# Are Pan-Diagnostic Molecular Tests a Panacea?

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# Disclosures

* Support from Hologic

# Objectives

* Learn about challenges in using multiplex versus pan-diagnostic molecular respiratory tests from a public health perspective
* Outline some lessons learned on how to manage workflow during the influenza season

# Background

* Laboratory processes described in this presentation are those used at the Saskatchewan Disease Control Laboratory (SDCL)
* Studies using the Hologic Panther Fusion real-time PCR assays were performed at the Saskatchewan Disease Control Laboratory

# Evolution of Respiratory Molecular Testing at SDCL

* Pre-pandemic testing
* 2009 influenza pandemic
* Post-pandemic multiplex testing of outbreaks only
* Pan-diagnostic testing using LDT platform
* Evaluation of commercial random access PCR assays

# Pre-Pandemic Testing

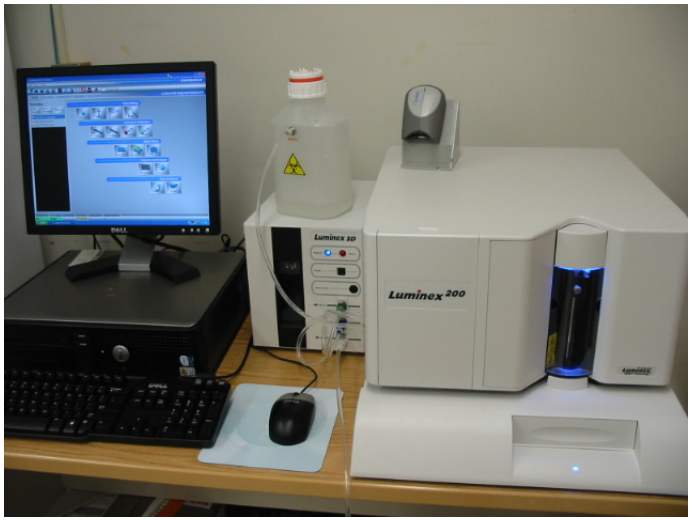
* Traditional virology: DFA, culture
* Singleton PCR assays for influenza A and typing on outbreak specimens
* Run on 96 well thermal cyclers
* Manual processing
* Pandemic preparedness: validated pre-amplification processing on commercial open platform (Abbott *m*2000)

# 2009 Influenza Pandemic

* Rapid scaling-up to include simultaneous testing of 5 flu A targets, using CDC primers
* Addition of two temporary staff positions and two thermal cyclers
* Testing volume rapidly surpassed capacity of the automated platform
  * Continued with manual specimen processing
  * Repetitive strain injuries
* Diagnostic culture in virology essentially ceased during pandemic due to PHAC biosafety advisory
  * Delay in detection of other viruses
  * Possible effect on patient care?

# Post-Pandemic Multiplex Testing

* Validation of Luminex xTag multiplex testing
* Significantly enhanced respiratory outbreak testing
* High cost per sample restricted use to samples from institutional outbreaks
* Positive feedback from MHOs
* Multiple testing pathways for specimens from different patients

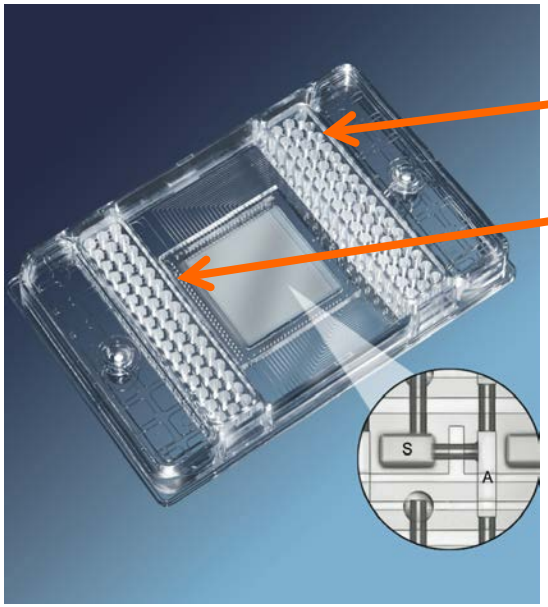


# What Led to the Development of an In-House Pan-Diagnostic Testing Platform?

* Concerns about:
  * Treating some specimens differently ("better" or "worse") than others
  * Multiple touches of same specimen
* Seeking efficiency via government-wide LEAN approach
* Aim to treat every specimen the same way
* Evaluated commercial multiplex PCR assays
  * High cost, did not cover all target viruses

# Development of an In-House Pan-Diagnostic Testing Platform

* Selected high-throughput PCR using Taqman chemistry on a microfluidic 48.48 chip using a BioMark HD system (Fluidigm)




48 wells for primers and probes

48 wells for template

Fluidigm Real-Time PCR Analysis User Guide www.fluidigm.com

# Microfluidic PCR Process

* Samples aliquoted into lysis buffer
* Total nucleic acid extraction using a Kingfisher extractor
* Each extract subjected to combined RT and pre-amplification step in a “pre-amp soup”
* After pre-amplification, pipetted into digital integrated fluidic circuit (the chip) along with reagents
* Loaded onto an IFC controller with prepares the chip for thermal cycling
* Chip transferred into the Biomark HD thermal cycler
* Post-amplification analysis and reporting

# Advantages

* Treat every specimen the same
  * 48 individual assays on every sample
  * Multiple targets per pathogen
  * Influenza typing on every specimen
  * *B. pertussis* included
  * Novel pathogens can be run on every specimen (eg: MERS)
* Extremely low cost per specimen
  * 48 assays for the cost of a single assay in a 96 well format

Antonishyn *et al.*, 2014 (CACMID abstract G02)

# Disadvantages (1)

* High capital cost ~ $250,000 in 2014
* No redundancy
  * single instrument leaves process vulnerable to equipment failure
* Extensive validation required, for each assay and for all assays combined
* Huge quality control undertaking
* Difficult to change the panel quickly
* Extensive manual pipetting: repetitive strain injuries
* Pipetting of pre-amplified template into chip
  * Potential for contamination
* Manual data transfer and analysis post-amplification

# Disadvantages (2)

* Reaction volume ~ 9 nL
  * Potential for stochastic effects
  * Performance in PT samples designed to challenge limit of detection
* Process requires 2 FTEs
* Six hour process from sample processing to results
* Difficult to meet same day turn around time
* Has led to a one day delay in testing most specimens, multiple days during peak flu season
* Outbreak specimens are tested by DFA or by rapid antigen tests, depending on time of receipt

# BCCDC Respiratory Testing

* Samples aliquoted into lysis buffer
* Nucleic acids extracted using MagMax extractor
* Influenza A/B + RSV +RNaseP  4-plex PCR
* Influenza typing PCR
* If negative for flu/RSV, outbreaks/in-patients/children/ immunocompromised tested using Luminex NxTAG respiratory panel
* Enterovirus D68 PCR upon request
* Algorithm adjusted seasonally
* Interfacing to LIS!

# Is There a Better Approach?

* Can this manual process be automated?
* Off the shelf automation cannot perform all the steps required
* Custom liquid handler was designed to de-cap specimen and process up to the extraction step
* Estimated cost US $400,000
* Perhaps unsurprisingly, this was not funded

# Is There a Better Approach?

* If lab-developed high throughput testing creates its own workflow problems, are there commercial platforms that will provide an alternative?
* SDCL has been using Hologic (formerly GenProbe) instruments for STI testing for about 10 years
* In fall 2017, a new Panther was installed for a viral load assay pre-qualification study
* Presented an opportunity to evaluate Panther Fusion respiratory virus assays

# Panther Fusion PCR Assays

* Random access, real-time PCR platform
* Manual transfer from UTM tube to lysis buffer
* Capacity to amplify 60 assays in sealed tubes, in 12 independent rows
* Three respiratory panels
  * Flu A/B/RSV
  * Parainfluenza 1/2/3/4
  * Adeno/hMPV/Rhinovirus
* All three panels can be run on a single sample extraction
* Time to first result ~2.5 hr, followed by five results every 5 min

# Performance of Panther Fusion versus Microfluidic LDT

* Tested 939 specimens during 2017-18 flu season
* Positive agreement between tests:
  * 99-100% for flu A, flu B and para-flu
  * 96% for RSV
  * 57% for adenovirus (11 specimens)
  * 100% for hMPV (Fusion assay detected 35% more positives)
* Difficult to compare rhinovirus (Fusion) versus entero/rhino (LDT)
* No coronavirus assay in Fusion panels

# Workflow of Panther Fusion versus Microfluidic LDT

* Ran three days of real time testing in parallel with LDT, as specimens arrived in the lab
* One day as an example:
* 116 specimens received, 147 minutes hands-on time
- 🕓 8:00 am cleaning benches and loading instrument
- 🕓 9:15 am first specimens arrived at lab
- 🕓 9:35 am first rack of specimens loaded
- 🕓 3:30 pm all results on 73 samples (Fusion) vs 37 samples (LDT)
- 🕓 4:00 pm all Fusion results on 79 samples
- 🕓 8:00 am next day, all Fusion results complete on 116 samples
- 🕓 3:30 pm all results complete on LDT
* Note: This comparison was done at the tail-end of the influenza season, when specimen numbers were decreasing

# Microfluidic LDT versus Panther Fusion Respiratory Assays

* Panther Fusion generated results faster than microfluidic LDT
* Much lower hands-on time
* Smaller range of viral targets on Panther Fusion
* Lack of influenza typing
* Flexibility for testing influenza versus whole panel
* Reflex testing without additional extractions
* Potential for combination of commercial products and LDT on the same Panther Fusion instrument

# Conclusions

* Pan-diagnostic testing using microfluidic PCR seemed like a good idea 5 years ago
* With hindsight, manual process involving extensive pipetting exacerbated RSI issues
* Increased workload created delays in testing during peak influenza seasons
* Commercial random-access panels offer gretaer flexibility compared with batch-based fixed panels
* Consider cost per reportable, including hidden costs
* Centralized testing may not serve the needs of largely rural populations
  * Distributed testing for influenza and RSV in local labs?
* Need to think far ahead when planning a testing strategy

# Acknowledgements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