A Publication of Geisinger Medical Laboratories

RESPView Pathogen Surveillance 2012-2013

- In 2013 CDC week 12, ending April 14, human metapneumovirus, fluB, and rhinovirus predominate
- The diversity of viruses is high with representatives from all groups.
- 39% of samples submitted were positive for at least one virus.

Remember no laboratory test method reaches 100% sensitivity or specificity. As the season begins to wane, test specificity may decline as the population transitions to low viral prevalence.

10X Essentials: New Rapid Testing Platform for MRSA and C. difficile and Enterovirus

Beginning April 29, 2013, the Microbiology Laboratory at GMC and GSACH will begin rapid testing for MRSA active surveillance, as well as diagnostic testing for enterovirus and Clostridium difficile. Specific information is listed below and in the C. difficile attachment.

MRSA PCR

Clinical and Diagnostic Utility

- PCR is the most sensitive method to identify patients colonized with methicillin resistant Staphylococcus aureus (MRSA), both community and healthcare associated strains.
- Sensitivity and specificity are generally above 95-100% compared to other molecular methods.
- Do NOT submit multiple specimens for testing. No improvement to yield (test positive predictive value) is achieved by testing multiple specimens.
- The test delivers nearly on-demand results (TAT is < 4 hours from receipt in laboratory (on first and second shift. Expansion to 3rd shftt will follow).
- Assay detects SCCmec types I-Iva

Testing Criteria New test code/test name: MRSAP, MRSA Screen PCR (old test code/test name: AMRSA, MRSA Screen PCR)

- Limit testing to patients that require active surveillance for MRSA
- Due to the high sensitivity of the PCR assay, we recommend only 1 specimen be tested per patient admission, with exceptions for some units where are “screened” on certain days of the week for all patients currently on unit, ICU’s, etc.)
- Specimens and Stability Nares swab collection (white cap molecular swab): Tilt patient’s head back. Insert both dry swabs approximately 1–2 cm into each nostril. Rotate the swabs against the inside of the nostril for 3

“Make it the best.” - A. Geisinger
seconds. Apply slight pressure with a finger on the outside of the nose to help assure good contact between the swab and the inside of the nose. Using the same swabs, repeat for the second nostril, trying not to touch anything but the inside of the nose. Remove the plastic transport tube. Twist off the tube cap and discard it. Place the swabs into the labeled plastic transport tube. The swabs should go all the way into the tube until they rest on top of the sponge at the bottom of the tube. Make sure the white cap is on tightly. **Note:** The swabs should stay attached to the white cap at all times.

- **Transport/Stability:** Transport to the laboratory at room temperature (15–30°C), Stable for 24 hours at room temperature (15-30°C) or 5 days refrigerated at 2-8°C.
- **Unacceptable:** Any specimen source other than nares, nose, and nasal swab.

**Cautions/Limitations to Testing**

- An assay positive result does not rule out the presence of other pathogens
- Because the detection of MRSA is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- A positive test result does not necessarily indicate the presence of viable organism. It is however, presumptive for the presence of MRSA DNA.
- Test results might also be affected by concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new/unknown variants resulting in a false negative result.
- Potentially **interfering substances** include blood, mucus and nasal sprays

**ENTEROVIRUS RT-PCR**

**New test code/test name:** EVP, Enterovirus PCR (old test code/test name: PCREV, Enterovirus PCR)

**Specimens/Stability:** All specimens must be labeled with patient’s name and collection date.

- Cerebrospinal Fluid (CSF): 350ul to 3 ml collected in a sterile tube.
- Do not place in Universal Transport Media (UTM).
- Transport to the laboratory at 2-8°C (room temperature, 15-25°C, is acceptable <24 hours). Stable for 3 days refrigerated at 2-8°C or ≥ 2 weeks at ≤-80°C.
- Keep CSF specimens at 2-8°C until testing or freeze specimens at -80°C if test will not be performed within 72 hours of collection. Do not freeze and thaw the specimens more than two times. Centrifugation is not recommended.
- Minimum volume = 350 µl CSF

**Limitations**

- Extremely high white blood cell counts, protein, whole blood, and hemoglobin in the CSF may interfere with the assay.

**Unacceptable Conditions:**

- Specimens other than CSF or samples outside the required parameters for specimen stability.

**Questions:** For newsletter questions, contact Christy Attinger at (570) 271-6338 or me. **Best regards,** Donna M. Wolk, MHA, Ph.D., D(ABMM), GML System Director of Microbiology
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- **adenovirus**: 1 1 0 0 2 2 3 5 3 1 1 0 1 2 4 2 3 5 2 1 1 2
- **coronavirus**: 2 4 2 7 8 17 19 24 25 21 15 36 31 27 25 29 30 18 26 11 4 5 6
- **human metapneumovirus**: 0 0 1 0 2 3 3 2 8 4 4 8 9 4 9 4 5 5 5 11 13 16 9 16
- **influenza A**: 0 1 0 1 24 49 155 221 218 322 284 208 132 87 87 52 35 15 9 7 1 8 1
- **influenza B**: 0 1 0 1 5 10 16 11 9 17 11 16 16 6 36 30 48 49 50 40 12 17 13
- **parainfluenza**: 11 17 8 15 13 8 4 3 20 5 5 2 5 2 2 4 5 2 5 4 4 6 4
- **respiratory syncytial virus**: 13 9 21 19 24 35 47 55 45 45 56 65 55 57 39 41 41 37 37 31 24 15 9
- **rhinovirus**: 33 41 26 33 28 44 48 40 37 30 21 26 17 14 13 17 20 29 27 26 25 25 16

- **% Positive Rollup**: 37% 51% 39% 44% 45% 52% 56% 63% 50% 44% 41% 46% 40% 37% 38% 39% 39% 41% 45% 43% 34% 37% 39%
Clinical and Diagnostic Utility

- PCR is the preferred method to detect *C. difficile* and its hypervirulent B1/NAP1 strains. Sensitivity and specificity are above 95%.
- Do NOT submit multiple specimens for testing. No improvement to yield (test positive predictive value) is achieved by testing multiple specimens.
- Traditional testing methods, are of limited use. Enzyme immunoassay (EIA), Glutamate dehydrogenase (GDH), and cytotoxicity assays (CT) range from 32-84% in sensitivity.
- Do NOT attempt laboratory test of cure for *C. difficile* infection (CDI).

Testing Criteria

New test code/name: **CDIFP, *C. difficile*/Epi PCR**; (old test code/test name: **CDIF, *C. difficile* PCR**)

- Limit testing to patients with risk factors for CDI and significant diarrhea (≥ 3 loose stools/day for ≥ 1 day).
  - False positive results can occur when testing patients without significant diarrhea, as the microbe can be present is some people’s GI tract without causing disease, especially patients < 1-2 yrs. of age.
- Due to the high sensitivity of the PCR assay, we recommend only 1 specimen be tested during each 5 day period.
  - For special circumstances, call the Microbiology Laboratory for consultation or to request a policy waiver. When your patient shows signs of ileus in the absence of bowel movements, use clinical judgment for atypical disease presentation and empiric antibiotics as warranted.

Specimens and Stability

- Liquid/Unformed stool specimens (feces), minimum ≥ 0.5ml, collected in a sterile cup or tube without preservatives
- Transport/Stability: Transport to laboratory at 2-8°C, Stable for 5 days refrigerated at 2-8°C.
- Unacceptable: Formed stool, rectal swabs, or stool preserved in 10% formalin, SAF, or PVA are NOT accepted.

*dmw: cdiff*  
*ver. 1, 2/14/2013*  
**ALERT:** Institute “**Contact Precautions**” for *C. difficile* positive patients  
For inpatient positive samples, request an Infectious Disease consult
Background on hypervirulent *C. difficile* strains

- A rise in the incidence and the virulence of CDI links to the emergence of **hypervirulent strains**, (most commonly B1/NAP1/027)
- NAP1 strains have more *efficient sporulation* and production of *toxins*. The presence of binary toxin genes serve as a presumptive biomarker of this hypervirulent strain.
- NAP1 strains are associated with increased severity, need for surgery, mortality, and higher relapse and dissemination rates.
- An expanded repertoire of antibiotic resistance includes resistance to vancomycin, fidaxomicin, and fluoroquinolones, which offers a competitive advantage for transmission in healthcare settings and may require combination or alternate therapy.
- Strains can cause disease in **non-traditional patient populations**, infecting young adults and pregnant women with no prior history of antibiotic use.

Cautions/Limitations to Testing

- The assay is inhibited by zinc oxide paste and Vagisil cream.
- Interpret results in conjunction with other laboratory and clinical data, e.g., radiologic and endoscopic abnormalities.
- An assay positive result does not rule out the presence of other pathogens, which are rarely known to cause pseudomembranous colitis and antibiotic-induced diarrhea.
- The toxin A gene is not detected; however, it is becoming increasingly evident that toxin B plays a much more important role in disease than Toxin A. Toxin A positive, B negative strains are exceedingly rare and not yet encountered in the US.
- The *C. difficile* NAP1 strain identification is reported as presumptive because this assay only detects the most common marker. Other hypervirulent strains such as 078/NAP7/BK, which can also carry the binary toxin and are also known to infect patients. The 078 strains will be identified only as *C. difficile* by the PCR, without any NAP designation.

**ALERT**: Institute “Contact Precautions” for *C. difficile* positive patients.