



ACL – Molecular Microbiology

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ACL Laboratories



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

Changing Pathogen Identifications in Microbiology

TABLE 1 Viruses Detected by Conventional Virology (2004) vs. Molecular Methods (2013)

	% POSITIVE SPECIMENS*		Comments
	2004	2013 [†]	
<u>Viruses</u>			
★ Herpes simplex	22	21.4	2013 – HSV subtyped (HSV-1=11%; HSV-2 = 10.4%)
★ Cytomegalovirus	4	14	2004 –Diagnosis; 2013 -diagnosis and monitoring
★ Epstein Baar virus	N/A	11.3	
Enterovirus	10	2	2003 - throat, rectal, CSF; 2013- CSF only, yearly panel
★ Varicella zoster	<1	14.3	2004 – vesicles only; 2013 vesicles, CSF, plasma and urine
<u>Respiratory Viruses</u>			
Adenovirus	8	1.1	2013 Respiratory Viral Panel available throughout the year
Influenza A	9	8.4	2013 Respiratory Viral Panel available throughout the year
Influenza 2009 H1N1	N/A	3.4	2013 Respiratory Viral Panel available throughout the year
Influenza B	4	2.9	2013 Respiratory Viral Panel available throughout the year
Respiratory syncytial virus	31	7.4	2004 – seasonal; 2013 - RVP available throughout the year
Human Matapneumovirus	N/A	3.4	2013 Respiratory Viral Panel available throughout the year
Parainfluenza 1–3	10	2.9	2004 – seasonal; 2013 - RVP available throughout the year
Parainfluenza 4	N/A	1.2	2013 Respiratory Viral Panel available throughout the year
Coronavirus (4 serotypes)	N/A	2.0	2013 Respiratory Viral Panel available throughout the year
★ Enterovirus/Rhinovirus	N/A	17.5	2013 Respiratory samples only. Available through the year

Personal data, M. Costello and L. Mazur, Multiple hospitals, Chicago area and southeast Wisconsin.

▪ Traditional workflow Vs. Syndromic Panels

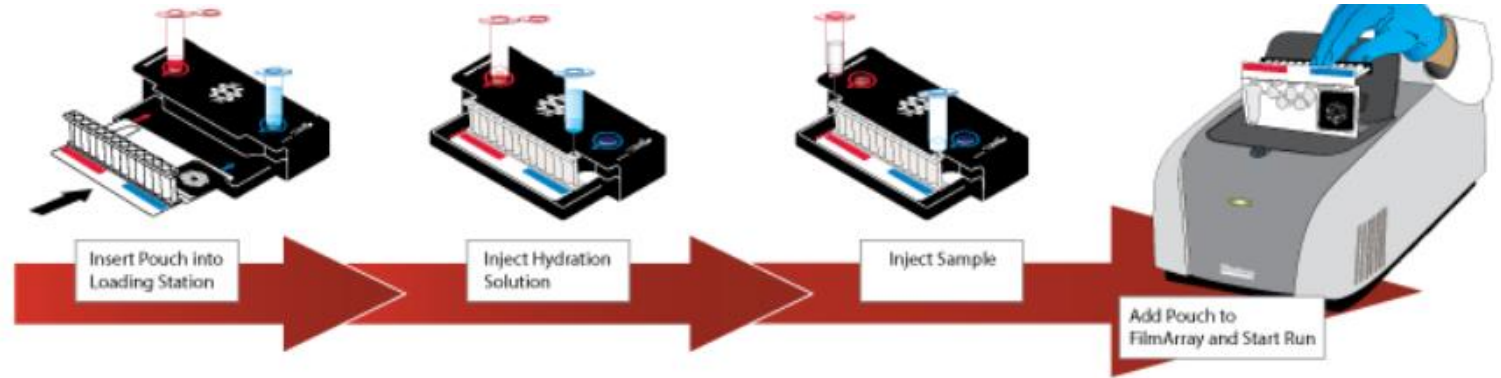
- Traditional workflow
 - Sequential traditional ordering of laboratory tests
 - Takes time
 - Less sensitive
 - Can be more expensive than molecular syndromic panels

Changing Workflow in Microbiology

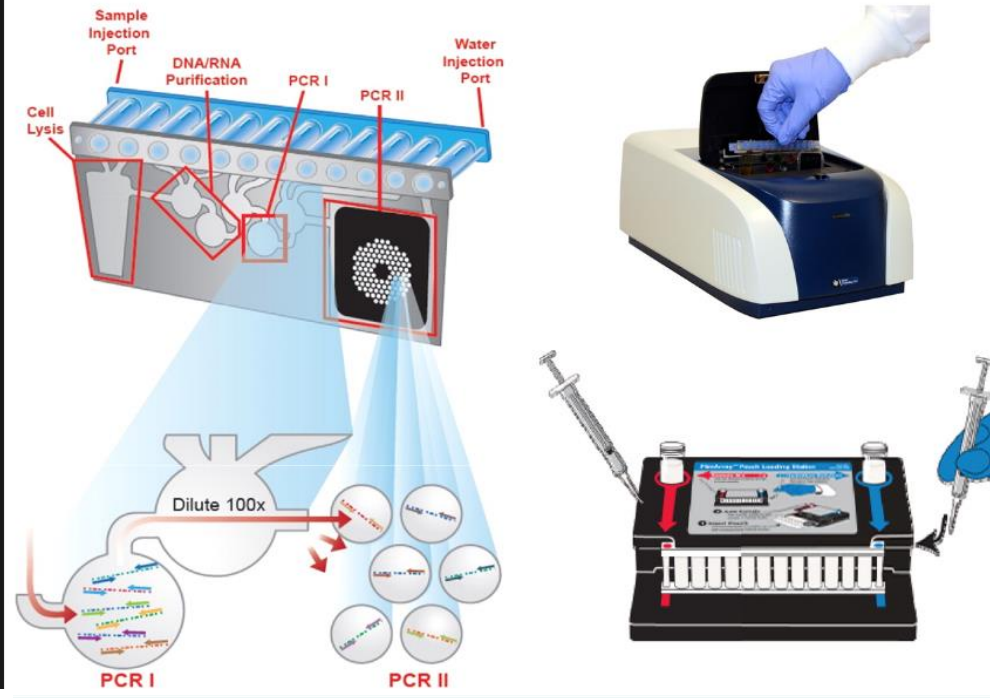
▪ Syndromic panels

- 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

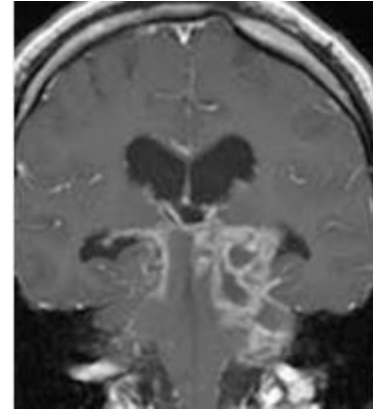
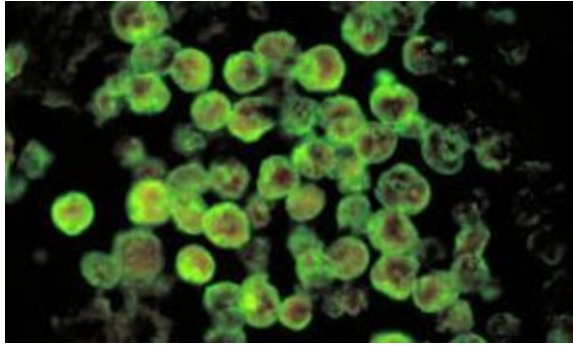


The FilmArray Pouch

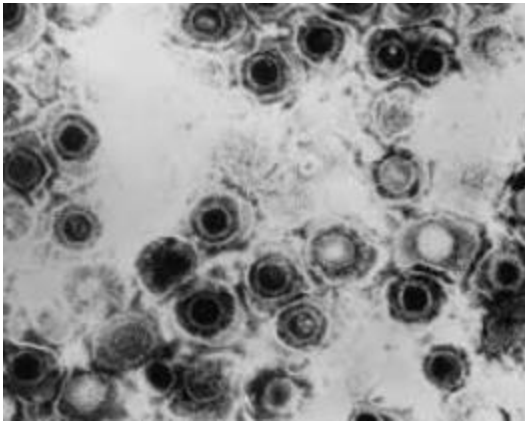


PCR II

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

FilmArray™ Meningitis/Encephalitis Panel

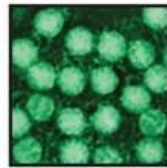
Currently in Development

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



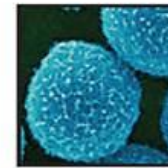
Bacteria

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



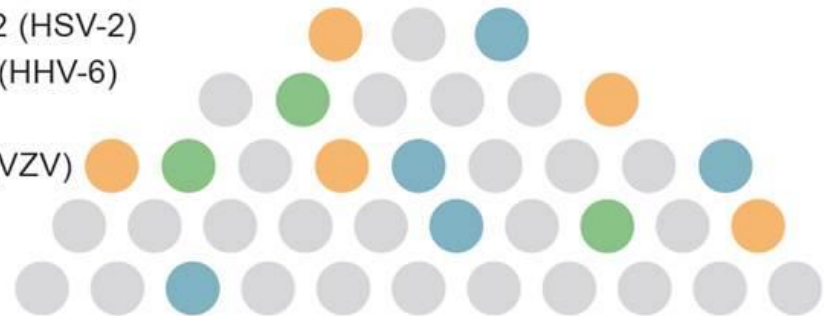
Viruses

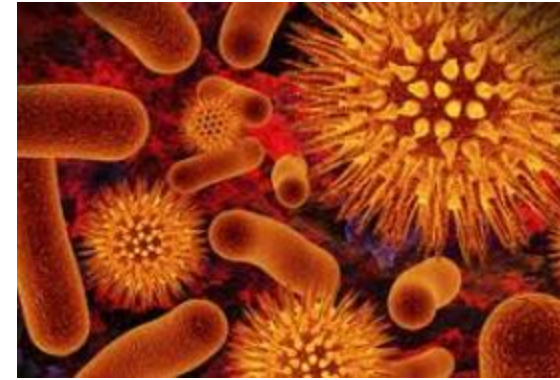
Cytomegalovirus (CMV)
Enterovirus
Epstein-Barr virus (EBV)
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Human herpesvirus 6 (HHV-6)
Human parechovirus
Varicella zoster virus (VZV)



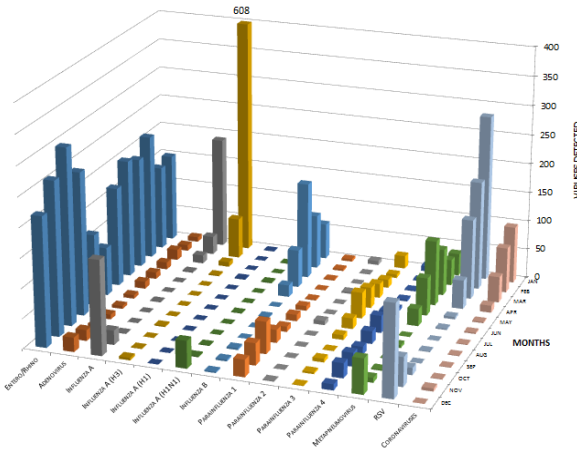
Yeast

Cryptococcus gattii
Cryptococcus neoformans





Respiratory Viral Panel



ACL RESP PANEL - Nov 01 2015 - Feb 20 2016															
InfA- UnSub	InfA-H1	InfA-H3	2009 H1N1	Inf B	RSV	All Para	EV / Rhino	Metapn eumo	Adeno	All Corona	Total Pos	POS PCR	Total	ACL %FLU	US %FLU
46		3	6	2	46	1	33	24	6	9	176	57	451	12.6	n/a
35	0	0	5	0	61	2	24	22	10	16	175	40	454	8.8	12.0
25	0	2	1	0	62	4	36	15	9	13	167	28	435	6.4	9.1
17	0	0	1	0	41	1	27	15	6	17	125	18	371	4.9	6.8
4	0	0	1	0	35	3	31	10	13	8	105	5	352	1.4	5.0
3	0	0	0	0	31	3	39	11	13	22	122	3	369	0.8	4.2
3	0	0	1	0	43	9	51	12	16	9	144	4	396	1.0	3.0
1	0	0	0	1	32	6	48	12	6	9	115	2	300	0.7	1.8
3	0	0	0	0	22	7	44	2	2	6	86	3	270	1.1	2.5
2	0	0	0	1	26	7	54	9	8	4	111	3	316	0.9	2.9
0	0	0	1	0	8	14	36	5	8	1	73	1	287	0.3	1.7
0	0	0	1	0	9	6	44	3	3	2	68	1	244	0.4	1.6
2	0	0	0	0	2	9	45	0	7	1	66	2	230	0.9	1.5
1	0	1	0	0	4	19	49	1	9	0	84	2	363	0.6	1.1
0	0	0	2	0	4	19	57	1	11	0	94	2	443	0.5	1.6
0	0	0	2	0	0	12	50	3	6	1	73	2	359	0.6	1.2

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

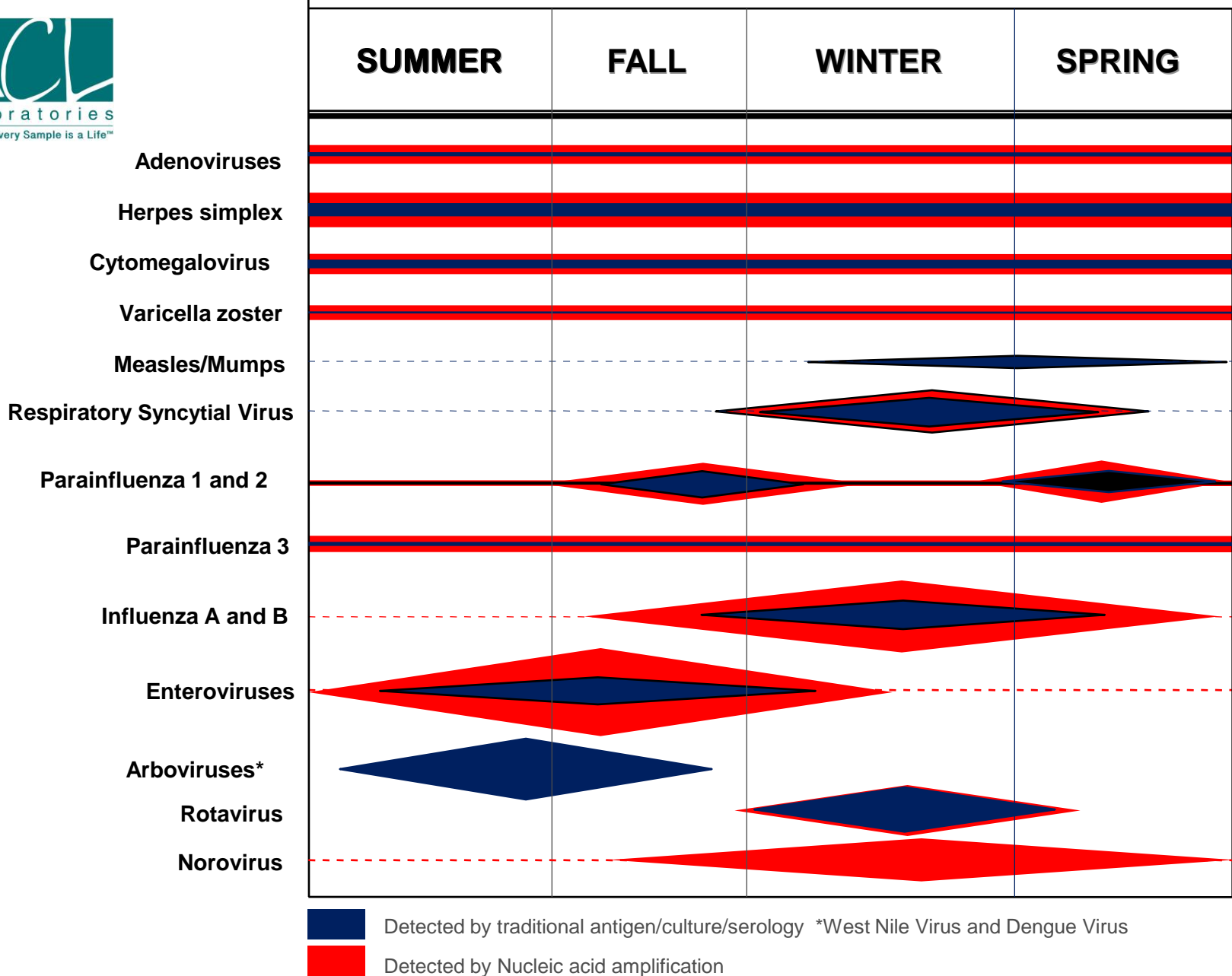
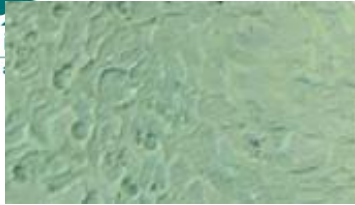
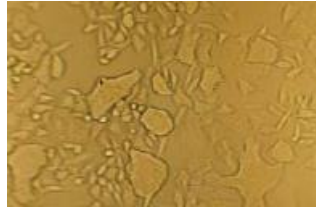


Figure 1. Seasonal variation of viral infections

Cytopathic Effect (CPE) in tube culture



Hep-2, uninfected

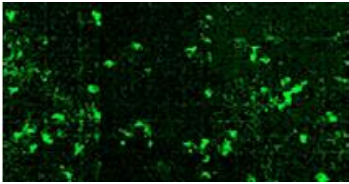


RSV/Hep-2

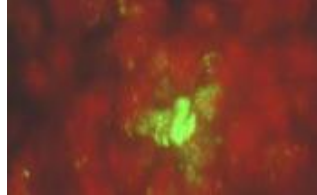


Adenovirus/Hep-2

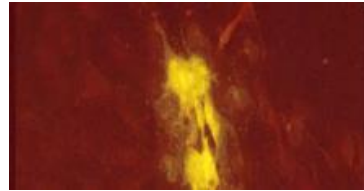
Shell vial monolayers stained with FITC-conjugated monoclonal antibodies



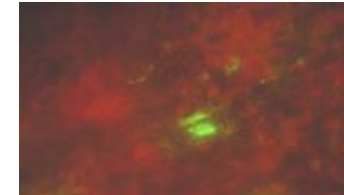
R-Mix shell vial
Influenza in mink lung



R-Mix shell vial
Parainfluenza in mink lung

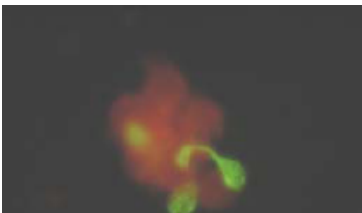


R-Mix shell vial
RSV in A549

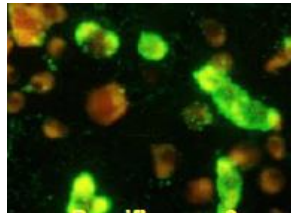


R-Mix shell vial
Adenovirus in A549

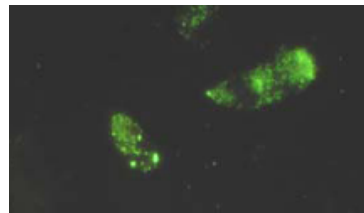
Patient respiratory cells stained with FITC-conjugated monoclonal antibodies



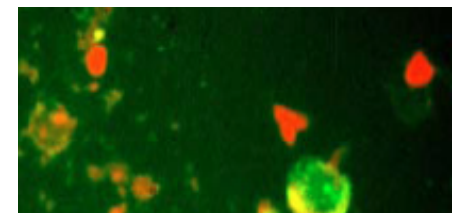
Influenza A
NP aspirate



Parainfluenza
NP aspirate



RSV
NP aspirate



Adenovirus
NP aspirate

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

Respiratory Panel

FDA-Cleared

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

[Download Product Sheet](#)

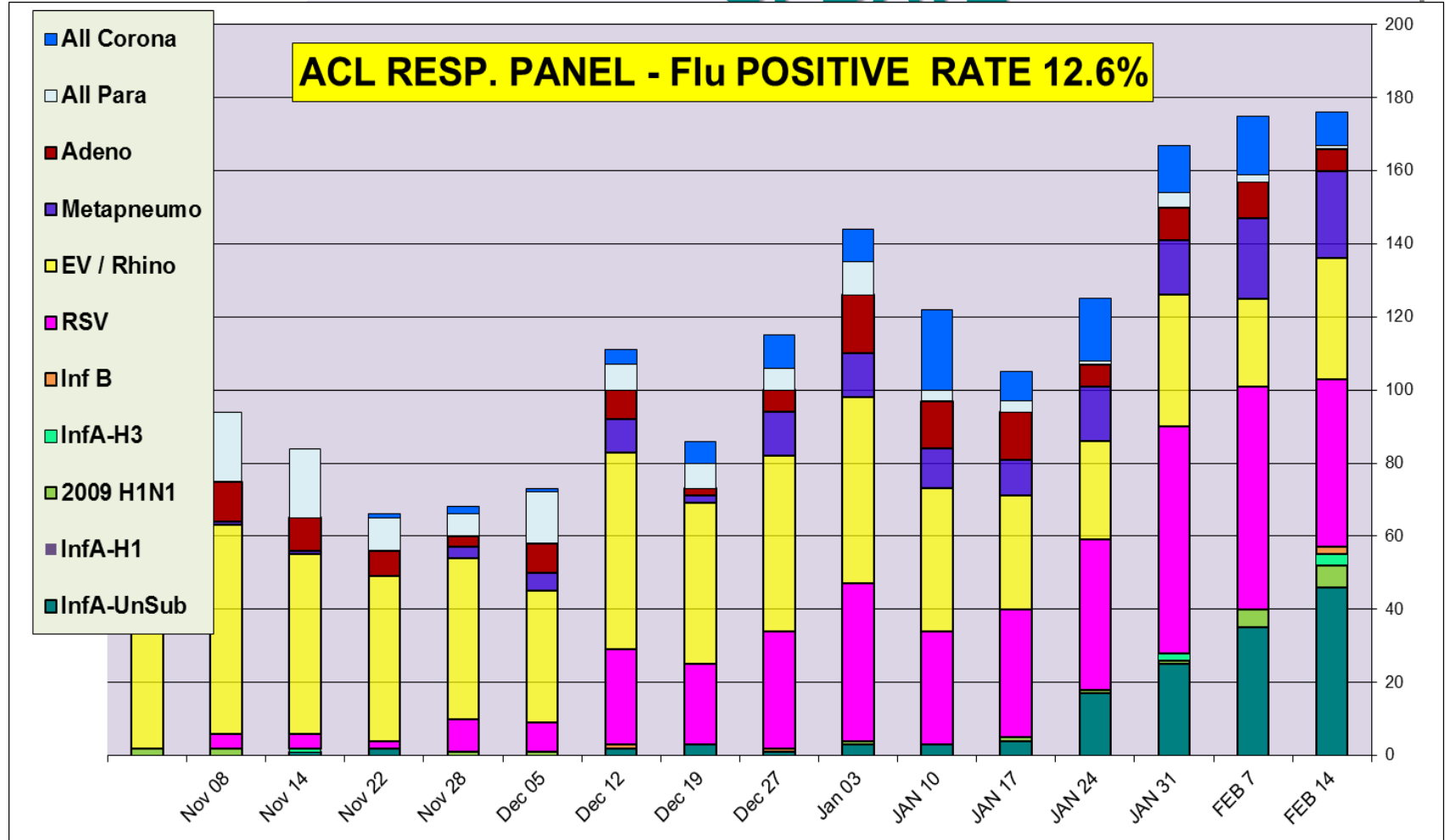
Viral Targets

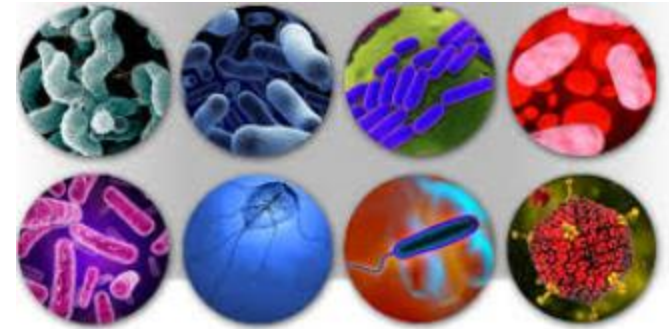
Adenovirus	Influenza A/H1
Coronavirus HKU1	Influenza A/H3
Coronavirus NL63	Influenza A/H1-2009
Coronavirus 229E	Influenza B
Coronavirus OC43	Parainfluenza Virus 1
Human Metapneumovirus	Parainfluenza Virus 2
Human Rhinovirus/Enterovirus	Parainfluenza Virus 3
Influenza A	Parainfluenza Virus 4
	Respiratory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

Bacterial Targets

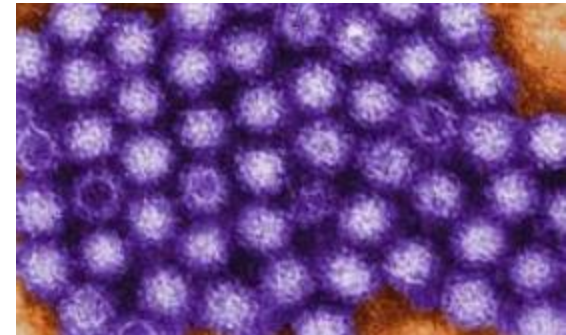
Bordetella pertussis
Chlamydomphila pneumoniae
Mycoplasma pneumoniae

MOLECULAR PATHOLOGY UPDATE





Gastrointestinal Panel



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. sonnei, S. flexneri and S. dysenteriae), Campylobacter spp (C. jejuni, C. coli and C. lari only), Shiga-like Toxin producing E. coli (STEC) stx 1/stx 2, 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 Carry-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

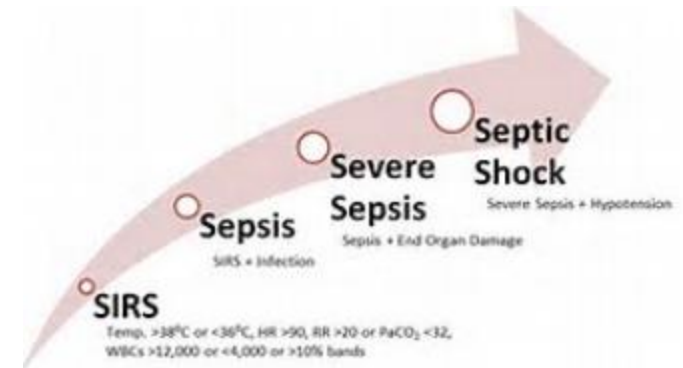
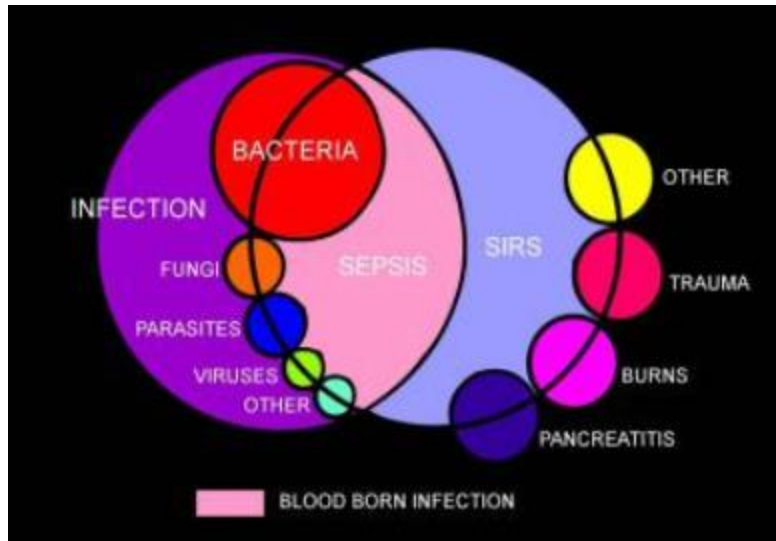
Performed: Weekdays

Reporting Time: Final within 3 Days

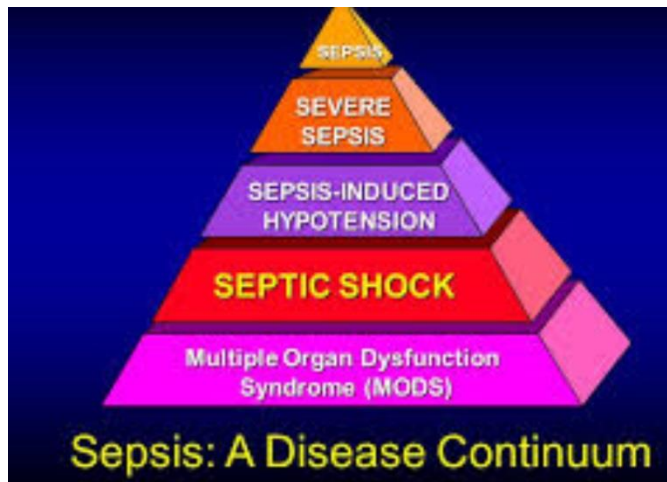
Performing Labs: ACL IL Central Laboratory - Rosemont, EMR/Interfaced Flagged "Client" Orderable test

CPT Codes

87507



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!**
 - 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
 - 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**
 - Tests must be clinically significant and cost effective
 - Most benefits will be “downstream”