



City of Milwaukee Health Department Laboratory

www.milwaukee.gov/healthlab

Phone: (414) 286-3526

Bevan K. Baker,
Commissioner of Health
www.milwaukee.gov/health

Steve Gradus, PhD, D(ABMM)
Laboratory Director

March 2011, Vol. 16, No. 3

Sanjib Bhattacharyya, PhD
Chief Molecular Scientist

Updates from MHDL

MHDL assay for the highly virulent 027/NAP/BI epidemic strain of *Clostridium difficile*

Public health investigations of enteric diseases by the Milwaukee Health Department may include ruling out a variety of etiologic agents including bacterial, culturable viruses, norovirus, shiga-toxin producing *E. coli* and now *Clostridium difficile* associated disease. In the U.S. in recent years *C. difficile* has been more severe and has been attributed to the more virulent NAP1 strain (synonyms: B1 or ribotype 027) (J.G. Bartlett Ann. Intern Med. 2006;145:758). MHDL now utilizes the NAP-1 strain PCR assay for enteric disease investigations, especially when more common agents are ruled out such as Norovirus.

Clostridium difficile infection (CDI) is a serious healthcare-associated infection that may result in severe diarrhea, colitis, sepsis, and in rare occasions, even death. Current screening tests may lack sensitivity and may take up to 72 hours. CDI, the most common cause of hospital acquired diarrhea, has been found to be increasing in incidence and severity, and is associated with increased hospital stay, treatment costs, morbidity and mortality.

C. difficile is a spore-forming, gram-positive anaerobic bacillus that produces two exotoxins: toxin A and toxin B and accounts for 15-25% of all episodes of antibiotic-associated diarrhea (AAD). Most of the current molecular assays detect the presence of toxin-producing *C. difficile* DNA, but do not detect North American Pulsed Field Type I (NAP-1) strains. This new epidemic strain (PFGE type BI/NAP1, also called ribotype 027) appears to be more virulent, with the ability to produce greater quantities of toxins A and B.

The MHD laboratory has recently validated PCR assays for detection of Toxin B, Binary Toxin, and *tcdC* deletion targets to provide superior coverage and offers presumptive identification of the 027/NAP/BI epidemic strains.

Laboratory Confirmed Influenza (by Week) at the City of Milwaukee Public Health Laboratory

| Laboratory Confirmed Influenza by Week: City of Milwaukee Public Health Laboratory | | | | | |
|--|------------|-----------|-------------------|----------|----------|
| October 1, 2010 to March 19, 2011 | | | Positives by Type | | |
| Week Ending | No. Tested | No. Pos. | 2009 A/H1N1 | A/H3 | B |
| 10/01/10 to 01/01/11 | 59 | 0 | 0 | 0 | 0 |
| 01/08/11 | 0 | 0 | 0 | 0 | 0 |
| 01/15/11 | 7 | 2 | 2 | 0 | 0 |
| 01/22/11 | 4 | 1 | 1 | 0 | 0 |
| 01/29/11 | 10 | 5 | 2 | 2 | 1 |
| 02/05/11 | 32 | 28 | 22 | 3 | 3 |
| 02/12/11 | 42 | 16 | 13 | 3 | 0 |
| 02/19/11 | 17 | 6 | 5 | 0 | 1 |
| 02/26/11 | 18 | 9 | 8 | 0 | 1 |
| 03/05/11 | 21 | 8 | 8 | 0 | 0 |
| 03/12/11 | 12 | 8 | 7 | 0 | 1 |
| 03/19/11 | 13 | 5 | 3 | 1 | 1 |
| Total | 235 | 88 | 71 | 9 | 8 |

SUMMARY OF CONFIRMED INFECTIONS

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The March 2011 issue presents the laboratory diagnosis of some of the infectious diseases and the reference microbiology work done in this laboratory during February 2011 and new cases of syphilis in the City of Milwaukee during February 2011.

Syphilis

| Test | Total | Test | Total |
|---------------|-------|--------------------|-------|
| RPR Reactive | 1 | TPPA Reactive | 11 |
| VDRL Reactive | 24 | Darkfield Positive | 0 |

New Cases of Syphilis

| Stage | Number of Cases | |
|--------------------|-----------------|---------------|
| | February 2011 | February 2010 |
| Primary syphilis | 0 | 0 |
| Secondary syphilis | 0 | 1 |
| Early latent | 1 | 2 |
| Late latent | 0 | 7 |
| Total | 1 | 10 |

Source: Wisconsin Division of Health

Gonorrhea Antimicrobial Susceptibility Testing

| Number Tested | Decreased Susceptible (DS) / Resistant (R) Antibiotics | | | |
|---------------|--|----------|-------------|--------------|
| | Ciprofloxacin | Cefixime | Ceftriaxone | Azithromycin |
| 38 | 2 (R) | 0 | 0 | 0 |

Isolates Other Than *N. gonorrhoeae*

| Organism | Site | Number Isolates | Organism | Site | Number Isolates |
|-------------------------------|---------|-----------------|---------------------------|---------|-----------------|
| <i>Ureaplasma urealyticum</i> | Genital | 6 | <i>Mycoplasma hominis</i> | Genital | 2 |

Enteric Parasites Identified

| Age | Sex | Parasite |
|-----|-----|-----------------------------|
| 31 | M | <i>Blastocystis hominis</i> |
| 26m | F | <i>Blastocystis hominis</i> |
| 16 | M | <i>Blastocystis hominis</i> |
| | | <i>Entamoeba coli</i> |
| 44 | F | <i>Endolimax nana</i> |
| 43 | F | <i>Entamoeba coli</i> |
| 39 | F | <i>Entamoeba coli</i> |
| 15 | F | <i>Entamoeba coli</i> |
| | | <i>Giardia lamblia</i> |
| | | <i>Iodamoeba buetschlii</i> |
| 77 | F | <i>Entamoeba coli</i> |
| 9 | M | <i>Giardia lamblia</i> |
| 20 | F | <i>Giardia lamblia</i> |

Mycobacterial Infections

| Age | Sex | Test Results | | | | Identification |
|-----|-----|--------------|---------|-----------|---------|-------------------------|
| | | Sputum Smear | Culture | DNA Probe | MTB-PCR | |
| 48 | M | - | + | ND | - | <i>M. abscessus</i> |
| 66 | F | - | + | + | - | <i>M. avium</i> complex |
| 20 | F | - | + | + | - | <i>M. avium</i> complex |
| 46 | F | - | + | + | - | <i>M. avium</i> complex |
| 56 | M | - | + | + | - | <i>M. avium</i> complex |

Reference Cultures

| Age | Sex | Source | Identification |
|-----|-----|---------------|--|
| | | Pleural fluid | <i>Bacillus</i> species, NOT <i>Bacillus anthracis</i> |
| 83 | M | Paracentesis | <i>Desulfovibrio</i> species |
| 19 | F | Vagina | <i>Neisseria gonorrhoeae</i> |
| 19 | M | Rectum/Throat | <i>Neisseria meningitidis</i> |
| 27 | M | Rectum/Throat | <i>Neisseria meningitidis</i> |
| 81 | M | Hand | <i>Paenibacillus amycolyticus</i> |
| 30 | M | Stool | <i>Plesiomonas shigelloides</i> |
| 72 | F | Stool | <i>Salmonella</i> Newport |
| 4 | M | Stool | <i>Salmonella</i> Enteritidis |
| 71 | F | Stool | <i>Salmonella</i> Enteritidis |
| 22 | M | Whole Blood | <i>Salmonella</i> Enteritidis |
| 55 | F | Blood | <i>Streptococcus salivarius</i> |

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Virus Isolations from Clinical Specimens

| Age | Sex | Source | Symptoms | Agent |
|-----|-----|---------------|---|-----------------------|
| 19 | M | Throat and NP | Fever, headache, cough, sore throat | 2009 influenza A H1N1 |
| 18 | M | Throat | Fever, headache, cough, sore throat | 2009 influenza A H1N1 |
| 22 | F | Throat | Fever, headache, cough, sore throat, URI | 2009 influenza A H1N1 |
| 18 | F | Throat and NP | Fever, cough, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat | Fever, headache, cough, sore throat, URI | 2009 influenza A H1N1 |
| 19 | F | Throat | Fever, headache, cough, sore throat, nausea | 2009 influenza A H1N1 |
| 21 | F | Swab | ARD, cough | 2009 influenza A H1N1 |
| 28 | M | Throat | Fever, cough, congestion | 2009 influenza A H1N1 |
| 25 | M | Throat and NP | ARD, cough | 2009 influenza A H1N1 |
| 22 | M | Throat and NP | Fever, headache, cough | 2009 influenza A H1N1 |
| 19 | F | Throat and NP | Fever, bronchitis, cough, sore throat | 2009 influenza A H1N1 |
| 23 | M | Throat and NP | Fever, headache, cough, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat | Fever, headache, URI, cough | 2009 influenza A H1N1 |
| 18 | F | Throat | Fever, URI, cough | 2009 influenza A H1N1 |
| 24 | M | Throat | Fever, headache, cough, sore throat, URI | 2009 influenza A H1N1 |
| 21 | M | Throat and NP | Fever, headache, cough | 2009 influenza A H1N1 |
| 24 | M | Throat and NP | Fever, cough, sore throat | 2009 influenza A H1N1 |
| 24 | M | Throat and NP | Fever, lymphadenopathy, cough, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat and NP | Fever (103°), cough | 2009 influenza A H1N1 |
| 21 | M | Throat and NP | Fever (101.3°), headache, myalgia, cough | 2009 influenza A H1N1 |
| 22 | M | Throat and NP | Fever, headache, myalgia, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat and NP | Fever, myalgias, cough, sore throat | 2009 influenza A H1N1 |
| 18 | F | Throat and NP | Fever, cough, sore throat | 2009 influenza A H1N1 |
| 20 | F | Throat and NP | ARD | 2009 influenza A H1N1 |
| 21 | F | Throat and NP | ARD | 2009 influenza A H1N1 |
| 26 | M | Throat and NP | Fever (101-102°), myalgia, cough | 2009 influenza A H1N1 |
| 27 | M | Throat | Fever, headache, URI, cough, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat and NP | Fever, headache, bronchitis, cough, sore throat | 2009 influenza A H1N1 |
| 20 | F | Throat | Fever, headache, cough | 2009 influenza A H1N1 |

| | | | | |
|----|---|---------------|--|-----------------------------|
| 22 | M | Throat and NP | ARD, cough, sore throat | 2009 influenza A H1N1 |
| 25 | M | Throat and NP | Fever, headache, ARD, URI, cough, sore throat | 2009 influenza A H1N1 |
| 19 | F | Throat and NP | Fever, pleurodynia, cough, sore throat | 2009 influenza A H1N1 |
| 18 | F | Throat and NP | ARD | 2009 influenza A H1N1 |
| 21 | F | Throat and NP | ARD | 2009 influenza A H1N1 |
| 24 | M | Throat and NP | Fever, myalgia, cough | 2009 influenza A H1N1 |
| 4 | F | Throat and NP | Confirm influenza A | Influenza A (H3) |
| 19 | F | Throat | Headache, URI, cough, sore throat | Influenza A (H3) |
| 19 | F | Throat and NP | Fever (102.6°), myalgia, cough | Influenza A (H3) |
| 20 | M | Throat | Fever, cough, ILI | Influenza A (H3) |
| 30 | M | Throat and NP | Fever, cough | Influenza A (H3) |
| 19 | F | Throat and NP | Fever, headache, cough, sore throat | Influenza A (H3) |
| 22 | M | Throat | Fever, headache, URI, cough, sore throat | Influenza B |
| 22 | F | Throat and NP | R/O influenza | Influenza B |
| 19 | F | Throat | Cough, sore throat, vomiting, nausea | Coronavirus |
| 21 | M | Throat | URI, cough | Coronavirus |
| 28 | F | Throat and NP | ARD, sore throat, myalgia | Coronavirus |
| 19 | F | Throat | Fever, headache, URI, cough, sore throat | Coronavirus |
| 22 | F | Throat and NP | Fever, headache, URI, sore throat | Parainfluenza virus type-3 |
| 25 | M | Throat and NP | ARD | Respiratory Syncytial Virus |
| 22 | F | Throat and NP | Fever, cough | Respiratory Syncytial Virus |
| 24 | F | Throat and NP | Fever, headache, myalgia, fatigue, vomiting, diarrhea, URI, cough, sore throat | Respiratory Syncytial Virus |
| 20 | M | Throat | Fever, cough, sore throat | Rhinovirus |
| 18 | M | Throat | Fever, cough, sore throat | Rhinovirus |
| 19 | M | Chest | R/O zoster | Varicella zoster virus |

Influenza Real-time RT-PCR Testing

| Samples Tested | 2009 Influenza A (H1N1) Positive | Influenza A (H3) Positive | Influenza B Positive |
|----------------|----------------------------------|---------------------------|----------------------|
| 76 | 35 | 6 | 1 |

Herpes Simplex Virus Isolations

| Agent | Number of Isolates |
|-----------------------|--------------------|
| Herpes Simplex type 1 | 7 |
| Herpes Simplex type 2 | 4 |

Molecular Amplification and PCR

| Agent | Method | Tested | Positive | % Positive |
|---|------------------|--------|----------|------------|
| Norovirus | RT-PCR | 3 | 2 | 66.7% |
| <i>Bordetella pertussis/parapertussis</i> | RT-PCR | 2 | 0 | 0% |
| <i>Parainfluenza</i> | RT-PCR | 1 | 1 | 100% |
| <i>Chlamydia trachomatis</i> | Gen-Probe Aptima | 515 | 71 | 15.7% |
| <i>Neisseria gonorrhoeae</i> | Gen-Probe Aptima | 714 | 47 | 6.6% |

Respiratory Virus Surveillance:

| Respiratory Virus Panel Test Results | | |
|--------------------------------------|-----------|---------|
| Virus | Positives | Percent |
| Human Rhinovirus (HRV) | 13 | 6.9% |
| Parainfluenza virus 2 (PIV2) | 4 | 2.1% |
| Parainfluenza virus 3 (PIV3) | 6 | 3.2% |
| Adenovirus Type E (Adeno E) | 3 | 1.6% |
| Coronavirus OC43 | 7 | 3.7% |
| Coronavirus NL63 | 3 | 1.6% |
| Respiratory Syncytial virus (RSV) | 4 | 2.1% |

Samples tested: 188 (Sept. 1, 2010 – March 8, 2011)

DNA Sequencing: The MHD laboratory uses 16S rRNA and the D2 region of the 26S rRNA genes for DNA sequence-based microbial identification of selective reference bacteria and fungal isolates.

| Reference Microbe | Target gene | Final Identification |
|-------------------|-------------|------------------------------------|
| Bacteria | 16S rRNA | <i>Desulfovibrio</i> species |
| Bacteria | 16S rRNA | <i>Paenibacillus barcinonensis</i> |
| Bacteria | 16S rRNA | <i>Streptococcus salivarius</i> |