ACL – Molecular Microbiology

Michael Costello, Ph.D.
ACL Laboratories
## Our Health Systems

### Advocate Health Care

**Largest Health System in IL**

- 12 Hospitals (~3,360 Beds)
  - 1 Integrated Children’s Network
  - 5 Level I Trauma
  - 2 Level II Trauma
  - 4 Teaching
- Advocate Medical Group
- Advocate Physician Partners
- Dreyer Medical Group
- Advocate at Home (Home Care/Hospice)
- 34,000 Employees
- $4.6 Billion Revenue

### Aurora Health Care

**Largest Health System in WI**

- 15 Hospitals (~3,000 Beds)
  - 1 Psychiatric
  - 5 Level II Trauma
  - 2 Teaching
- Aurora Medical Group
- Aurora Advanced Medical Group
- Aurora U.W. Medical Group
- Lakeshore Medical Group
- Aurora VNA (Home Care/Hospice)
- 30,000 Employees
- $4.3 Billion Revenue
ACL Laboratories Profile

- One of the largest hospital system laboratories in the US
- $300M Annual Operating Expense Budget
- Provides services to 27 Hospitals; 2 Central Laboratories; 110+ Clinics & Patient Service Centers (PSC)
- 2,700 Associates/Caregivers
- 90 Pathologists; Midwest Diagnostic Pathology (IL) and Great Lakes Pathologists (WI)
- 24M laboratory tests performed annually (50k a day)
- 5200+ Clients outside of our systems
- Couriers: 80+ vehicles, > 3.6 million miles annually
- Client Services handles ~1000 in-bound calls day
“Unique Molecular Test Challenges”

- **Size**
  - The Good
    - Spread cost over 27+ hospitals
  - The Bad
    - 27+ hospitals with unique testing requirements
  - The Ugly
    - Getting two large healthcare systems (Aurora and Advocate) to agree on anything
      - What testing is the most significant?
        - When
        - Where
    - Keeping everyone on the “same page”
      - Which page?
“Unique Challenges”

- Different patient populations
  - Urban – Milwaukee Vs. Chicago
    - Widest spectrum of pathogens
    - Increased antibiotic resistance
      - MRSA, VRE, ESBLs (*shv, tem* Vs. CTX-M), CRE
  - Suburban
    - Widest variation in pathogens and antibiotic resistance
  - Rural
    - Smallest variation in pathogens and antibiotic resistance

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Personal data, M. Costello and L. Mazur, Multiple hospitals, Chicago area and southeast Wisconsin.
Changing Workflow in Microbiology

- Traditional workflow Vs. Syndromic Panels
  - Traditional workflow
    - Sequential traditional ordering of laboratory tests
      - Takes time
      - Less sensitive
      - Can be more expensive than molecular syndromic panels
Changing Workflow in Microbiology

- Syndromic panels
  - To be performed as “stat” tests
    - Meningitis-Encephalitis (ME) Panel
    - Blood Culture Identification Panels
      - Molecular panel vs. MALDI-TOF
        - Ensure that someone acts on results
  - 2-24 hour turnaround time
    - Respiratory Panel
    - Gastrointestinal Panel
1-24 Hour Turnaround Time

All PCR reactions are 3X
CNS Workup
CSF Workup

- CSF for cell count, glucose, protein, Gram stain
- Nucleic acid tests for HSV 1 and 2, VZV, CMV, EBV, and Enteroviruses, Paraechoviruses
- Cell culture: Inoculation of super E-mix (engineered BGMK and A549 cells), HDF, PMK, Hep-2, RD, etc.
- Additional CSF/tissue: freeze at -20°C (-70°C for long term storage)
- Acute phases serum: freeze 1-2 mL at -20°C (-70°C for long term storage)
- Refer CSF and acute phase serum to public health or reference laboratory for seasonal West Nile Virus or other Arbovirus IgM* antibody testing.
- Collect convalescent serum, if required (10-14 days)

Diagnosis established

(+ ) Nucleic acid tests for HSV VZV, (CMV) or enterovirus
(+ ) Virus isolation in cell culture
(+ ) WNV/arbovirus IgM

No viral infection identified – further testing as clinically indicated

- Nucleic acid testing of stored (-20°C) CSF
- EBV, HIV, CMV, JC virus, etc.
- Collect convalescent serum
- Consult with public health/reference laboratory; provide Clinical and travel history. Send paired acute and convalescent sera and frozen CSF/tissue for additional testing

Aseptic meningitis
- Enteroviruses
- WNV, other regional arboviruses
- Travel-associated arboviruses*
- HIV
- HSV-2
- EBV
- VZV.
- Mumps,
- Adenovirus
- LCM
- Influenza A&B

Meningoencephalitis/encephalitis
- WNV or other arboviruses
- Travel-associated arboviruses (Dengue virus or Chikungunya)
- HSV
- Enteroviruses (paraechoviruses
- EBV
- VZV,
- Measles, Rabies
- RSV, hMPV,
- Influenza,
- Adenovirus

Immunocompromised patient
- CMV,
- HSV,
- VZV
- HIV
- EBV
- JC/BK virus
- Enteroviruses
- Parvo B19 virus
- HHV6,
- Toxoplasma gondii

* Consult with Public Health/Reference laboratory for volume of CSF needed for all nucleic acid tests and serology ordered.

Brain biopsy - 0.5 cm³ tissue biopsy usually sufficient for imprints, surgical pathology, comprehensive microbiology, and nucleic acid tests.

# Arboviruses include West Nile Virus,
BGMK=buffalo green monkey cells; HDF=human diploid fibroblasts; PMK=primary monkey kidney, RD= Rhabdomyosarcoma cells
Traditional Culture Vs. Syndromic Panels

- Traditional Culture - Sequential/separate diagnostic tests
  - Culture/serology
    - Different tests for bacterial, viral and fungal pathogens

- Meningitis-Encephalitis (ME) Panel
  - One test for viruses, bacteria and fungus
  - How will this panel be used?
    - Triaging ED patients – 1 hour TAT
      - HSV encephalitis admit and treat aggressively
      - Enteroviral meningitis – Send home
    - Rapid diagnosis
      - Antibiotic therapy
      - Viral vs. bacterial
Meningitis/Encephalitis Panel

1 Test. 16 Targets. All in about an hour.

**Bacteria**
- *Escherichia coli* K1
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

**Viruses**
- Cytomegalovirus (CMV)
- Enterovirus
- Epstein-Barr virus (EBV)
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Human herpesvirus 6 (HHV-6)
- Human parechovirus
- Varicella zoster virus (VZV)

**Yeast**
- *Cryptococcus gattii*
- *Cryptococcus neoformans*
Respiratory Viral Panel

ACL RESP PANEL - Nov 01 2015 - Feb 20 2016

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Respiratory Panel

- How will this panel be used?
  - Expense Vs. Utility?
  - Influenza season?
    - Reflex test?
  - In-patients Vs. out-patients
    - Syndromic panels for in-patients?
  - Admit or not to admit
  - Bacterial Vs. viral
Figure 1. Seasonal variation of viral infections

**SUMMER**
- Adenoviruses
- Herpes simplex
- Cytomegalovirus
- Varicella zoster
- Measles/Mumps
- Respiratory Syncytial Virus
- Parainfluenza 1 and 2
- Parainfluenza 3
- Influenza A and B
- Enteroviruses
- Arboviruses*
- Rotavirus
- Norovirus

**FALL**

**WINTER**

**SPRING**

*Detected by traditional antigen/culture/serology  
*West Nile Virus and Dengue Virus

Detected by Nucleic acid amplification
Cytopathic Effect (CPE) in tube culture

Shell vial monolayers stained with FITC-conjugated monoclonal antibodies

Patient respiratory cells stained with FITC-conjugated monoclonal antibodies

Figure 5. Viral growth in cell culture line and viral detection in patient samples.
Respiratory Panel

**RESPIRATORY PANEL**

The FilmArray Respiratory Panel tests for a comprehensive panel of 20 respiratory viruses and bacteria. The FilmArray instrument integrates sample preparation, amplification, detection and analysis into one simple system that requires 2 minutes of hands-on time and has a total run time of about 1 hour.

- **Simple**: 2 minutes of hands-on time
- **Easy**: No precise measuring or pipetting required
- **Fast**: Turnaround time of about 1 hour
- **Comprehensive**: 20 target respiratory panel

**Viral Targets**

- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H3
- Influenza A/H1-2009
- Influenza B
- Parainfluenza Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 4
- Respiratory Syncytial Virus

**Bacterial Targets**

- *Bordetella pertussis*
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*
MOLECULAR PATHOLOGY UPDATE

ACL RESP. PANEL - Flu POSITIVE RATE 12.6%
Gastrointestinal Panel
Luminex Assay

GPPNL  Gastrointestinal Pathogen Panel
Reported as: GASTRO PATHOGEN PNL
Also known as: Gastrointestinal Pathogen Panel by PCR, Viruses: Adenovirus 40/41, Rotavirus A, Norovirus (GI/GII includes Sydney 2012), Bacteria and bacterial toxins: Escherichia coli (E. coli) O157, Enterotoxigenic E. coli (ETEC) LT/ST, Salmonella spp, Shigella spp (S. boydii, S. sonnel, S. flexneri and S. dysenteriae), Campylobacter spp (C. jejuni, C. coli and C. lari only), Shiga-like Toxin producing E. coli (ETEC) stx1/stx2, Vibrio cholerae, Parasites: Giardia (G. lamblia only), Entamoeba histolytica, Cryptosporidium spp. (C. parvum and C. hominis only).

Specimen Requirements
- Patient Preparation: Stool specimens must not be collected after administration of barium, bismuth or oil.
- Collect: Stool in sterile container or in C&S (Cary-Blair) media
- Transport: Do not freeze.
- 5.0 mL (min: 1.0 mL) refrigerated
- Unacceptable Conditions: Frozen, Leaking container, Non-sterile container, Specimen not received in appropriate transport media, Rectal swabs.
- Stability: Ambient: 8 Hours / Refrigerated: 2 Days / Frozen: 2 Weeks (to be Frozen at ACL core lab only - for long term storage)

Ordering Instructions
- Order Remarks: This assay is FDA approved for use with unpreserved raw stool specimens and Cary-Blair media. Other type of collection media are not validated and will be rejected.

Clinical Significance
- Click here for more information

Lab Notes
- Frozen unpreserved stool will limit any additional culture and parasitology type testing due to stability.
- Stool specimens must not be collected after administration of barium, bismuth or oil.
- Throat swabs, vomitus and other stool transport devices will be rejected.
- Not suitable for test of cure on previously positive patient.

Test Performance
- Performed: Weekdays
- Reporting Time: Final within 3 Days
- Performing Labs: ACL IL Central Laboratory - Rosemont, EMR/Interfaced Flagged "Client" Orderable test

CPT Codes
- 87567
Blood Culture Identification Panel
Blood Culture Identification Panel

- Syndromic Panel Vs. MALDI-TOF
- Remote sites Vs. Non Remote sites
  - Considering molecular rapid ID methods for remote sites
In Summary
Outstanding Issues

- Validation/verification of syndromic panel assays
  - Need to validate all pathogens detected

- Controls
  - IQCP?
  - How often to run controls?
  - Single positive or multiplexed controls?
  - Prepare or purchase?

- How/what to charge for syndromic panels?

- Limit ordering of syndromic panels?
  - Influenza test Vs. whole syndromic panel
    - Limit testing by season?
  - Limit ME Panel to patients with abnormal protein, glucose and WBC counts?
Outstanding Issues

- Training
  - Training must be **extensive and continuous** if syndromic molecular panels are to be performed by non-Molecular trained technologists
  - Tests are more complicated than they appear!
  - Test setup **must include** proper precautions against contamination (including barrier and process precautions)
    - HSV-1 contamination example on Biofire
      - Two HSV-1 consecutive positive CSF samples
        - Positive HSV-1 not consistent with patient symptoms and other labs.
          - HSV-1 would have not been ordered if not in the panel
Outstanding Issues

• Think small!
  o Amplicon contamination
    ▪ Multiple glove changes
    ▪ One sample at a time
    ▪ Thoroughly clean after each sample with chemicals that denature nucleic acids
    ▪ No multi-tasking when performing amplified molecular assays.
  ▪ How do you know if you have amplicon contamination?
    ▪ Keep track of your positive samples
      ▪ Look for clusters
      ▪ Ask the physicians if your results makes sense
Summary - What Has Worked for Us

- Taking the time to assess the needs of our patients
  - Infectious Disease physicians
  - Pharmacists
  - ED physicians and others
    - Admit Vs. not to admit
      - ME panel
        - Children – Enterovirus positive – send home
        - Adults HSV-1 positive – Aggressive treatment
    - Respiratory Panel
      - Influenza Vs. others
        - Antivirals Vs. antibiotics
        - Immunosuppressed Vs. not immunosuppressed
Summary - What Has Worked for Us

• Blood Culture Panels
  o Antibiotic stewardship
    ▪ Right antibiotic, at the right time, for the right duration
    ▪ Antibiotic De-escalation
    ▪ Ensure that someone is listening, especially for stat syndromic panels
      ▪ Close the “loop”, labs do not function in a vacuum
  o Tests must be clinically significant and cost effective
• Most benefits will be “downstream”