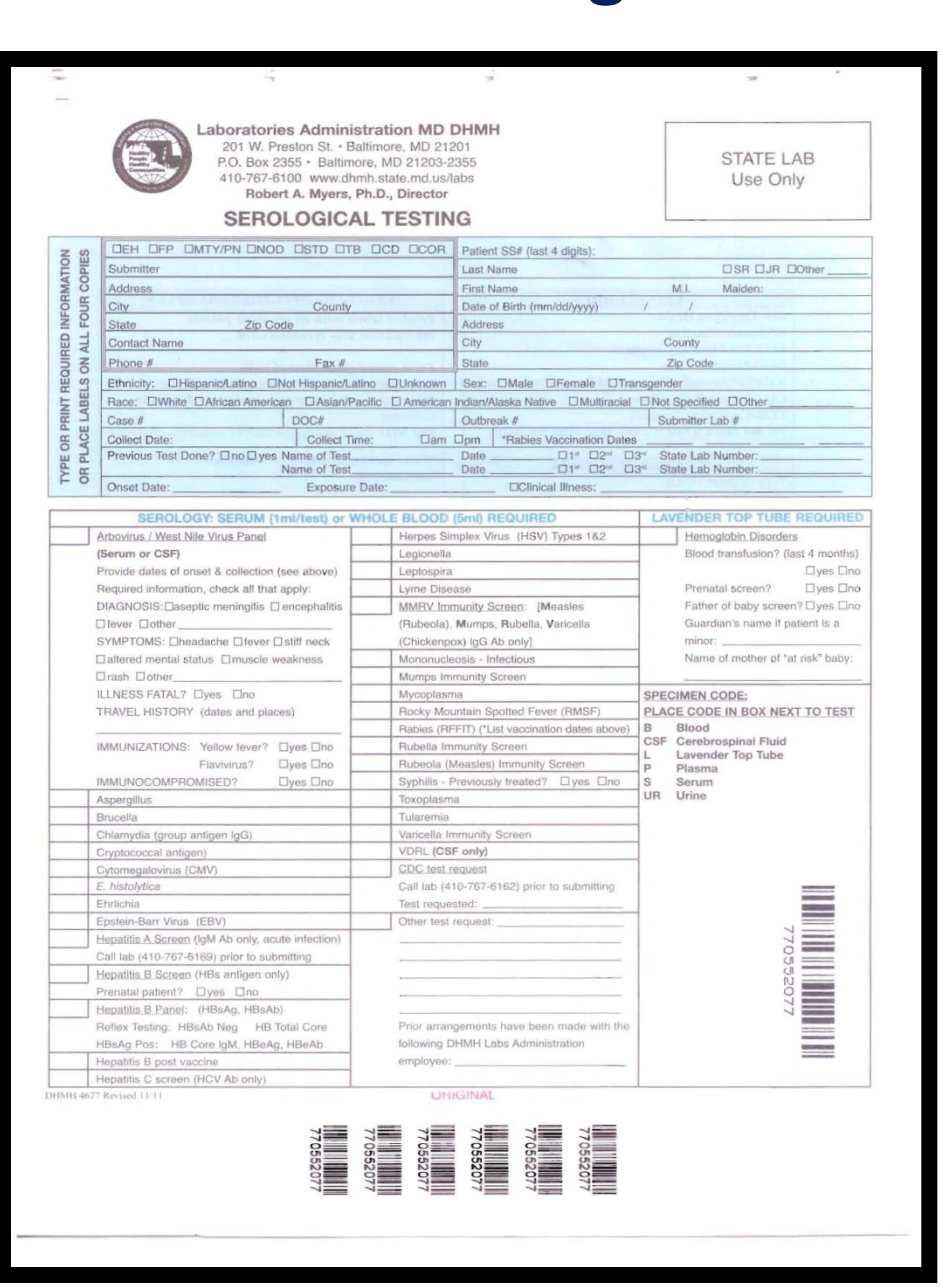
Prior arrangements have been made with the following DHMH Laboratories Administration employee:

SPECIMEN CODE:
PLACE CODE IN BOX NEXT TO TEST

B	Blood
BW	Bronchial Washing
CSF	Cerebrospinal Fluid
CX	Cervix/Endocervix
E	Eye
F	Feces
N	Nasopharynx/Nasal
P	Penis
R	Rectum
SP	Sputum
T	Throat
URE	Urethra
UFV	Urine (First Void)
UCC	Urine (Clean Catch)
V	Vagina
W	Wound
O	Other:

DHMH 4676 Revised 09/11

ORIGINAL



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SEROLOGICAL TESTING

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL FOUR COPIES

☐EH ☐FP ☐MTY/PN ☐NOD ☐STD ☐TB ☐CD ☐COR | Patient SS# (last 4 digits):
Submitter | Last Name ☐SR ☐JR ☐Other
Address | First Name M.I. Maiden:
City County | Date of Birth (mm/dd/yyyy) / /
State Zip Code | Address
Contact Name | City County
Phone # Fax # | State Zip Code
Ethnicity: ☐Hispanic/Latino ☐Not Hispanic/Latino ☐Unknown | Sex: ☐Male ☐Female ☐Transgender
Race: ☐White ☐African American ☐Asian/Pacific ☐American Indian/Alaska Native ☐Multiracial ☐Not Specified ☐Other
Case # | DOC# | Outbreak # | Submitter Lab #
Collect Date: | Collect Time: ☐am ☐pm | *Rabies Vaccination Dates
Previous Test Done? ☐no ☐yes Name of Test Date ☐1st ☐2nd ☐3rd State Lab Number:
Name of Test Date ☐1st ☐2nd ☐3rd State Lab Number:
Onset Date: | Exposure Date: | ☐Clinical Illness:

SEROLOGY: SERUM (1ml/test) or WHOLE BLOOD (5ml) REQUIRED
- Arbovirus / West Nile Virus Panel (Serum or CSF)
- Provide dates of onset & collection (see above)
- Required information, check all that apply:
- DIAGNOSIS:☐aseptic meningitis ☐ encephalitis ☐fever ☐other
- SYMPTOMS: ☐headache ☐fever ☐stiff neck ☐altered mental status ☐muscle weakness ☐rash ☐other
- ILLNESS FATAL? ☐yes ☐no
- TRAVEL HISTORY (dates and places)
- IMMUNIZATIONS: Yellow fever? ☐yes ☐no Flavivirus? ☐yes ☐no
- IMMUNOCOMPROMISED? ☐yes ☐no
- Aspergillus
- Brucella
- Chlamydia (group antigen IgG)
- Cryptococcal antigen)
- Cytomegalovirus (CMV)
- *E. histolytica*
- Ehrlichia
- Epstein-Barr Virus (EBV)
- Hepatitis A Screen (IgM Ab only, acute infection) Call lab (410-767-6169) prior to submitting
- Hepatitis B Screen (HBs antigen only) Prenatal patient? ☐yes ☐no
- Hepatitis B Panel: (HBsAg, HBsAb) Reflex Testing: HBsAb Neg HB Total Core HBsAg Pos: HB Core IgM, HBeAg, HBeAb
- Hepatitis B post vaccine
- Hepatitis C screen (HCV Ab only)
- Herpes Simplex Virus (HSV) Types 1&2
- Legionella
- Leptospira
- Lyme Disease
- MMRV Immunity Screen: [Measles (Rubeola), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only]
- Mononucleosis - Infectious
- Mumps Immunity Screen
- Mycoplasma
- Rocky Mountain Spotted Fever (RMSF)
- Rabies (RFFIT) (*List vaccination dates above)
- Rubella Immunity Screen
- Rubeola (Measles) Immunity Screen
- Syphilis - Previously treated? ☐yes ☐no
- Toxoplasma
- Tularemia
- Varicella Immunity Screen
- VDRL (CSF only)
- CDC test request
- Call lab (410-767-6162) prior to submitting
- Test requested: _____
- Other test request: _____

Prior arrangements have been made with the following DHMH Labs Administration employee: _____

LAVENDER TOP TUBE REQUIRED
- Hemoglobin Disorders
- Blood transfusion? (last 4 months) ☐yes ☐no
- Prenatal screen? ☐yes ☐no
- Father of baby screen? ☐yes ☐no
- Guardian's name if patient is a minor: _____
- Name of mother of "at risk" baby: _____

SPECIMEN CODE:
PLACE CODE IN BOX NEXT TO TEST

B	Blood
CSF	Cerebrospinal Fluid
L	Lavender Top Tube
P	Plasma
S	Serum
UR	Urine

DHMH 4677 Revised 11/11

ORIGINAL

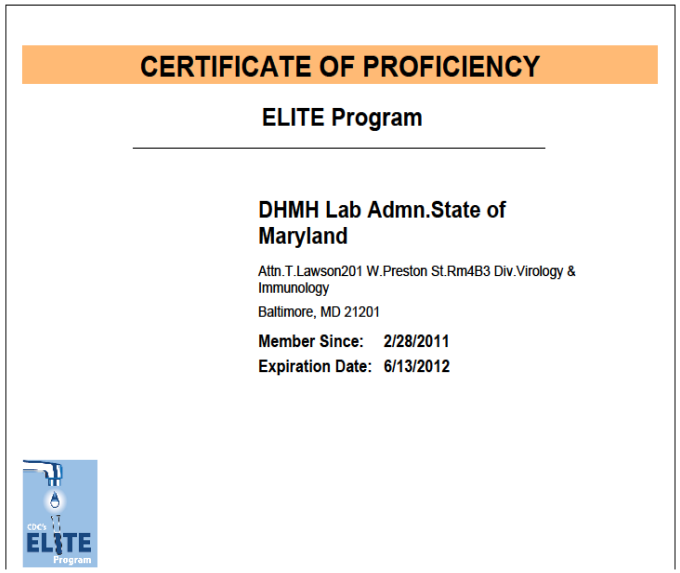
Environmental Tests for Legionella Performed at the MD DHMH Laboratories

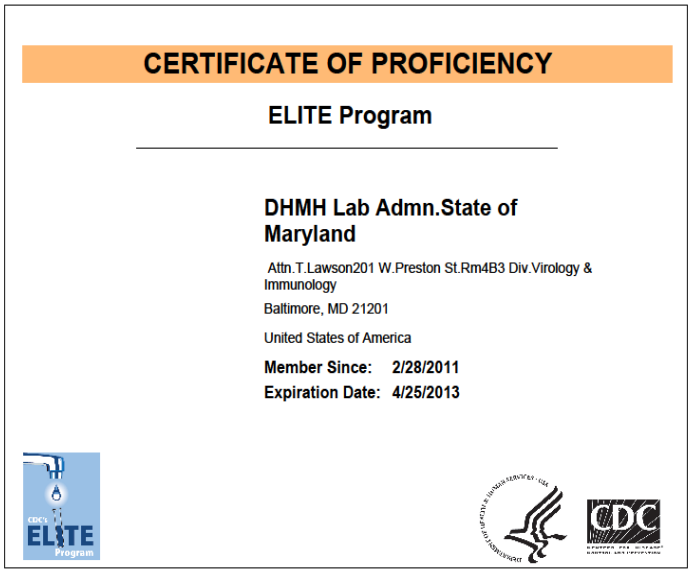
Isolation by Culture

Identification by Direct Fluorescent Antibody (DFA) Test

MD DHMH Laboratories Administration – **Certified by CDC**

ELITE (Environmental Legionella Isolation Techniques Evaluation)





Legionella Possible Case Scenario in Maryland: The Call

- Lab receive call from State Epi
- Preapproval
- Pre-remediation
- Post-remediation
- Coordination with Epi, HDs
- Collection Sites in Facility
- Collection Kit
- Specimen Submission Policy

Sample Collection Kit

- Sterile 1 L bottles
- Sodium thiosulfate
- Swabs
- Sterile 5ml tubes
- Sterile water
- DHMH 4676 Culture Detection Submission Forms

➢ Storage Condition for collection kit: 4°C

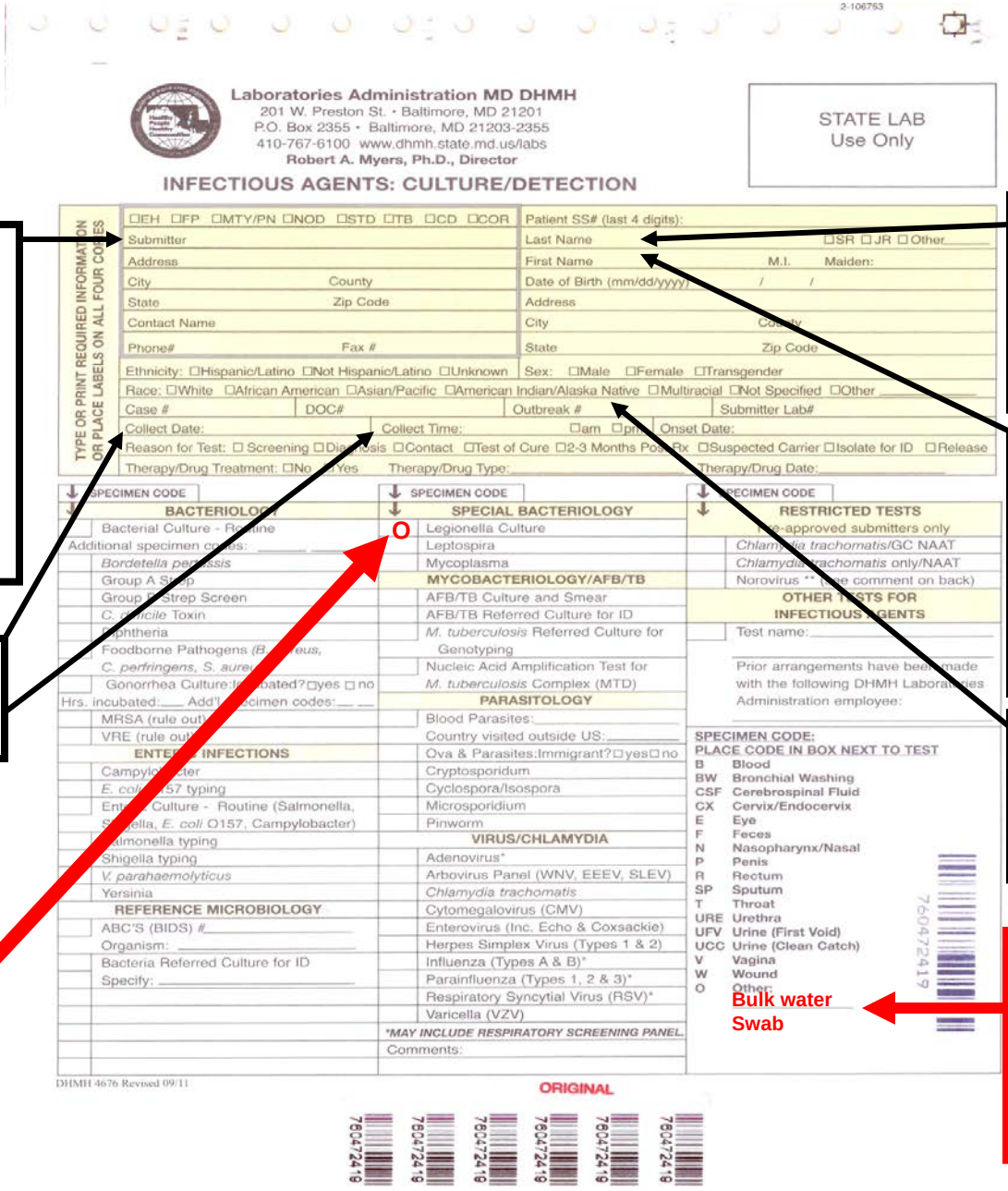
Bulk Water Sample Collection

- **Bulk water: Minimum of 1 liter**
- **Add 0.5 ml of 0.1N sodium thiosulfate to each 1L water bottle.**

Swab Sample Collection

- **Swabs of faucet aerators and shower heads should be taken before water samples from these sites.**
- **The sample should be taken with the aerator or shower head removed if possible.**
- **Submerge in 3-5 ml of water taken at the same time to prevent drying during transport to the laboratory.**

Environmental Test Request Submission Form for Legionella



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TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL FOUR CORNERS

☐EH ☐FP ☐MTY/PN ☐NOD ☐STD ☐TB ☐CD ☐COR
Submitter
Address
City County
State Zip Code
Contact Name
Phone# Fax #

Patient SS# (last 4 digits):
Last Name ☐SR ☐JR ☐Other
First Name M.I. Maiden:
Date of Birth (mm/dd/yyyy)
Address
City County
State Zip Code

Ethnicity: ☐Hispanic/Latino ☐Not Hispanic/Latino ☐Unknown
Sex: ☐Male ☐Female ☐Transgender
Race: ☐White ☐African American ☐Asian/Pacific ☐American Indian/Alaska Native ☐Multiracial ☐Not Specified ☐Other
Case # DOC# Outbreak # Submitter Lab#
Collect Date: Collect Time: ☐am ☐pm Onset Date:
Reason for Test: ☐ Screening ☐Diagnosis ☐Contact ☐Test of Cure ☐2-3 Months Post Rx ☐Suspected Carrier ☐Isolate for ID ☐Release
Therapy/Drug Treatment: ☐No ☐Yes Therapy/Drug Type: Therapy/Drug Date:

SPECIMEN CODE	BACTERIOLOGY
	Bacterial Culture - Routine
	Additional specimen codes:
	Bordetella pertussis
	Group A Strep
	Group B Strep Screen
	C. difficile Toxin
	Diphtheria
	Foodborne Pathogens (*B. cereus, C. perfringens, S. aureus*)
	Gonorrhea Culture: Incubated? ☐yes ☐no
	Hrs. incubated: Add'l specimen codes:
	MRSA (rule out)
	VRE (rule out)
	ENTERIC INFECTIONS
	Campylobacter
	E. coli O157 typing
	Enteric Culture - Routine (Salmonella, Shigella, *E. coli* O157, Campylobacter)
	Salmonella typing
	Shigella typing
	V. parahaemolyticus
	Yersinia
	REFERENCE MICROBIOLOGY
	ABC'S (BIDS) #
	Organism:
	Bacteria Referred Culture for ID
	Specify:

SPECIMEN CODE	SPECIAL BACTERIOLOGY
O	Legionella Culture
	Leptospira
	Mycoplasma
	MYCOBACTERIOLOGY/AFB/TB
	AFB/TB Culture and Smear
	AFB/TB Referred Culture for ID
	M. tuberculosis Referred Culture for Genotyping
	Nucleic Acid Amplification Test for *M. tuberculosis* Complex (MTD)
	PARASITOLOGY
	Blood Parasites:
	Country visited outside US:
	Ova & Parasites: Immigrant? ☐yes ☐no
	Cryptosporidium
	Cyclospora/Isospora
	Microsporidium
	Pinworm
	VIRUS/CHLAMYDIA
	Adenovirus*
	Arbovirus Panel (WNV, EEEV, SLEV)
	Chlamydia trachomatis
	Cytomegalovirus (CMV)
	Enterovirus (Inc. Echo & Coxsackie)
	Herpes Simplex Virus (Types 1 & 2)
	Influenza (Types A & B)*
	Parainfluenza (Types 1, 2 & 3)*
	Respiratory Syncytial Virus (RSV)*
	Varicella (VZV)
	*MAY INCLUDE RESPIRATORY SCREENING PANEL
	Comments:

SPECIMEN CODE	RESTRICTED TESTS
	Pre-approved submitters only
	Chlamydia trachomatis/GC NAAT
	Chlamydia trachomatis only/NAAT
	Norovirus ** (see comment on back)
	OTHER TESTS FOR INFECTIOUS AGENTS
	Test name:
	Prior arrangements have been made with the following DHMH Laboratories Administration employee:

SPECIMEN CODE:
PLACE CODE IN BOX NEXT TO TEST

B	Blood
BW	Bronchial Washing
CSF	Cerebrospinal Fluid
CX	Cervix/Endocervix
E	Eye
F	Feces
N	Nasopharynx/Nasal
P	Penis
R	Rectum
SP	Sputum
T	Throat
URE	Urethra
UFV	Urine (First Void)
UCC	Urine (Clean Catch)
V	Vagina
W	Wound
O	Other: Bulk water Swab

DHMH 4676 Revised 09/11

ORIGINAL

7604724 19

Complete submitter information and include the name of the authorized person requesting the test (note prior approval needed).

Enter date and time sample was collected.

Under specimen code, indicate "Legionella Culture"

Include unique identifier that matches sample ID (e.g. A-1).

Include specific sample collection location (e.g. Bathroom Sink).

Complete the outbreak number field.

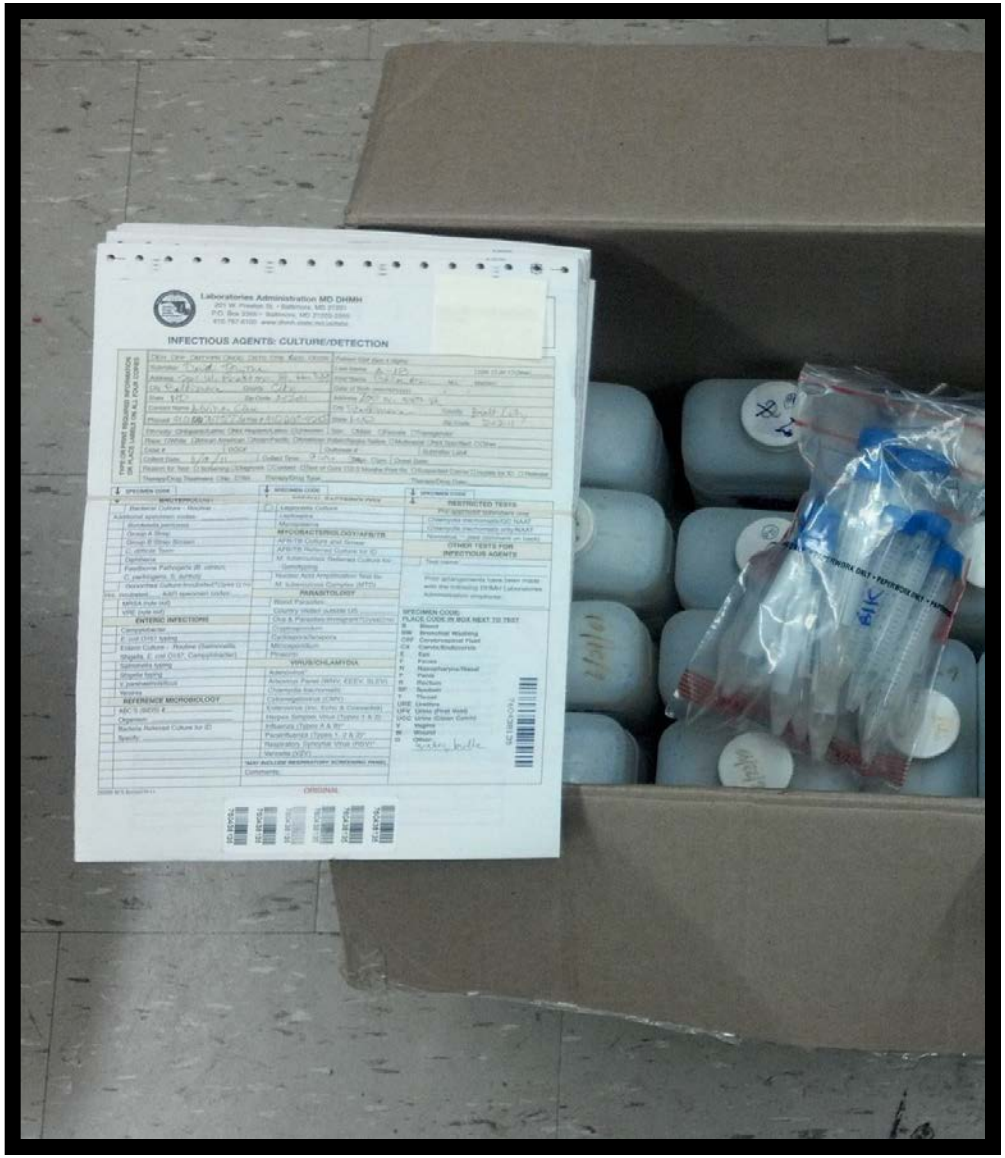
Use field to provide the source: bulk water, swab

Specimen Packaging

- **Collected Samples**

  Ensure that the water bottles and tubes are tightly closed.

- **Completed Submission Forms (DHMH 4676 Culture Detection)**



Sample Handling and Delivery

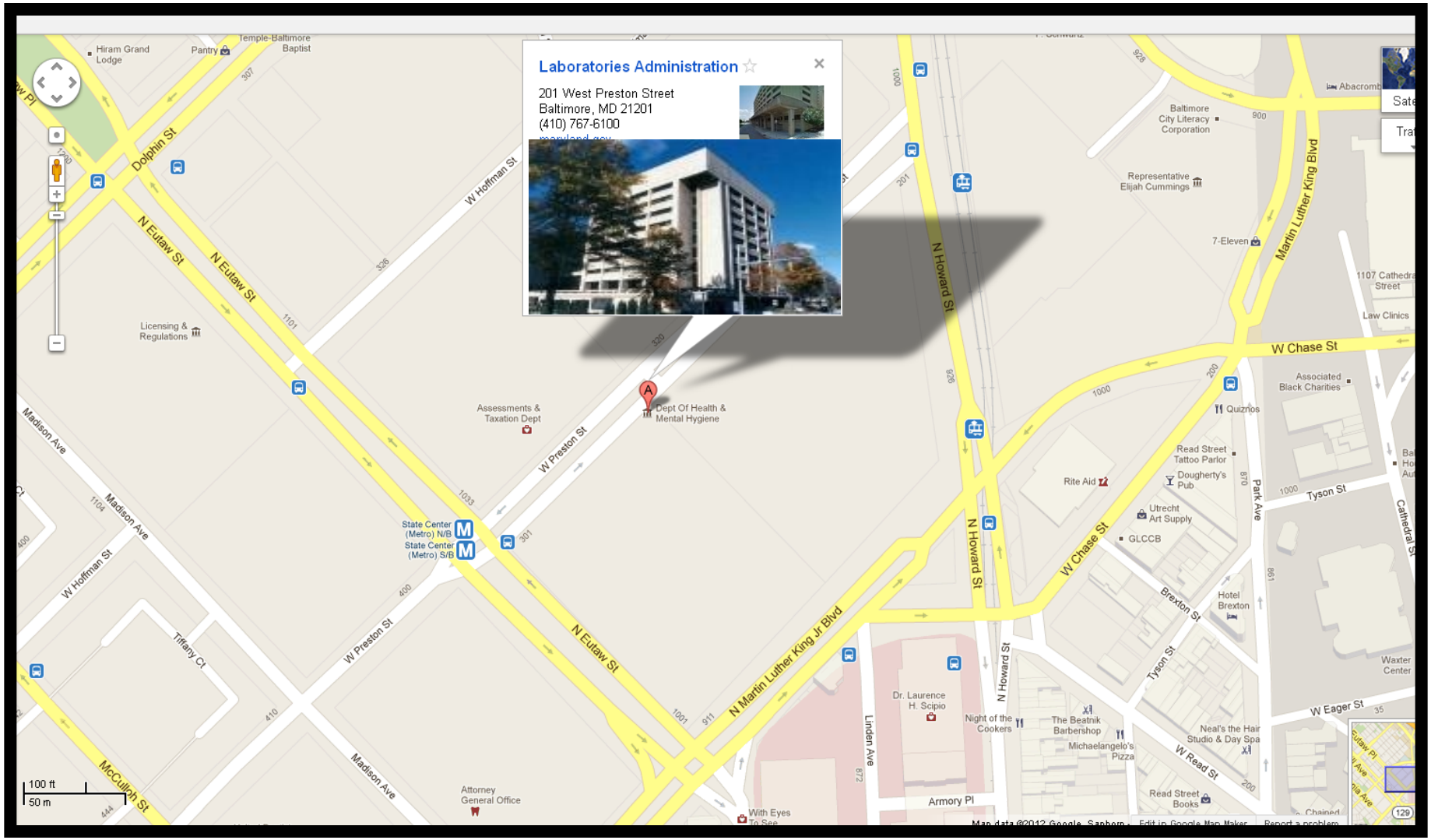
Sample delivery MUST be coordinated with the laboratories prior to submission. Please call 410-767-6162 (Supervisor: Thomas Lawson).

If samples cannot be delivered directly to the lab after collection, store at 4°C.

Samples must be received within 48 hours after collection.

MD DHMH Laboratories Administration

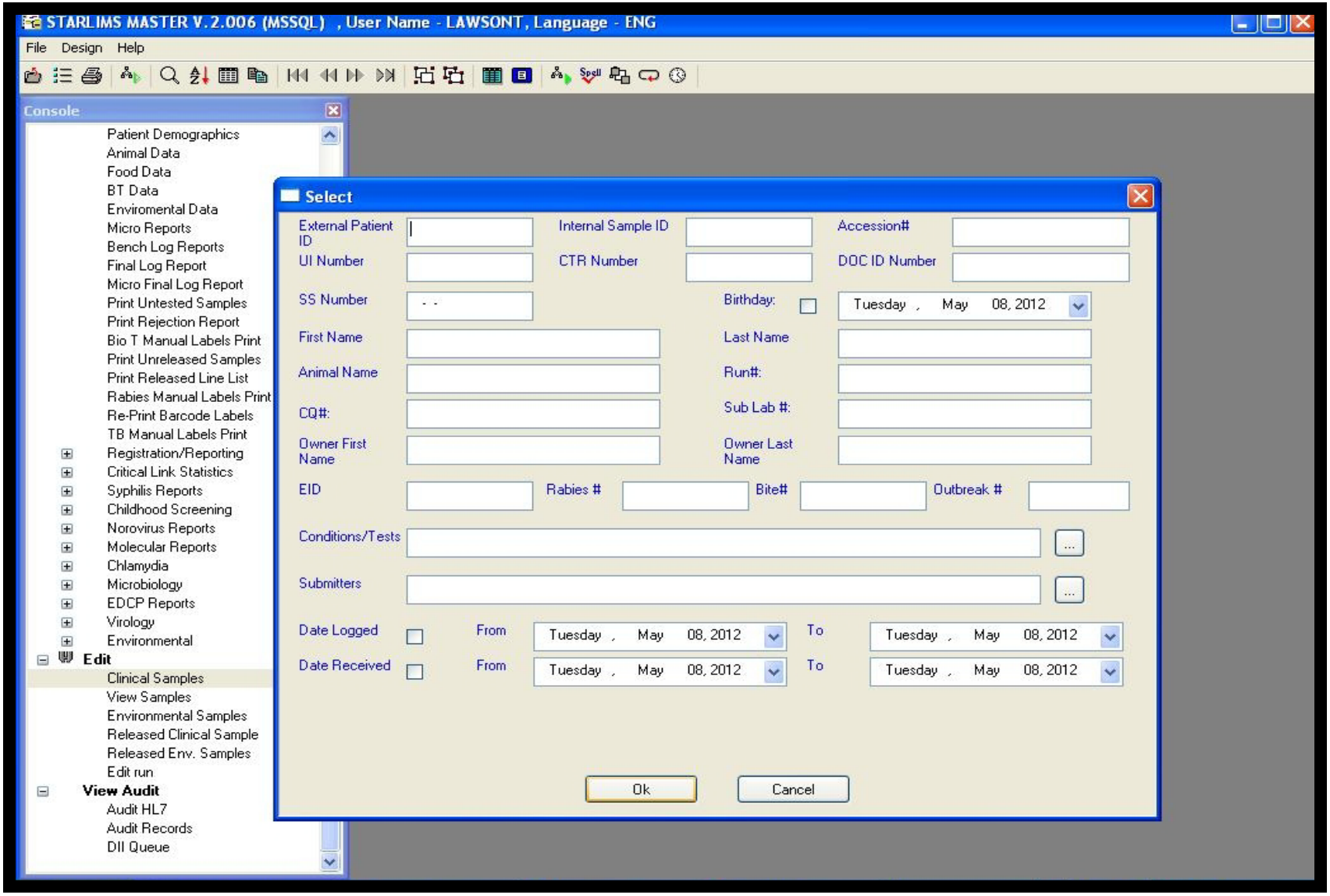
http://dhmh.state.md.us/laboratories



Samples Received at the Lab
Samples Acceptability Criteria

- **Collected samples**
- **Completed DHMH 4676 Culture Detection Submission Forms**
- **Printed/e-mail Sample Log**

Laboratory Information Management System
STARLIMS



Accessioning

Sample Processing

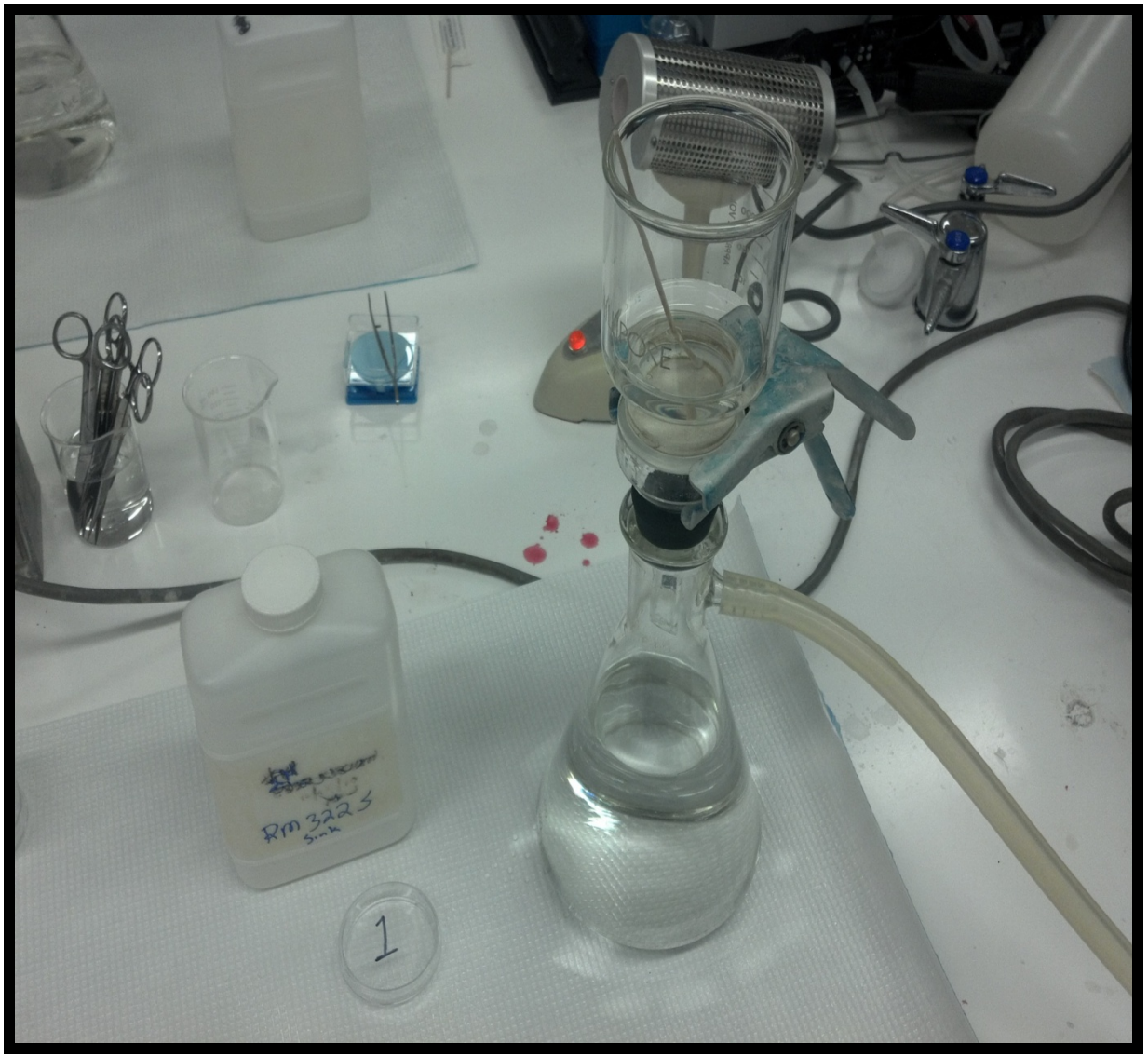
Bulk Water

Filtration

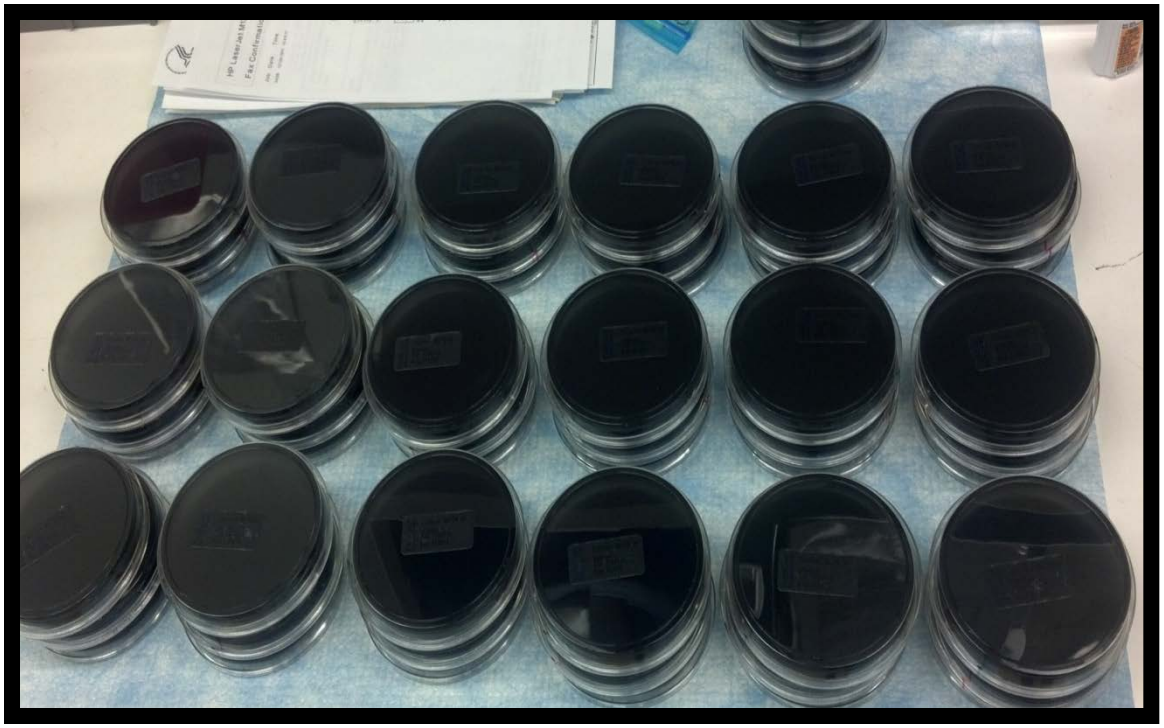
- 1L bulk water
- 0.2 uM polycarbonate filter

Swab

- Direct plating



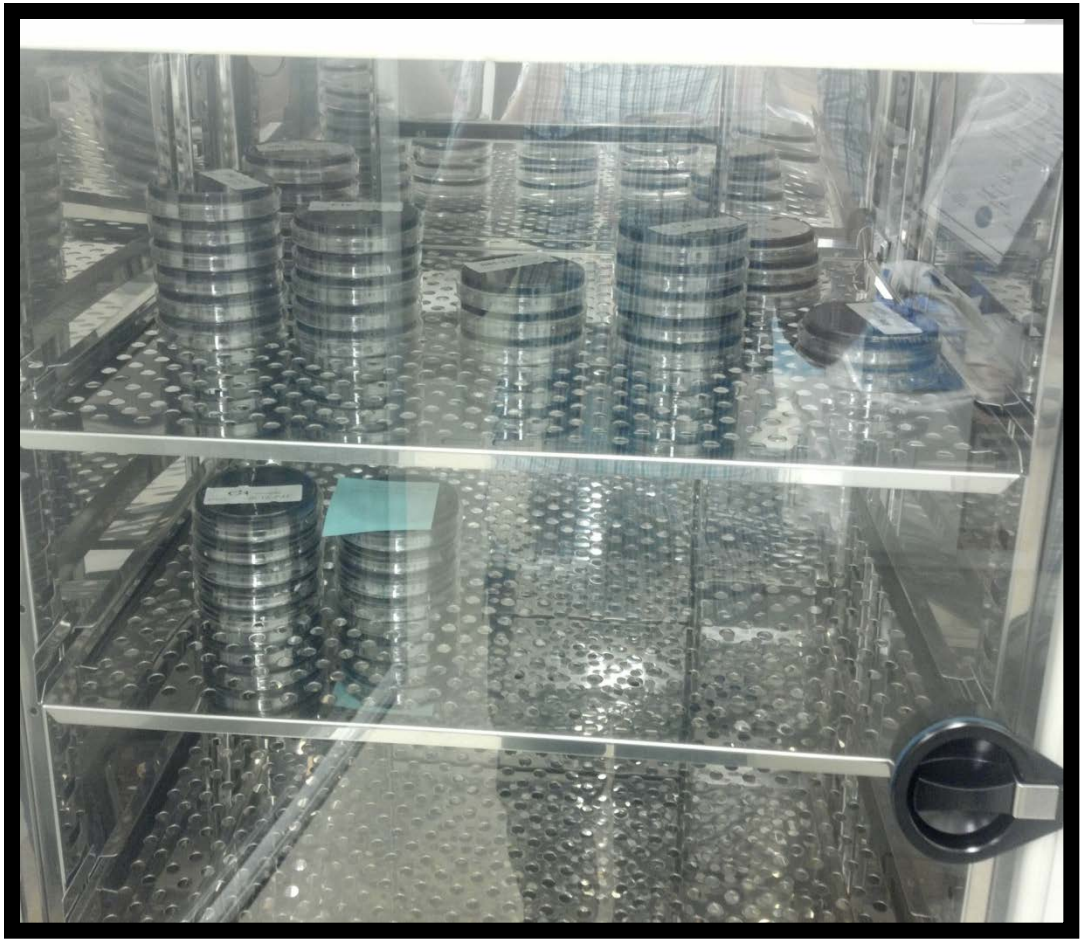
Inoculation



Inoculate into 3 types of culture plates.

1. **PAV (Polymyxin -B Anisomycin Vancomycin)**
2. **EBCYE (Environmental Buffered Charcoal Yeast Extract)**
3. **BCYE (Buffered Charcoal Yeast Extract)**

Incubation



37°C Incubation

2.5% CO_2

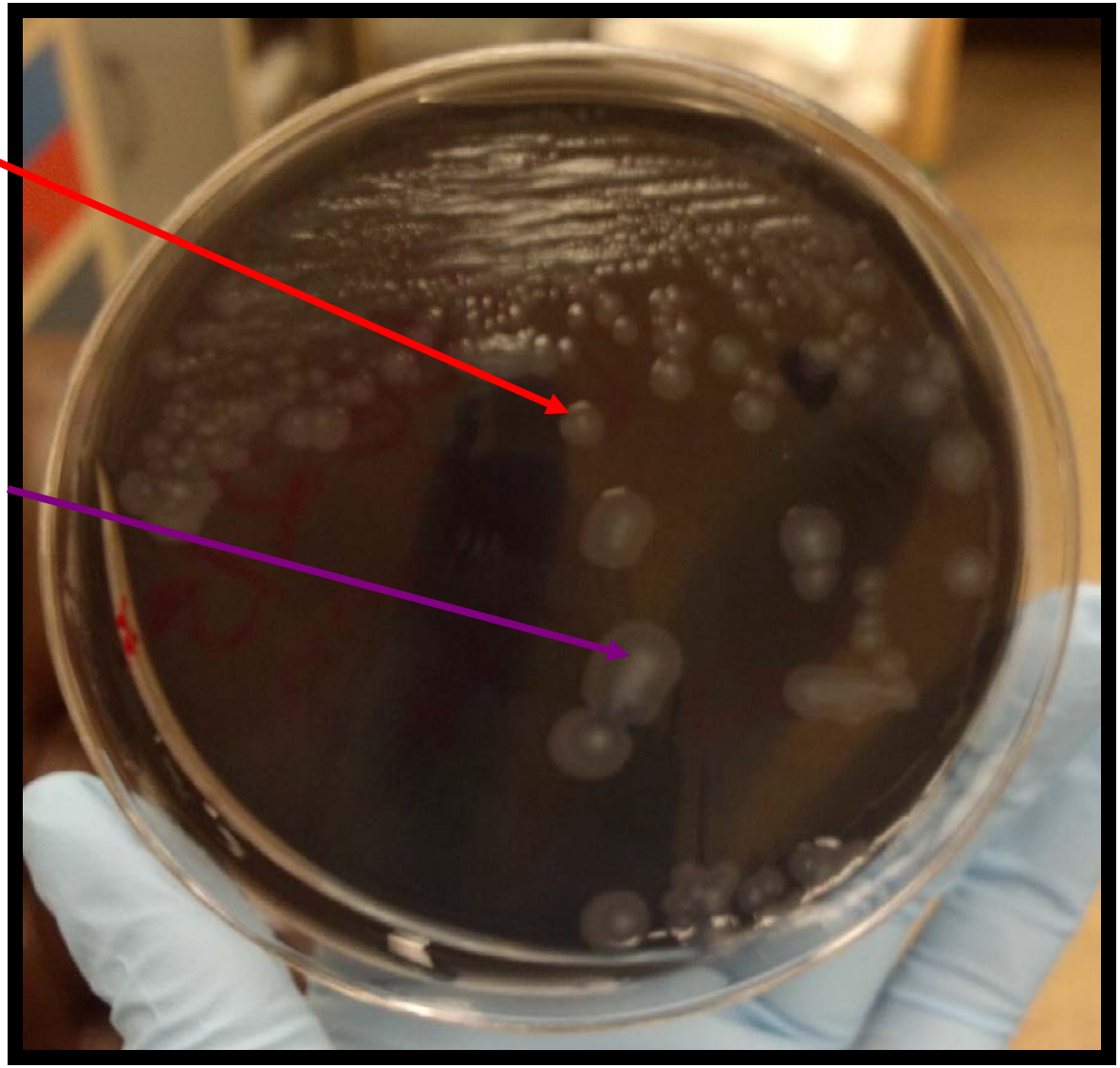
Plates checked daily after Day 2

Examination of Culture Plates
Collection of Cultures for Legionellae

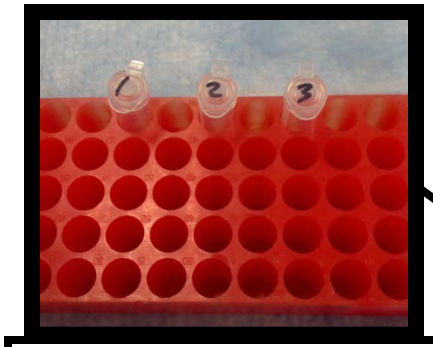
Colonies convex round with entire edges

Center of the colony usually bright white with textured edges

Once identified, individual colonies are collected with loop

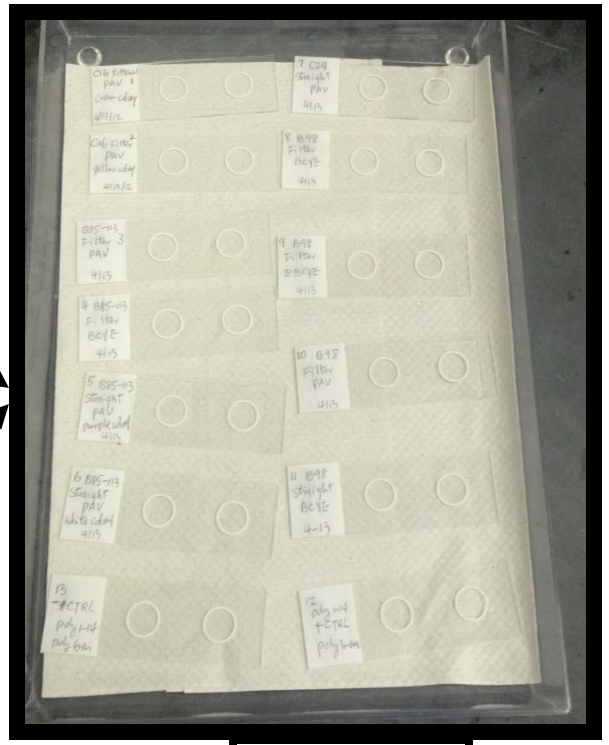


Colony Screen: DFA (Direct Fluorescent Antibody)



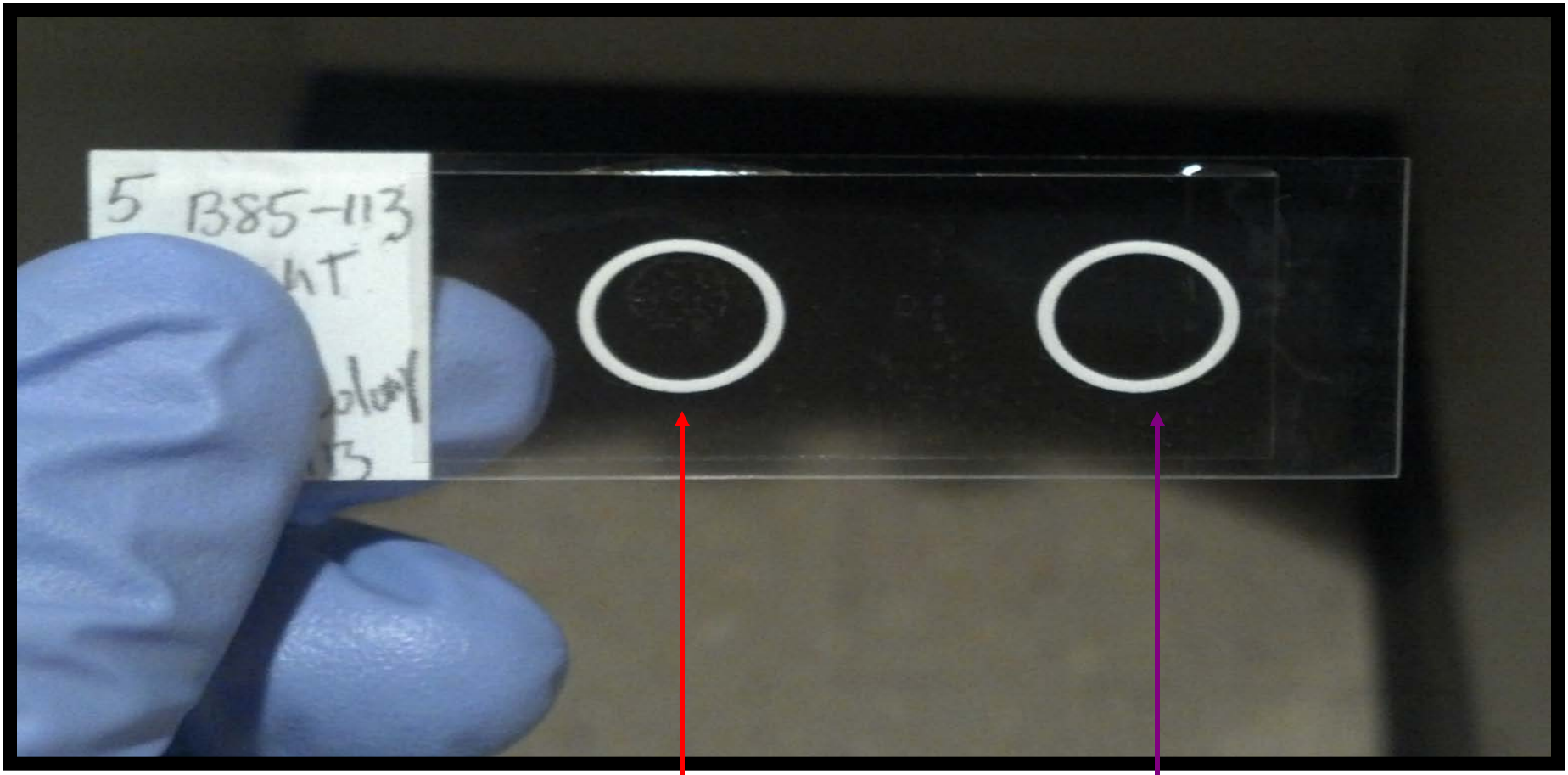
Cell Suspensions

Antibodies and Antigen Controls



Staining

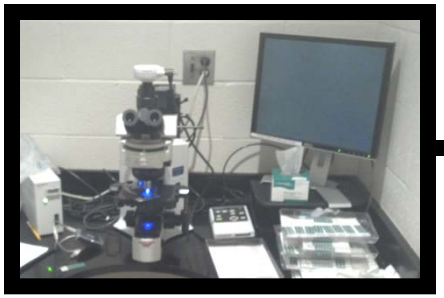
Initial Screen

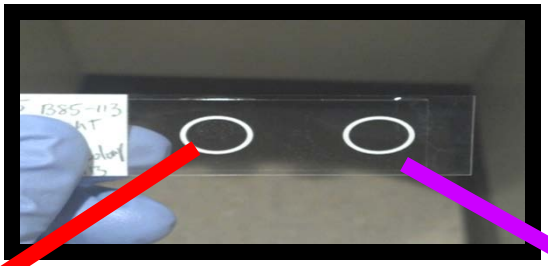


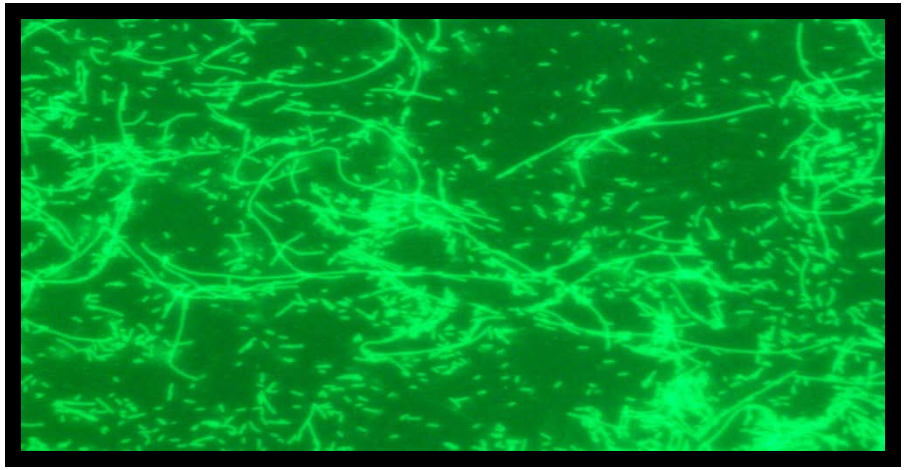
Legionella pneumophila **serogroups 1-14**

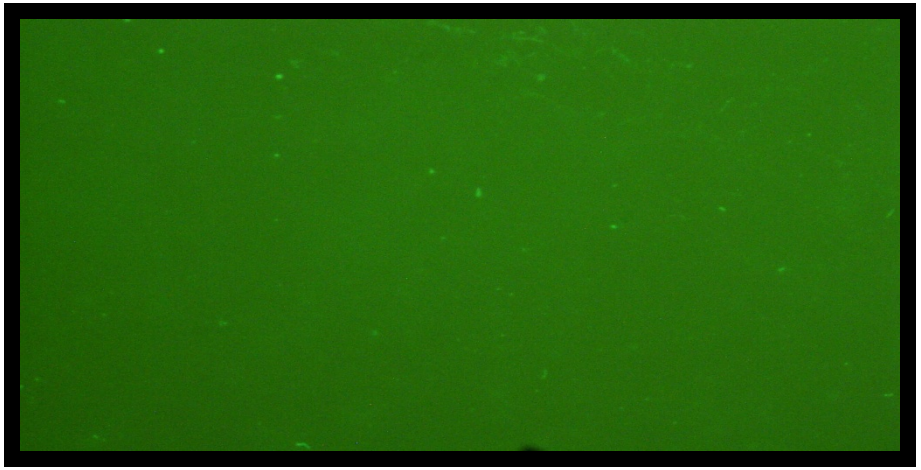
Legionella non-pneumophila spp. **(b-m)**

DFA Fluorescent Microscopy









Reactive

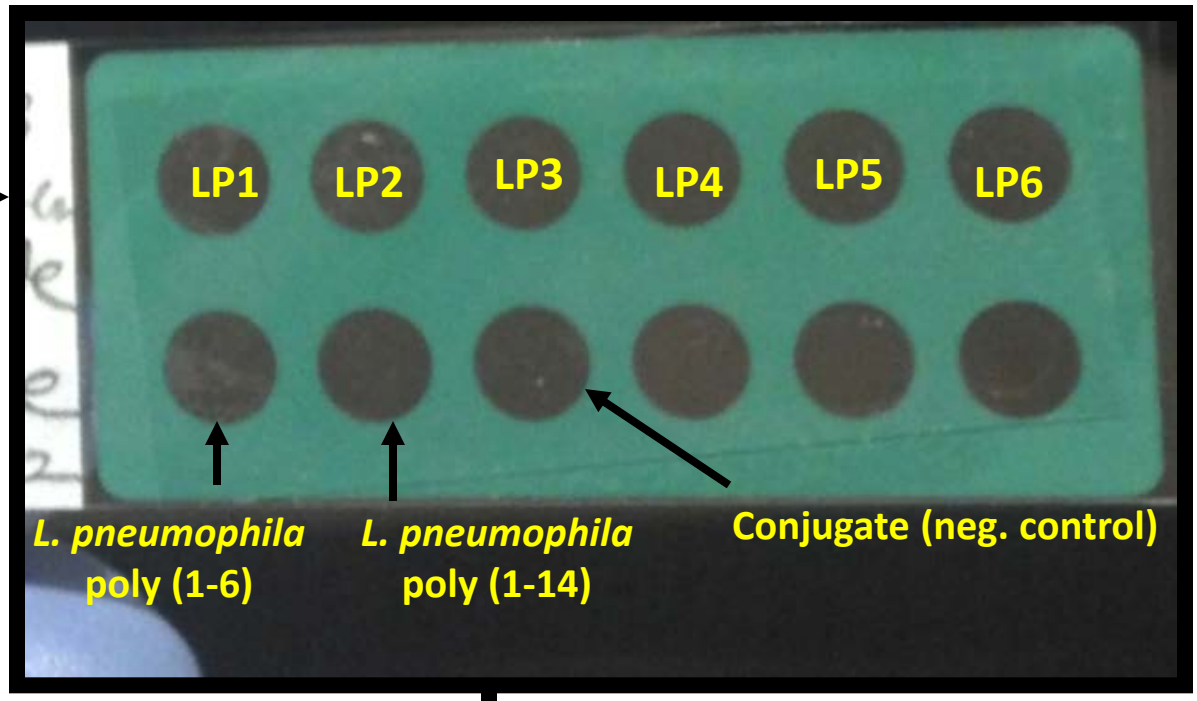
***Legionella pneumophila* Serogroups 1-14**

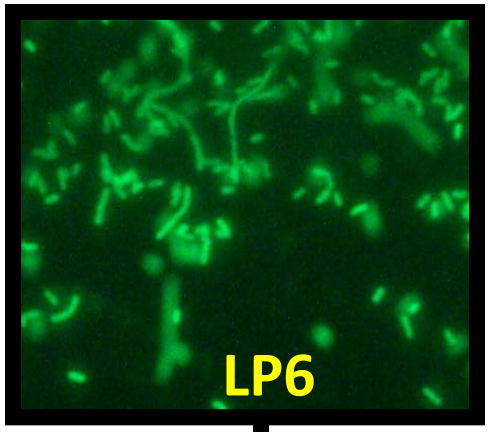
Continue with *L. pneumophila* subculture, serotyping, blood agar plate (BAP)

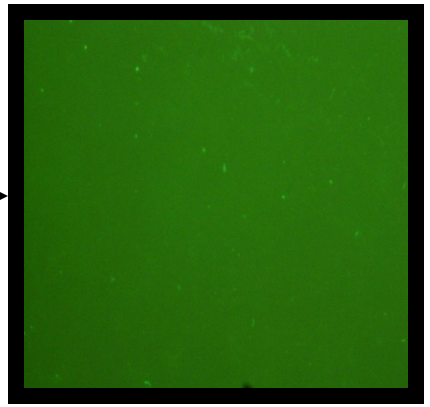
Non-Reactive

***Legionella non- pneumophila species* (b-m)**

Legionella pneumophila Serotyping

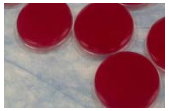




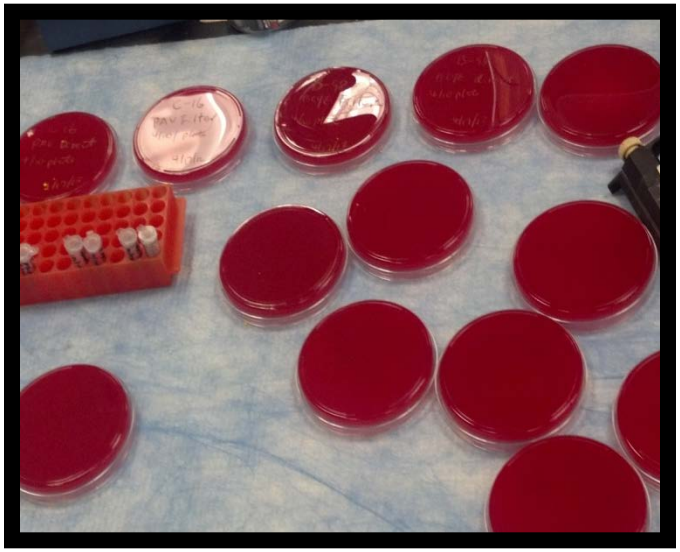


Non-Reactive

Continue with blood agar plate inoculation



Blood Agar Plate (BAP) Confirmation



24 hour incubation

Growth on BAP
Absence of Legionella

No Growth on BAP
Presence of Legionella

Calculate CFU/ml on original plates

SAMPLE REPORT

State of Maryland
Department of Health and Mental Hygiene
LABORATORIES ADMINISTRATION
Central Laboratory
201 West Preston Street
Baltimore, MD 21201
Robert Myers, Ph.D., Director
www.dhmh.state.md.us/labs

201 W PRESTON STREET
3RD FLOOR, O'CONNOR BUILDING
BALTIMORE, MD 21201

Specimen Number:
Patient Name:
Patient Id:
Birth Date:
Sex:
SSN:
Patient Address:

Internal Lab Number:
Outbreak Number:
Specimen Source:
Date Collected:
Date/Time Received:
Submitter Lab #

Comments: LEGIONELLA OUTBREAK INVESTIGATION - SWAB

Test Name	Results	Date Reported
Legionella Culture Isolation	L. pneumophila serogroup 4 isolated after 10 days incubation.	
Legionella Culture Isolation	L. pneumophila serogroup 5 isolated after 10 days incubation.	
Legionella Culture Isolation	L. pneumophila serogroup 6 isolated after 10 days incubation.	
Legionella Culture Isolation	No L. pneumophila serogroup 1 to 6 or L. micdadei isolated after 10 days incubation.	
Legionella Culture Isolation	L. pneumophila isolated after 10 days incubation.	
Legionella Culture Isolation	Legionella species isolated after 10 days incubation.	

Comments:
CFU/ML = 3.39

Conclusions

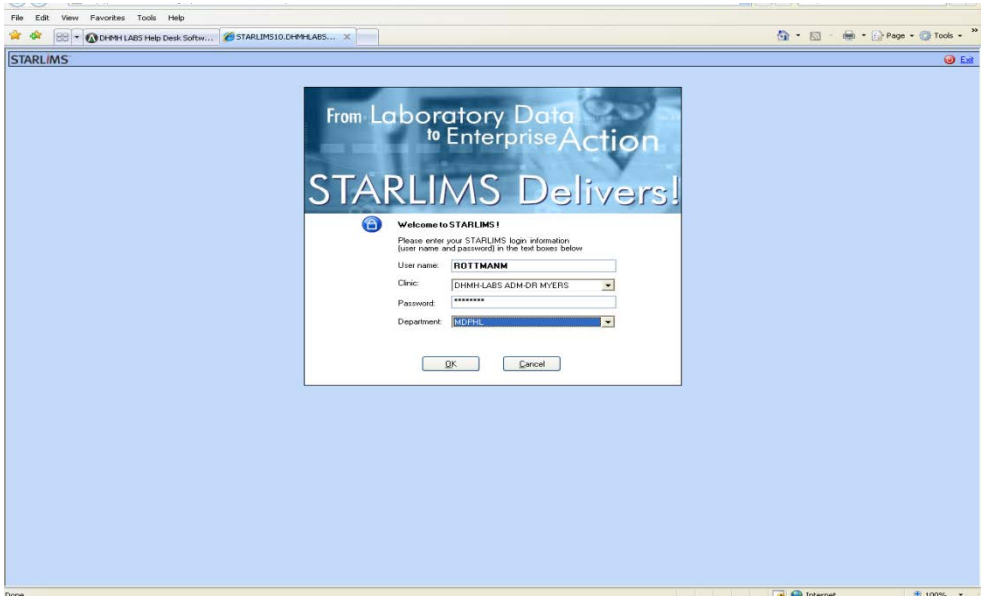
- **Proper specimen collection and submission are essential for quality assurance testing.**
- **Clinical and Environmental Case Linkage**
- **Strong coordination and collaboration with Epi and Local Health Departments are critical.**

Future Directions

Collaboration with Molecular on developing a PCR assay for Lp1.

Legionella Laboratory Website

MyLIMS Legionella Roll-Out



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