Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Multiplex Syndromic Testing's Effect on Public Health

Molecular Testing & Emerging Technology- 2016

WCLN Workshop

April 28, 2016

Objectives

- Learn what current multi-target testing activities are ongoing in WI in clinical and public health laboratories
- Better understand the impact that multiplex PCR assays are having on public health
- Become aware of potential issues that may arise with the use of multiplex assays
- Share experiences (good and bad) with multi-target assay use in your laboratory and/or jurisdiction

Respiratory CIDT- Ahead of the Game

- Nature of respiratory disease diagnostics lent itself to early focus for development of CIDT and multi-target assays
- Difficult to culture/ detect
- If not Flu…then what?

WSLH Multi-Target Assay Implementation

- Respiratory virus panels
  - EraGen Multicode
  - Qiagen ResPlex
  - Genmark RVP
  - Luminex NxTAG RPP (Current)
- GI pathogen panel
  - Luminex xTAG GPP (Current)
- Multiplex PCR (small-scale)

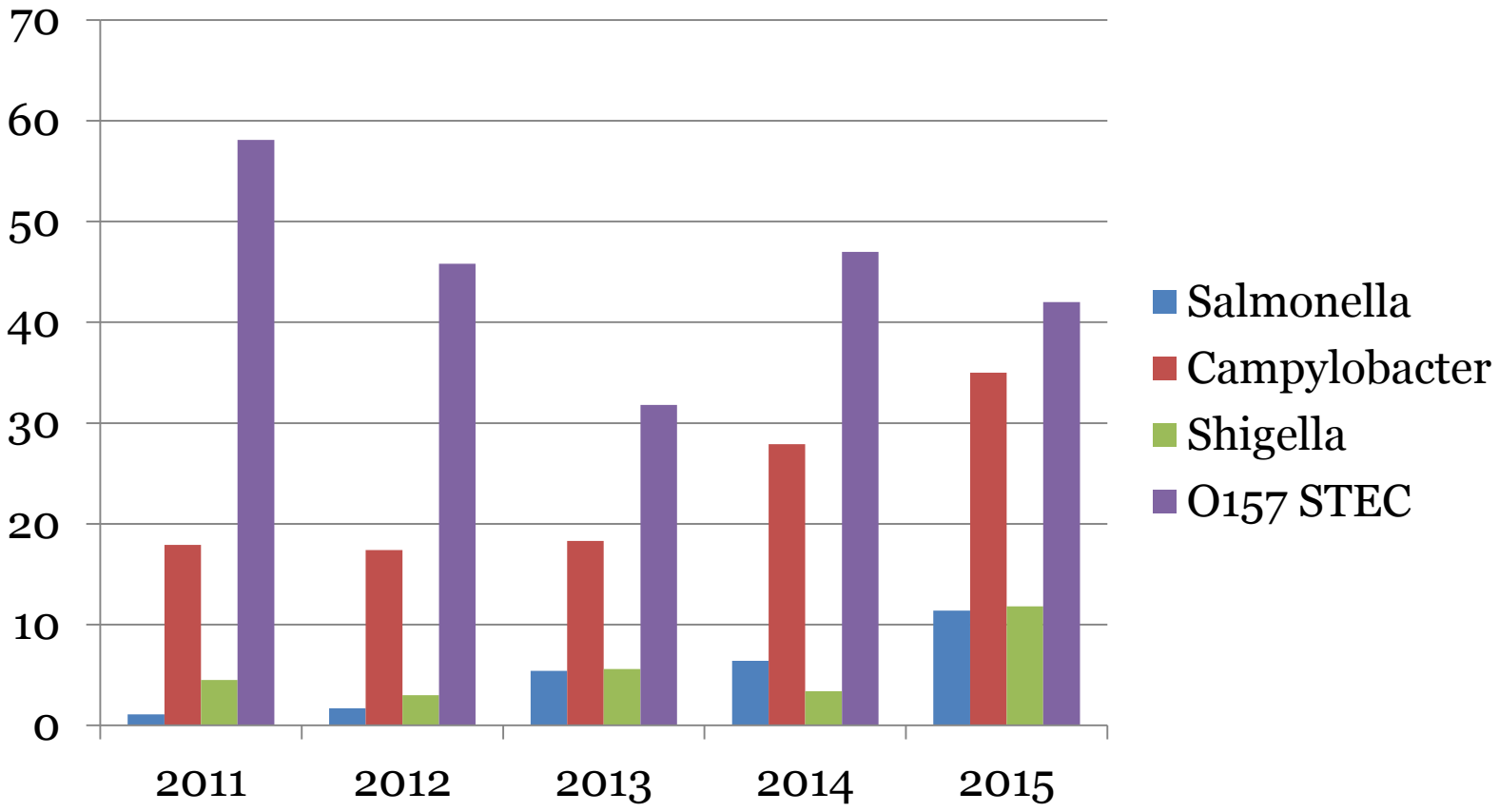
WSLH Multi-Target Assay Applications

- Luminex NxTAG RPP
  - Outbreak specimens
  - Flu negative cases (WDPH request)
  - Respiratory virus surveillance
- Luminex xTAG GPP
  - Outbreak specimens (unknown etiology)
  - Gastrointestinal pathogen surveillance

What Has Been the Effect of Multi-Target CIDT's on Public Health?

- Improved public health surveillance
  - Sensitive and specific pathogen detection
  - Better understanding of "opportunistic" or "less virulent" pathogens (mixed infections ???)
- Epidemiologic case definition changes
  - Historically, case definitions based upon isolation of pathogen(s)
- Increase in public health laboratory resources used
  - Culture and isolation shift to PHL's (AST???)

Percent of Isolates Recovered from Stool at WSLH



WI State Epidemiologist Guidance for Local Health

Scott Walker
Governor

Kitty Rhoades
Secretary

State of Wisconsin

Department of Health Services

DIVISION OF PUBLIC HEALTH

1 WEST WILSON STREET
P O BOX 2659
MADISON WI 53701-2659

608-266-1251
FAX: 608-267-2832

dhs.wisconsin.gov

Date: January 20, 2016

To: Local and Tribal Health Departments

From: Jeffrey P. Davis, M.D.
Chief Medical Officer and State Epidemiologist for Communicable Diseases

RE: Changes in Test Methods for Detection of Enteric Pathogens

This memo provides guidance to local, regional, and tribal health department staff regarding important changes in laboratory methods being used by several clinical laboratories to detect enteric pathogens in patient stool specimens. The traditional method of detecting enteric bacteria has been culture of a stool specimen to grow and identify the pathogens. Multiple clinical laboratories serving Wisconsin residents have transitioned to using a culture independent diagnostic test (CIDT) method for diagnosis of bacterial enteric pathogens. The expectation is that this trend will continue with more laboratories moving to CIDT methods in the future.

WDPH Guidance- Cont'd

Appendix A. Specific pathogens that can be detected in one or more of the commercially available CIDT enteric pathogen panels. (https://www.dhs.wisconsin.gov/disease/index.htm)

Pathogen	Type of pathogen	Reportable	Public health case classification for PCR (+) cases
Campylobacter	Bacteria	Yes	Probable
Cryptosporidium	Parasite	Yes	Confirmed
Cyclospora	Parasite	Yes	Confirmed
E. coli O157	Bacteria	Yes	Suspect
Enteropathogenic E. coli (EPEC)	Bacteria	Yes	Suspect
Enterotoxingenic E. coli (ETEC)	Bacteria	Yes	Suspect
Giardia	Parasite	Yes	Confirmed
Salmonella	Bacteria	Yes	Suspect
Shiga toxin 1 or Shiga toxin 2	Bacteria	Yes	Suspect
Shiga toxin-producing E. coli (STEC)	Bacteria	Yes	Suspect
Shigella	Bacteria	Yes	Suspect
Shigella/Enteroinvasive E. coli (EIEC)	Bacteria	Yes	Suspect
Vibrio	Bacteria	Yes	Suspect
Vibrio cholerae	Bacteria	Yes	Suspect
Vibrio Group	Bacteria	Yes	Suspect
Yersinia entercolitica	Bacteria	Yes	Suspect
Adenovirus	Virus	No	Not Reportable as individual cases
Astrovirus	Virus	No	Not Reportable as individual cases
Clostridium difficile	Bacteria	No	Not Reportable as individual cases
Entamoeba histolytica	Parasite	No	Not Reportable as individual cases
Enteroaggregative E. coli (EAEC)	Bacteria	No	Not Reportable as individual cases
Norovirus	Virus	No	Not Reportable as individual cases
Plesiomonas shigelloides	Bacteria	No	Not Reportable as individual cases
Rotavirus	Virus	No	Not Reportable as individual cases
Sapovirus	Virus	No	Not Reportable as individual cases

CIDT Interim Guidelines

Submission of Enteric Pathogens from Positive Culture-Independent Diagnostic Test Specimens to Public Health

Interim Guidelines

BACKGROUND

Laboratory tests that detect the molecular or antigenic signature of pathogens independent of generating an isolate are rapidly being adopted by clinical laboratories. These culture-independent diagnostic tests (CIDTs) represent a major shift in clinical microbiology practices and have important implications for physicians, patients and public health. CIDTs include PCR-amplified, antigen-based, and/or multi-analyte panel tests that are often ordered based on a clinical syndrome rather than a specific suspected pathogen. A GI Panel for enteric pathogens is often available from companies that develop and market CIDTs.

http://www.aphl.org/AboutAPHL/publications/Documents/FS-Enteric_Pathogens_Guidelines_0216.pdf

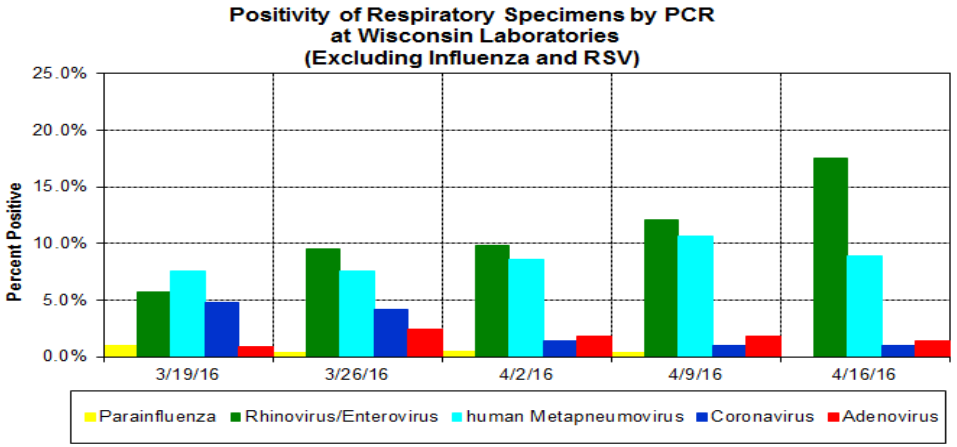
WSLH Guidance for Clinical Laboratories

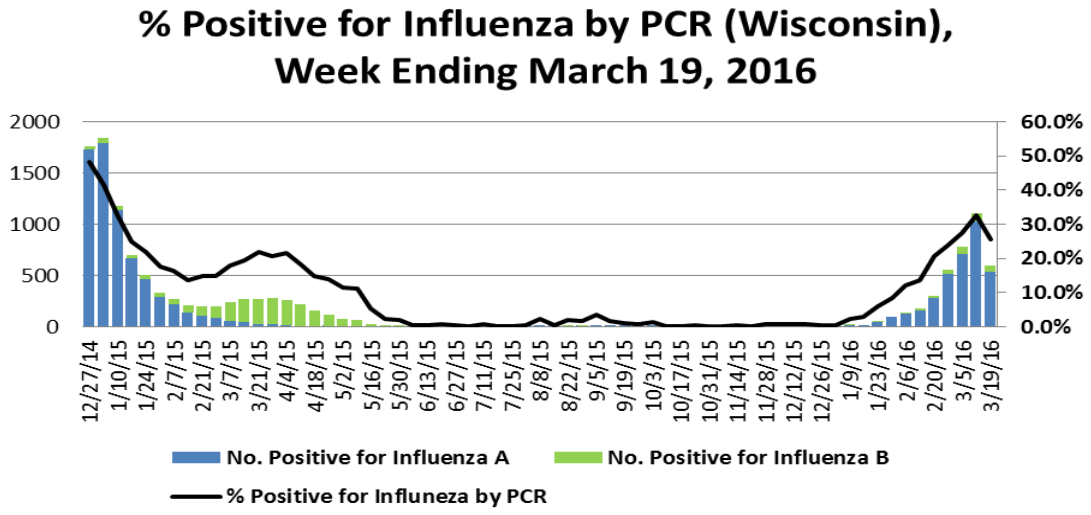
- When implementing a multiplex diagnostic assay:
  - In addition to conversations with physicians, reach out to local health and educate them about reporting changes
  - Notify WSLH to allow for planning and budgeting
  - Submit data to Erik Reisdorf or Mary Wedig
    - De-identified collated data to WSLH website
  - If discontinuing culture, promptly submit positive specimens to WSLH for further testing

What is WSLH Doing With Requested Specimens/ Data?

- Respiratory pathogen data collated and placed on WSLH website and in Newsletters
- Enteric pathogen data collated and reported in newsletters; Website page in progress
- Enteric pathogen-positive stool specimens
  - Viruses- genotyping, storage
  - Bacteria- isolation, subtyping, AST, storage
  - Parasites- Crypto genotyping, storage

WI Respiratory Disease Surveillance





Future of Public health Surveillance

- Test of Cure
  - Multi-target assays too much???
    - Too sensitive (below level of infective dose)?
    - Too expensive?
    - Too comprehensive (many more targets than needed)?
- Shift to point-of-care; Collating clinical lab data
  - What pathogens are healthcare systems seeing?
  - Has been ongoing in WI for respiratory pathogens
  - Now looking at GI pathogen data
  - Similar process for meningitis, antimicrobial resistance, invasive organisms (blood infections)?

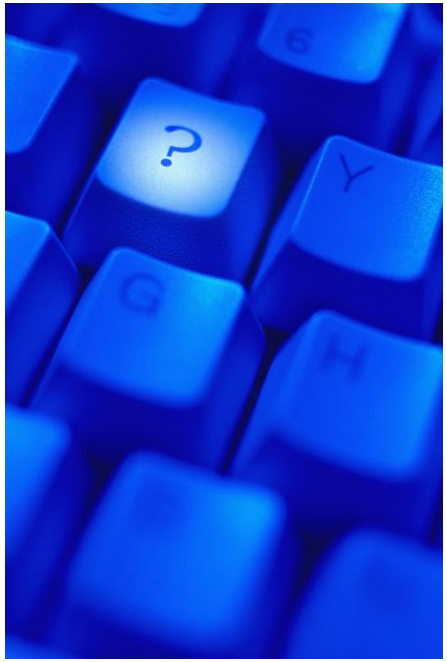
Future of Public health Surveillance- Cont'd

- Better understanding of multiple pathogen infections
- Better understanding of pathogenesis (EPEC, EIEC, ETEC, GI viruses other than Norovirus
- Discovery of novel pathogens
- "Culture-less" diagnostic world awaits…
  - 2016-17 Epidemiology and Laboratory Capacity (ELC) grant- Funding for Whole Genome Sequencing of all major enteric bacterial pathogens- Subtyping, serotyping, resistance

Multiplex PCR…what is there not to like?

Q1. Does your lab perform multiplex PCR testing?

A. Yes - 75%
B. No – 25%



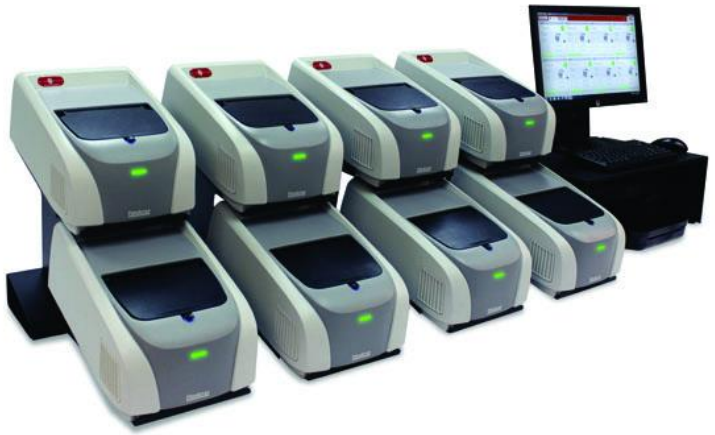
Q2. If no, why?

A. Cost of the instrument – 11%
B. Cost per test – 56%
C. Difficult to verify/validate - 6%
D. Lack of lab space – 11%
E. I don't know, but would like to! - 17%

Trends in Molecular Microbiology

- Syndromic Panels
- More is better ?
- Decrease complexity
- CLIA-waived
- Point of Care Tests
- Sample to Answer
- On demand, scalable
- IVD
- Decreasing Footprint

Footprints



Footprints

Syndromic Panels

- Respiratory Pathogen (21 targets)
- Gastrointestinal Pathogen (22 targets)
- Meningitis/CNS (14 targets)
- Blood Culture (27 targets)
- Lower Resp. Tract
- STI
- Sepsis
- CNS--Arbovirus

Q3. What Panel are you considering?

A. Respiratory – 9%
B. Gastropathogen – 26%
C. CNS – 13%
D. Blood/Sepsis – 6%
E. All of the above – 46%

The Players….a Short List

- BioFire (FilmArray)
- Luminex (xTAG, NxTAG)
- GenMark (eSensor)
- Nanosphere
- BD (Max)
- Hologic GenProbe
- Cepheid (Xpert)
- Seegene (MagicPlex, AllPlex)
- Focus Diagnostics (Simplexa)

Advantages….

- Improved sensitivity
- High throughput
- Increased automation → accuracy
- Decreased labor burden
- “Shotgun” approach
- TAT

Aim to improve patient management

Impact on Patient Management

- Early initiation of antiviral therapies
- Antibiotic stewardship
- Decreased length of stays
- Provide definitive diagnosis

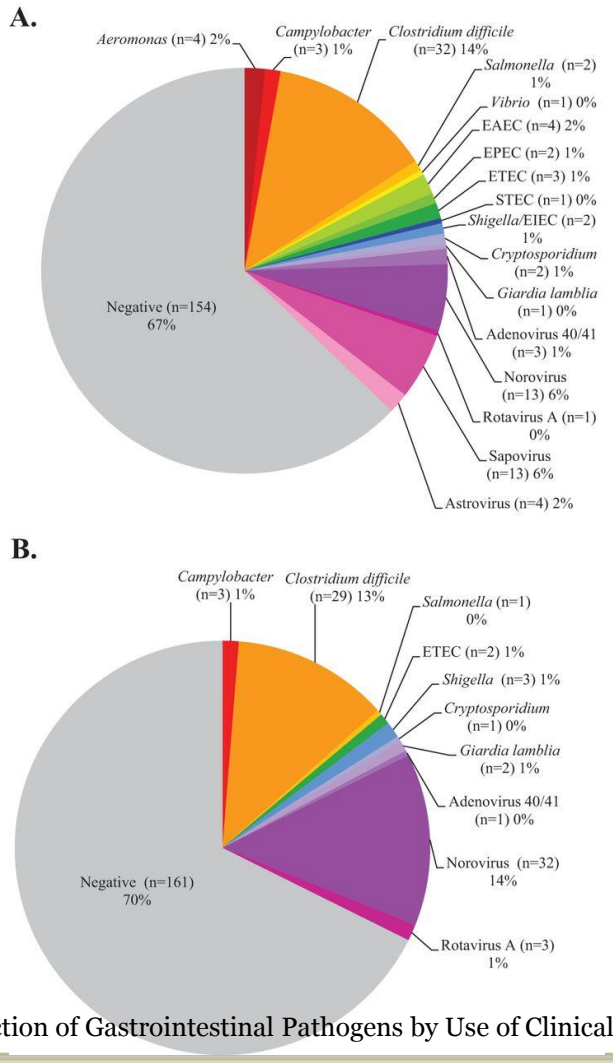
RVP Testing

Woo et. al., documented a **decrease** in length of stay (0.9 days), **decrease** days of antibiotic usage (2.6 days), and **decrease** in number of ancillary tests ordered (1.7 per patient investigation).

Kanack K. (2014) Rapid Respiratory Panel Testing Influences Patient Management and Clinical Outcomes. MLO. Dec. 2014.

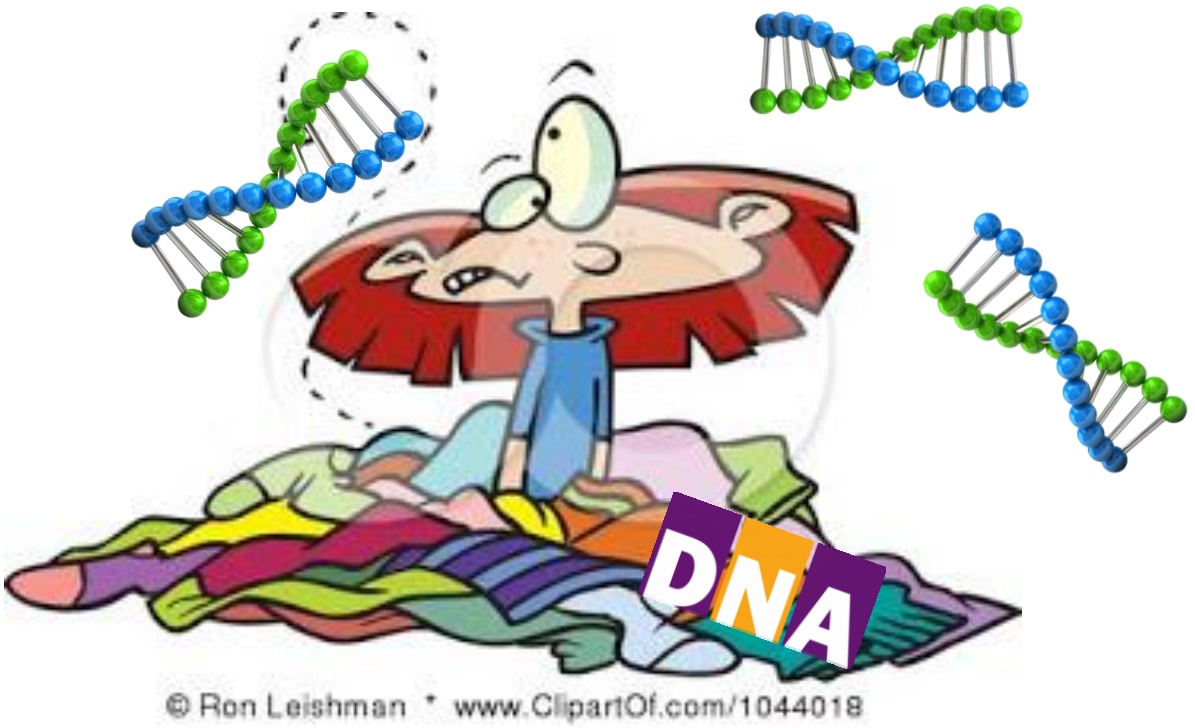
Gastropathogen Panels

- Detect >90% of commonly detected pathogens.
- No special media required
- Results in the same day
- No special tests
- Candy for the clueless clinician!



Comparative Evaluation of Two Commercial Multiplex Panels for Detection of Gastrointestinal Pathogens by Use of Clinical Stool Specimens
Khare R et. al., (2014) JCM

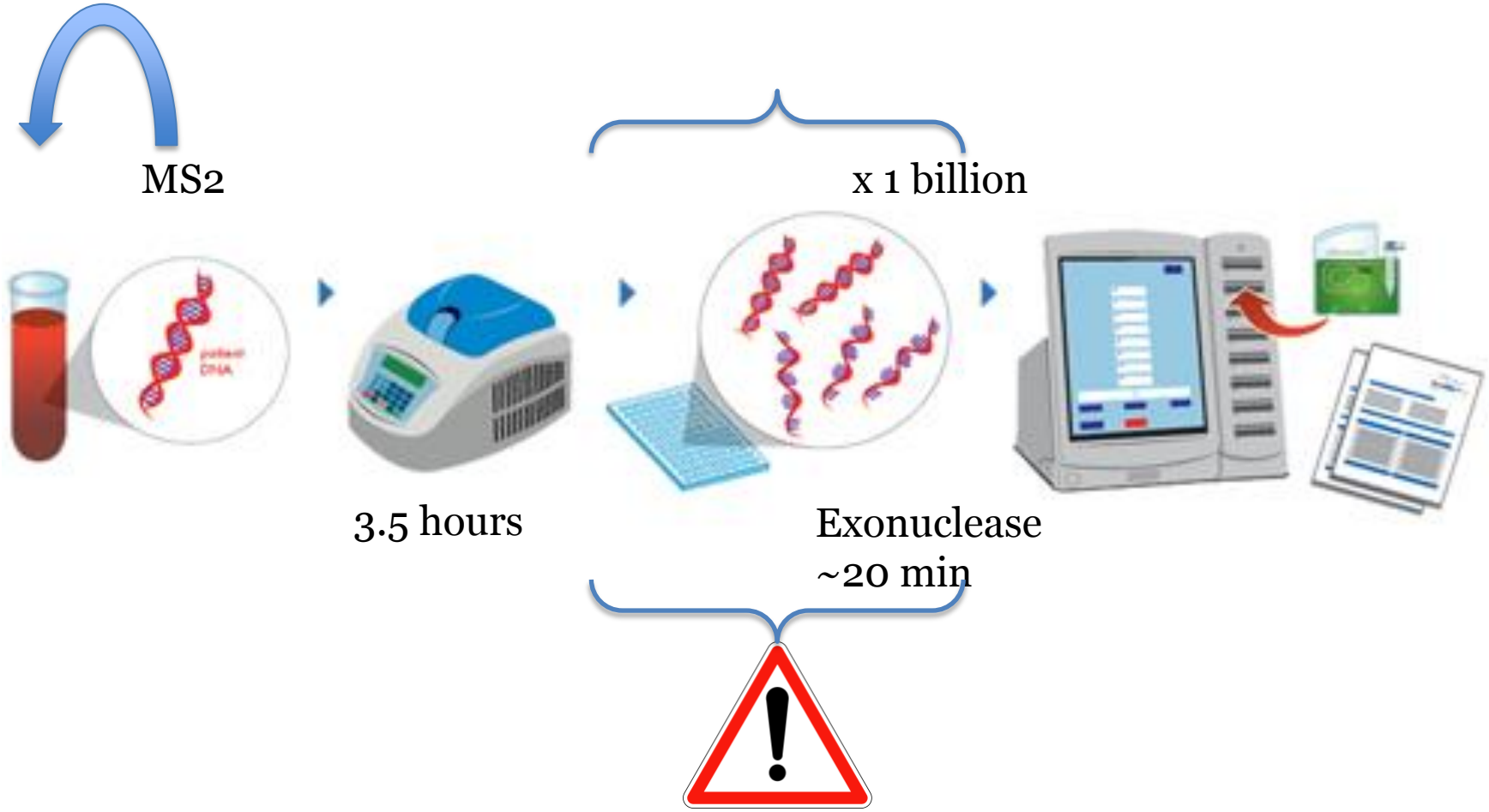
Now for the Challenges
"The good, the bad and the ***ugly***…"



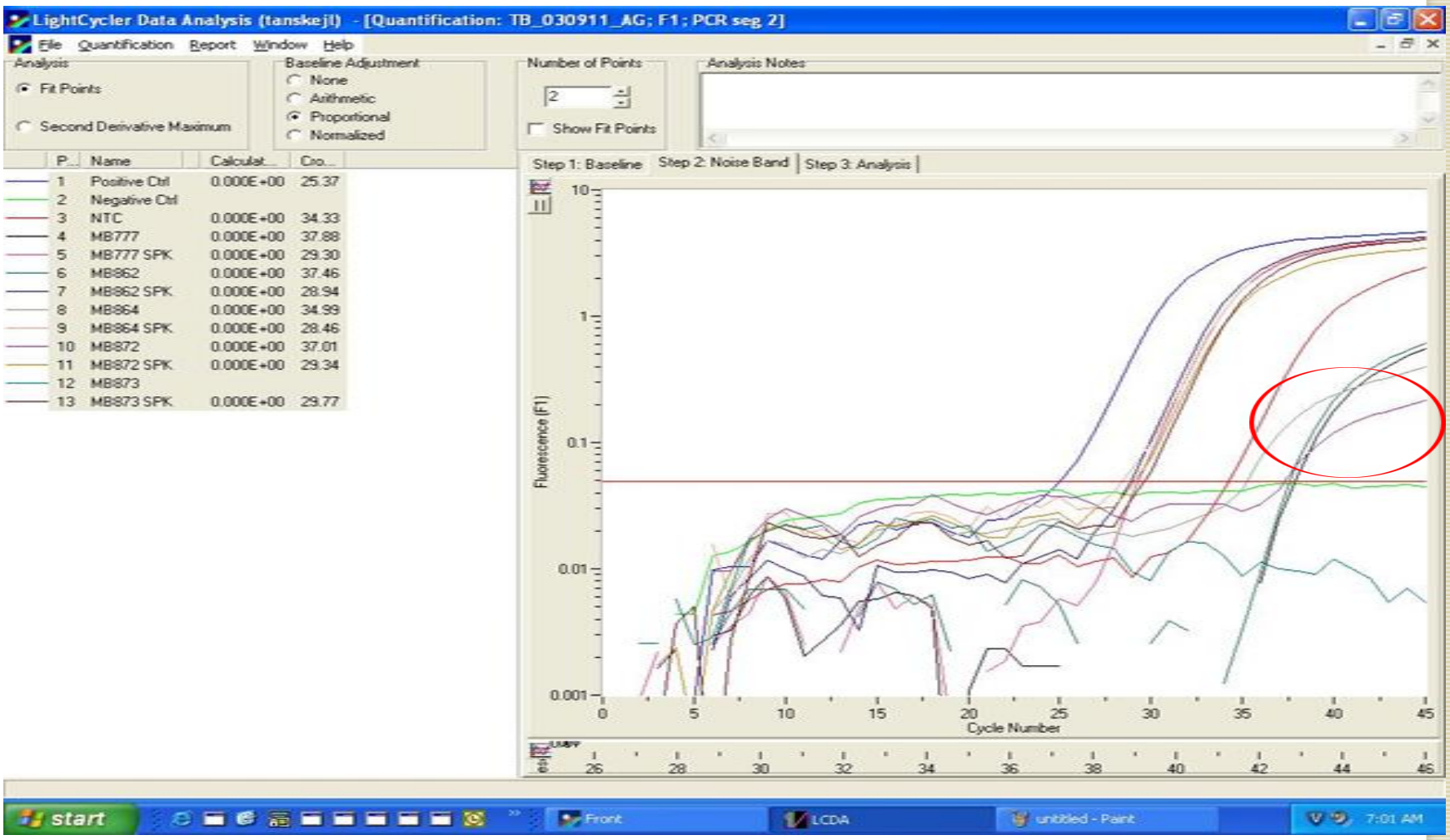
Q4. Have you had to troubleshoot a possible PCR contamination event?

A. Yes – 65%
B. No – 35%

“Open” System Workflow



What does contamination look like?

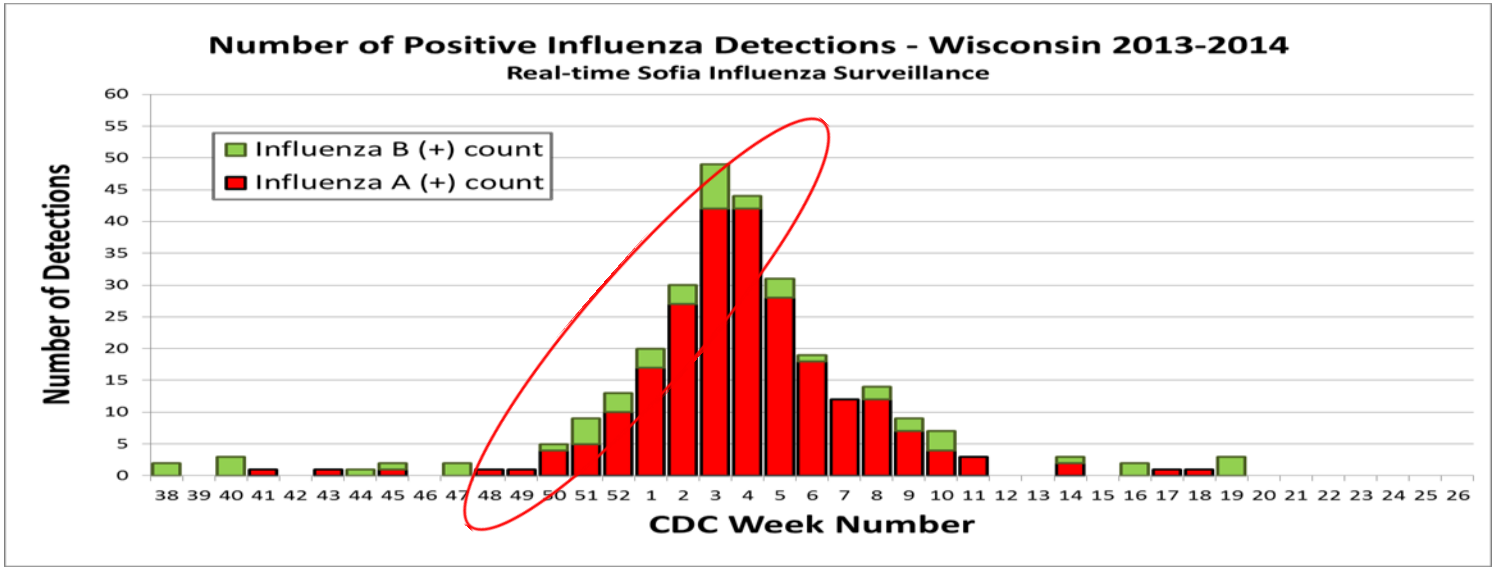


What does contamination look like?

Accessic	RSVA	PIV2	PIV1	INFB	PIV3	PIV4	NL63	RHV	CVEV	INFA	ADVB	HMPV
NTC	15	12	27	1	10	33	11	13	17	29	65	11
CON D	3215	20	10	17	21	13	20	11	13	13	7	13
VI1056	5199	30	29	22	23	6	24	25	21	19	73	10
VI1057	7	13	17	20	28	12	11	23	14	29	49	13
VI1307	12	16	12	25	17	13	25	16	16	32	334	10
VI1318	26	17	25	1	5	11	6	25	31	13	119	3
VI1332	2	18	3	13	24	16	0	33	295	28	127	11
VI1337	0	14	23	11	21	19	27	19	22	16	143	1
VI1349	11	4	7	12	15	14	16	23	15	15	166	1
VI1350	10	14	12	27	17	19	19	16	25	21	123	9
VI1356	24	15	11	6	15	26	17	26	14	19	153	11
VI1360	13	14	22	14	24	12	11	23	12	24	129	10
VI1363	13	5	21	9	13	16	4	4	12	10	53	17
VI1390	6	10	3	14	8	18	8	21	10	18	74	20
VI1402	12	15	12	21	10	25	12	1	16	208	68	22
VI1403	9	11	0	5	17	1	26	28	23	2270	51	10
VI1404	11	0	3	13	13	5	17	13	15	23	33	13
VI1405	8	0	13	7	13	0	16	21	9	10	4360	9
VI1413	12	22	8	1	22	20	5	12	10	14	1282	13
VI1426	14	7	18	15	16	15	11	32	21	23	213	15
VI1429	2	0	10	15	13	12	9	13	12	14	127	13
VI1431	10	17	3	9	4038	37	5	23	23	15	93	6
VI1434	8	11	4	16	13	11	338	14	11	16	137	15
VI1436	14	14	23	19	9	19	0	4	20	7	90	4
VI1437	10	6	0	0	23	11	0	14	14	17	84	801
VI1439	13	18	12	12	21	7	16	19	52	14	187	2
VI1441	3	5	19	3	21	2	1	8	81	10	143	10
VI1443	21	18	15	10	28	12	15	15	14	11	31	7
VI1452	0	10	16	19	6	23	3	16	18	10	29	7
VI1457	1	27	11	11	23	13	19	17	18	19	224	3
VI1480	17	17	4	9	6	8	16	26	19	11	70	17
VI2481	6	9	17	10	23	13	16	25	23	1	125	14
VI1484	11	17	14	5	22	18	26	22	15	13	62	4
VI1509	22	10	15	23	24	10	11	20	6	6	78	18
VI1510	1	12	8	16	21	0	18	11	9	5	147	20
VI1514	3	0	0	12	17	12	7	12	14	12	233	6
VI1516	12	9	16	7	15	12	18	11	7	9	145	7

Cut-off >85

False Positives



Contamination: Common Causes

- Breakage/defects of cartridges
- Transfer on gloves or hands
- Transfer of paper, pens, racks, lab coats, etc., between PCR areas
- Inadequate sealing
- Aerosol generation
- Waste handling
- Re-entry of clean areas

Manufacturing Problems

Reason for Recall

Focus Diagnostics is recalling Simplexa™ Herpes Simplex Virus 1 & 2 Direct and Simplexa™ Group A Strep Direct kits containing the Direct Amplification Discs due to poor lamination between the sample reaction wells. This poor lamination may lead to leakage into adjacent wells causing cross-contamination between samples, which could yield false positive, false negative, or invalid test results. Inaccurate diagnostic test results may lead to improper patient treatment for HSV or GAS and may cause serious adverse health consequences, including death.

Monitoring Activities

- Monitor co-infection rate
- Multiple low level positives
- Results that “don’t make sense”
- Monitoring surveillance reports
- Environmental ‘swipe’ tests
- Re-testing multiples
- Multiple NTC’s

WSLH as a Resource

- Provide confirmatory testing
- Provide verification panel
- WCLN List Serv
- Surveillance Reports
- Website graphs
- Technical assistance, troubleshooting
- Reports from other labs

Contact Information

- Erik Reisdorf
  - erik.reisdorf@slh.wisc.edu
- Tim Monson
  - timothy.monson@slh.wisc.edu

Questions, Comments and Robust Discussion