BC Centre for Disease Control
AN AGENCY OF THE PROVINCIAL HEALTH SERVICES AUTHORITY

Molecular Methods in Diagnostic Microbiology: Making the Great Discovery Greater?

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Presentation Objectives

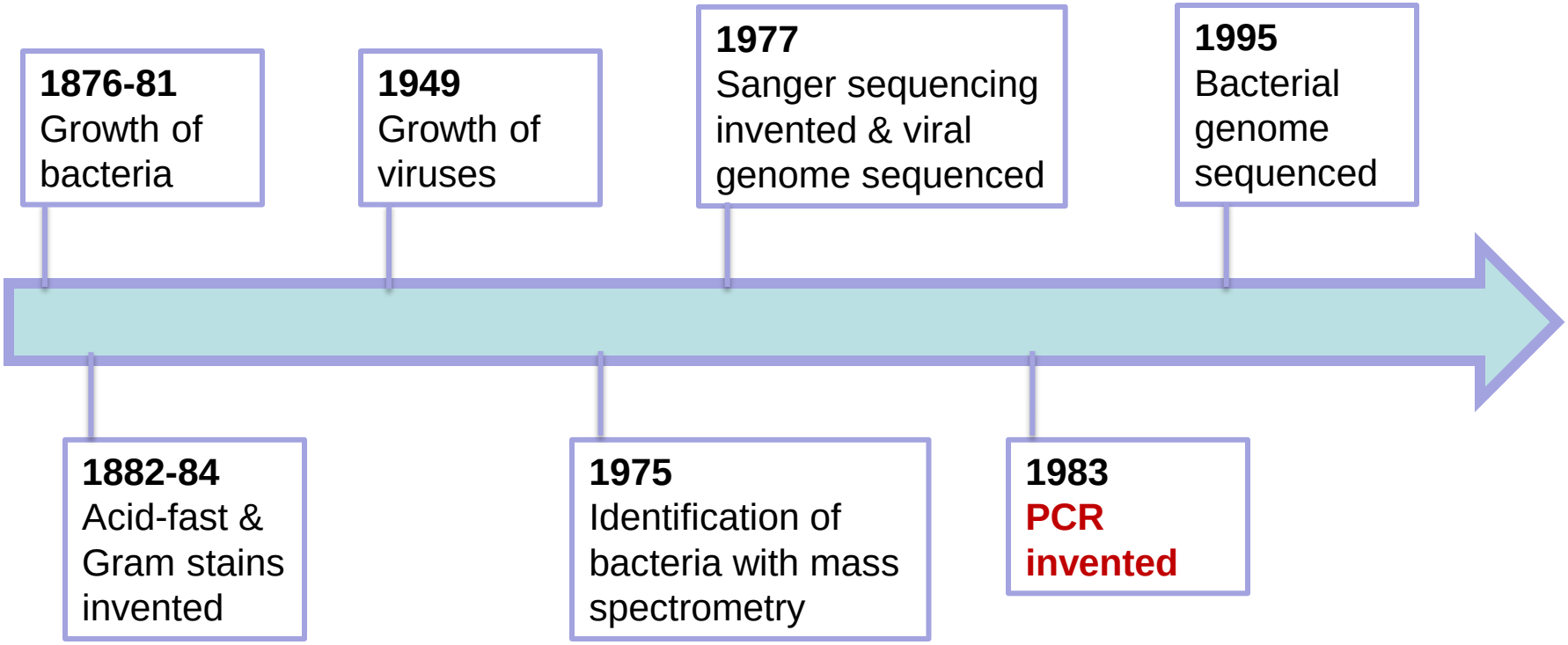
Describe the role of molecular methods (nucleic acid tests) in diagnostic microbiology

- **The past**: initial impacts
- **The present**: strategies to reduce cost and workflow pressures
- **Current limitations**: laboratory & technological
- **The future**: advances & impacts

Diagnostic Microbiology

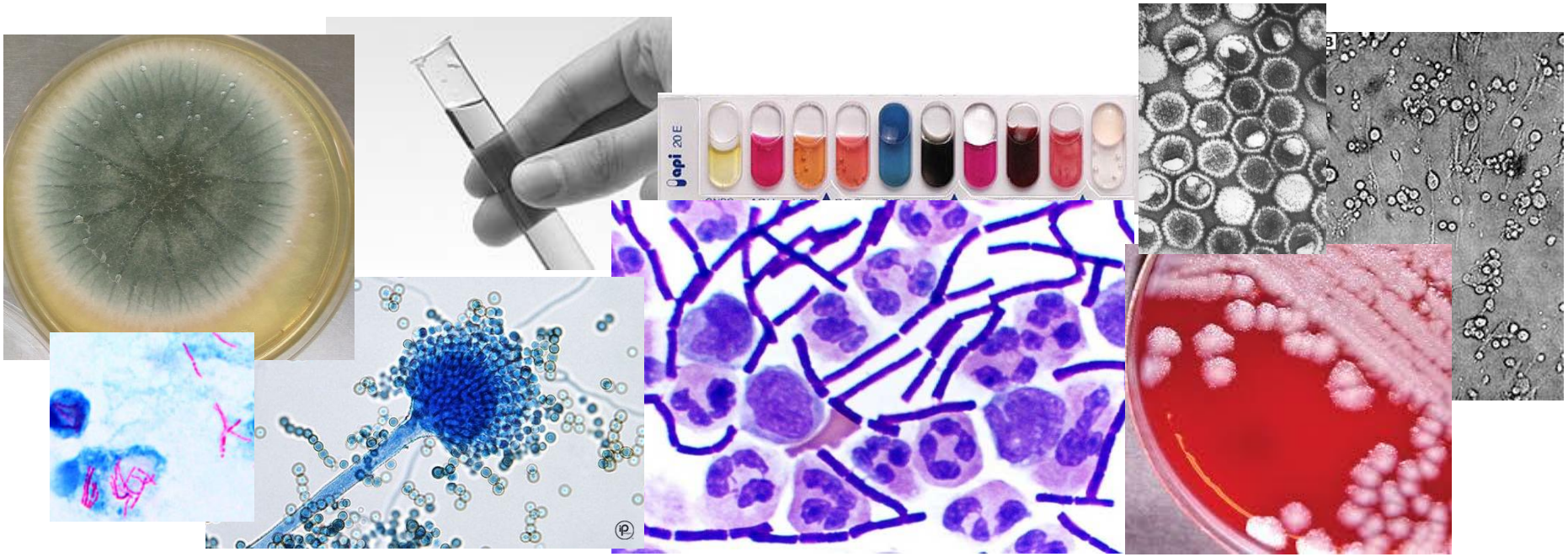
- Initial diagnosis of illness
- Disease progression
- Response to therapy
- Screening of carriers
- Surveillance & outbreak investigation

Detection of Microbes



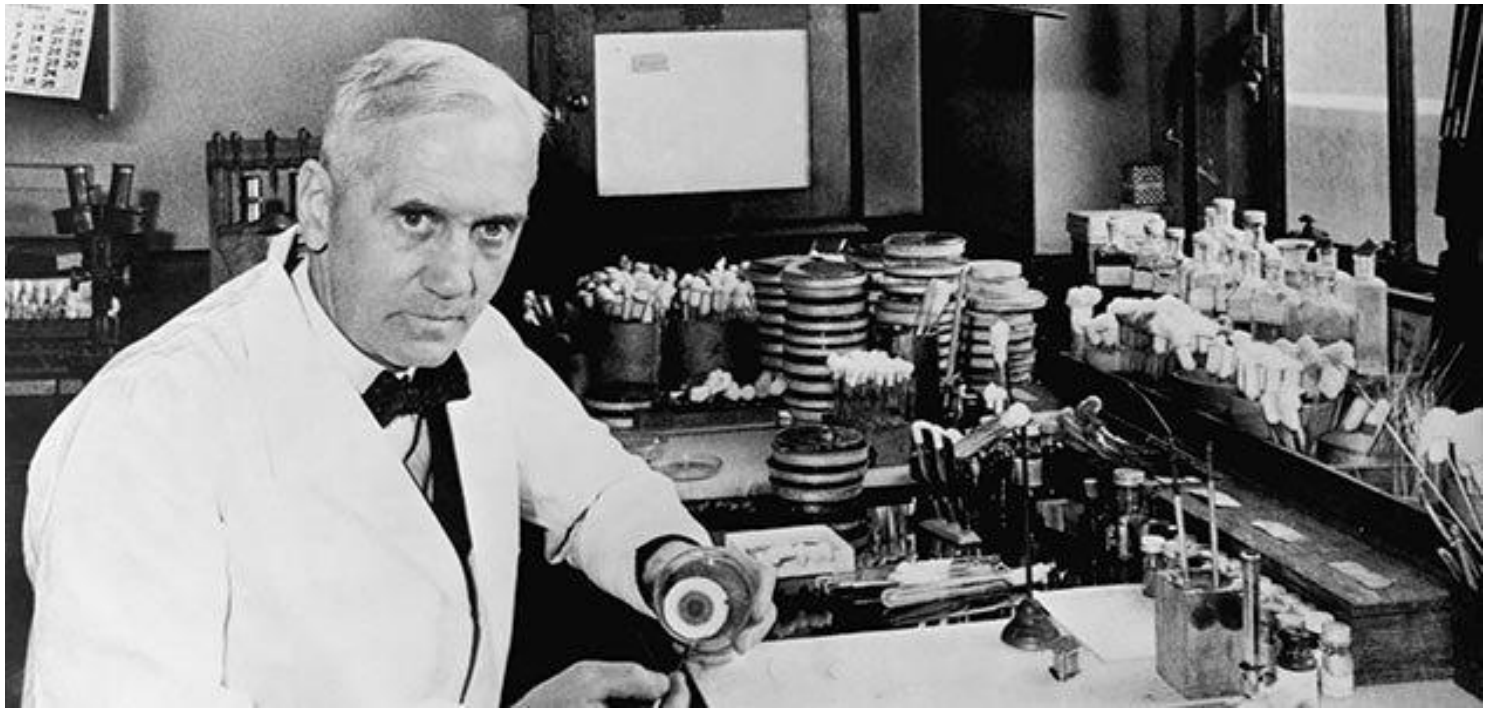
Conventional Methods

Direct detection & characterization via morphological & biochemical methods



Conventional Methods

Direct detection & characterization via morphological & biochemical methods

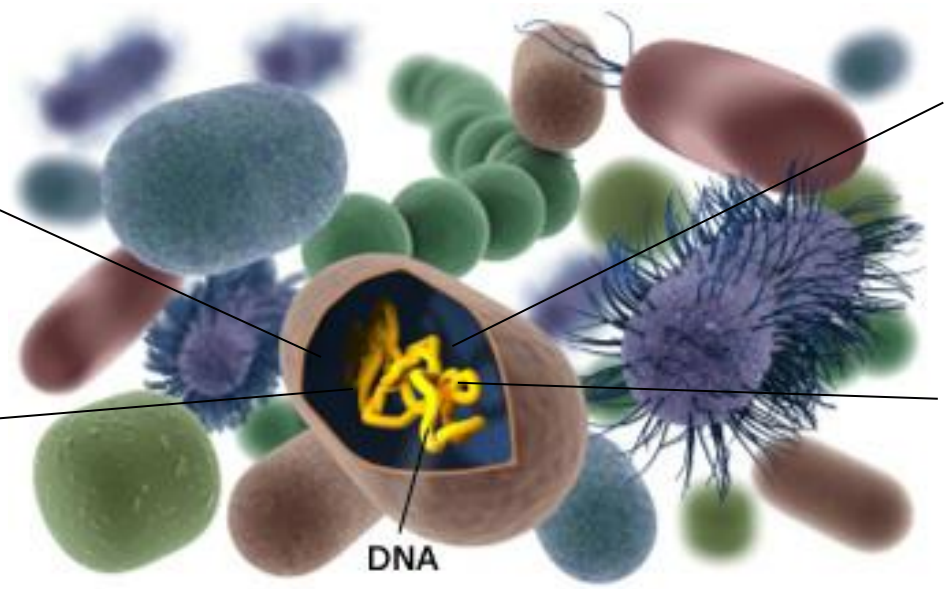


Nucleic Acid Testing (NAT)

Direct detection & characterization via molecular methods

Non-amplified nucleic acid probes

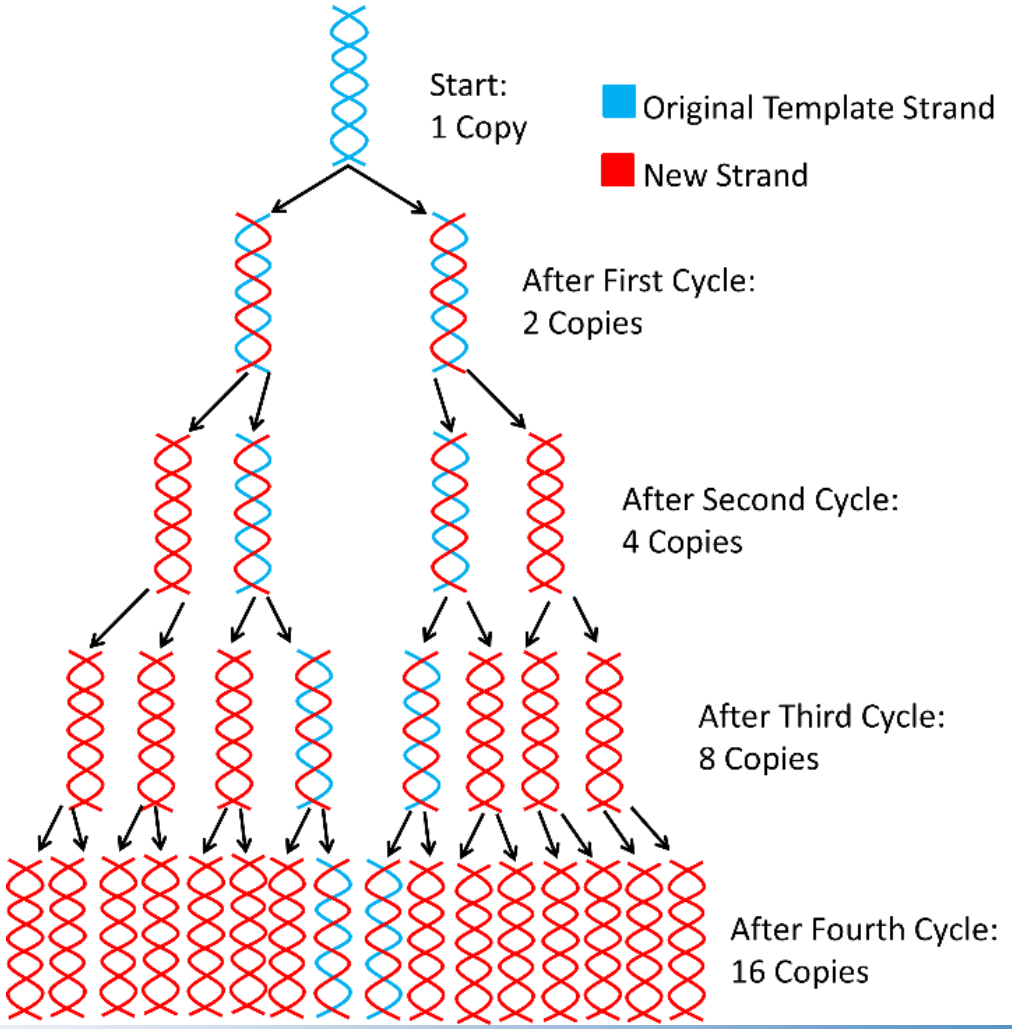
Signal amplification (hybrid capture)



Polymerase chain reaction

Isothermal amplification (transcription-based, loop-mediated)

PCR and sequencing

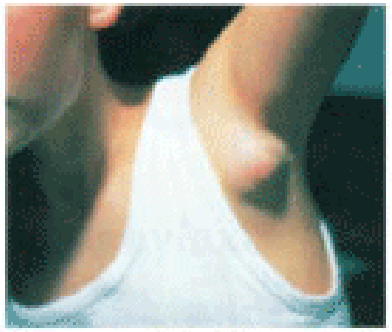


Nucleic Acid Testing (NAT)

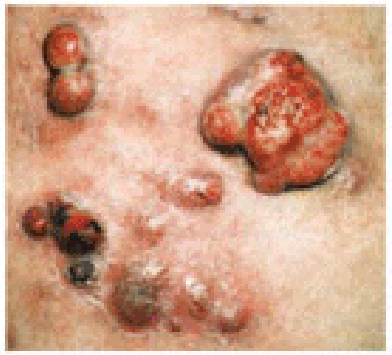
Direct detection & characterization via molecular methods

NAT: The Past

Patient-centric Impact of NAT



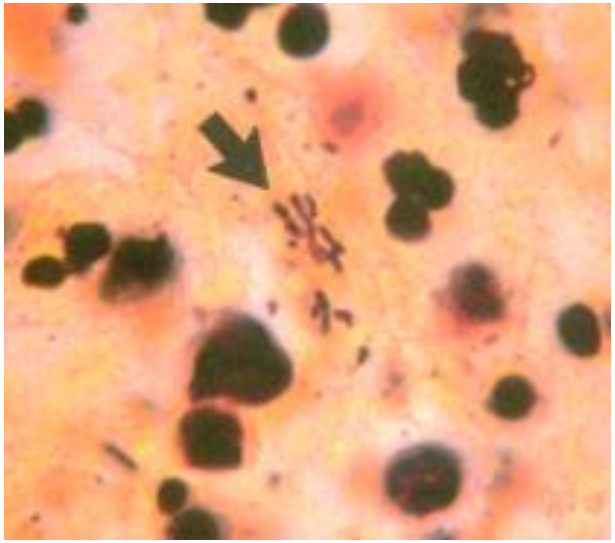
Cat-scratch disease in immunocompetent person

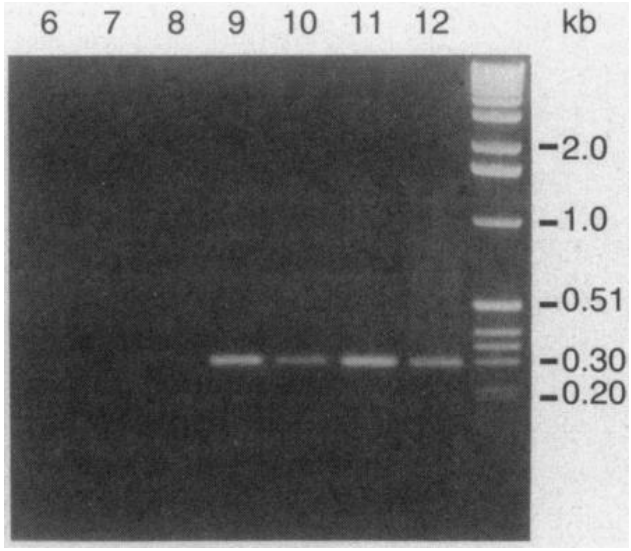


Bacillary angiomatosis in immunocompromised person

Dehio. 2005. Nature Reviews Micro. 3: 621-31

Identification of pathogens associated with clinical syndromes: *Bartonella* spp by 16S rRNA gene



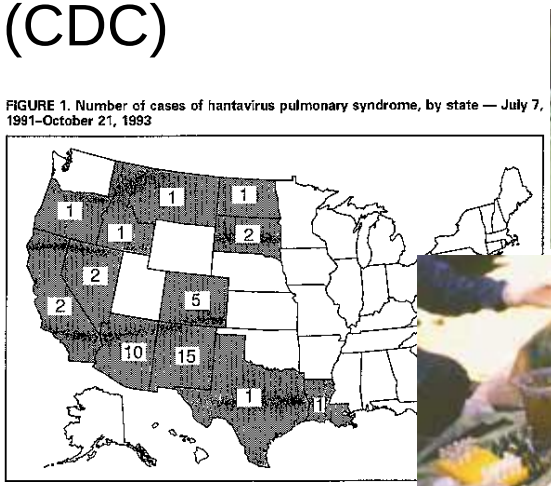


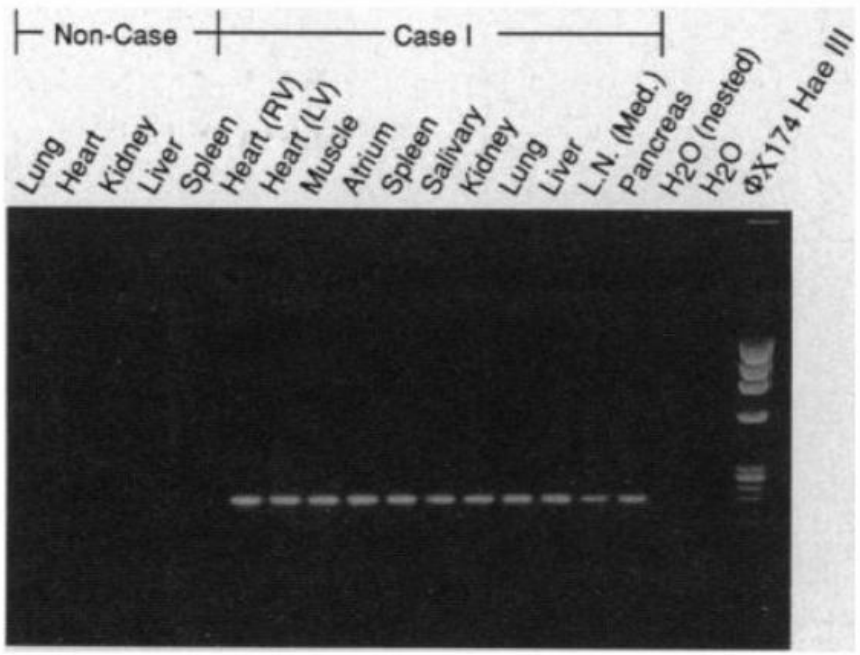
Relman *et al.* 1990. N Engl J Med. 323: 1573-80.

Public Health Impact of NAT

Identification of emerging pathogens:
Sin Nombre virus using conserved hantavirus gene

1993 outbreak of an unexplained pulmonary illness in "The Four Corners“ (CDC)





Nichol *et al.* 1993. Science. 262:914-917.

Impact of NAT on Infectious Diseases

Detection of organisms where microscopy and/or culture is not useful or preferred

- Unculturable organisms: Hepatitis C virus, EBV, HPV, Norovirus, *T. pallidum*, *M. pneumoniae, Borrelia* spp, *Rickettsia* spp… & many more
- Stains and cultures have low sensitivity: STIs caused by *N. gonorrhoeae*, *C. trachomatis*
- Culture is hazardous: Ebola virus, Hantavirus, *B. anthracis, Francisella* spp, dimorphic fungi

Impact of NAT on Infectious Diseases

Detection of organisms where microscopy and/or culture is not useful or preferred

- Quantification required: HIV, CMV & EBV, hepatitis B & C viruses
- Subtyping required*: Human papillomavirus, Hepatitis C, influenza, HIV, measles, shiga toxin-producing *E. coli*, *Campylobacter* spp, *C. difficile*

*including for molecular epidemiology

Impact of NAT on Infectious Diseases

Detection of organisms where microscopy and/or culture is not useful or preferred

- Clinical rapid response required: HSV/Enterovirus in CSF, *M. tuberculosis* (+resistance), influenza, pertussis, shiga toxin, malaria… & many more
- Emerging pathogens rapid response required: SARS/MERS-CoV, Ebola, measles, influenza, carbapenemase-producing organsims…

Nucleic Acid Testing (NAT)

- Initial diagnosis of illness
- Disease progression
- Response to therapy
- Screening of carriers
- Surveillance & outbreak investigation

Nucleic Acid Testing (NAT)

Increased sensitivity

+

Decreased turn-around-time

+

Increased applications

=

Increased demand

BCCDC PHL: 22% increase in overall expenditure over the last 8 years

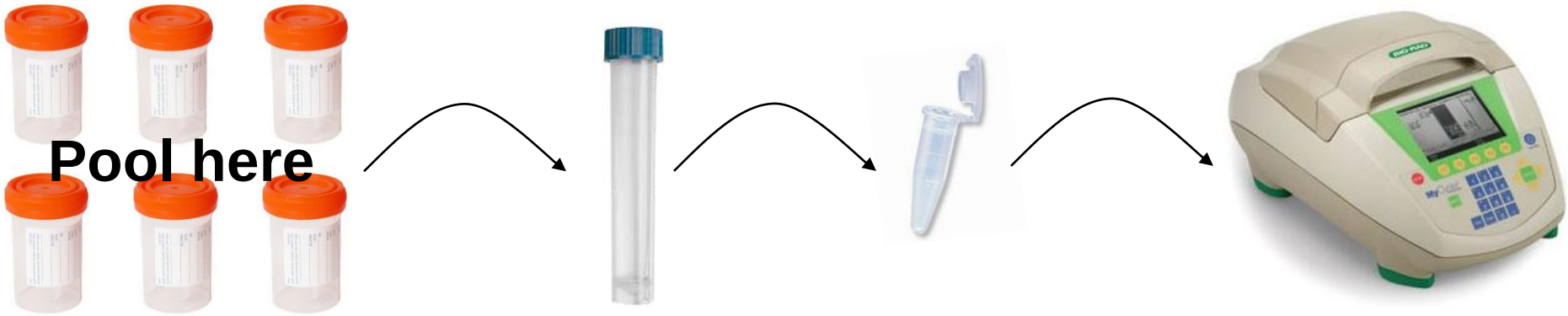
NAT: The Present

Strategies to Improve Lab Efficiencies

- Pooled Specimen NAT
- Pathogen Panel NAT
- Automation of NAT

Pooled Specimen NAT

A strategy to reduce the cost and labor of test



- Useful in low prevalence settings
- Decreases sensitivity of the test

Pooled Specimen NAT

Has been used as a strategy for detection of

- Malaria (*Plasmodium* spp)
  - surveillance; reduction of labor & costs by 95.5%

  (Hsiang *et al*. 2012. PLoS One 7:e29550)
- Acute HIV infection (currently at BCPHMRL)
  - Targeted for higher risk populations (MSM)
  - Cost savings from timely notification & care

  (Krajden *et al.* 2014. J Clin Virol. 61:132-7)
- **Shiga toxin+ *E. coli*** (validated at BCPHMRL**)**

Shiga Toxin-Producing *E. coli*

Leading cause of bacterial enteric infections

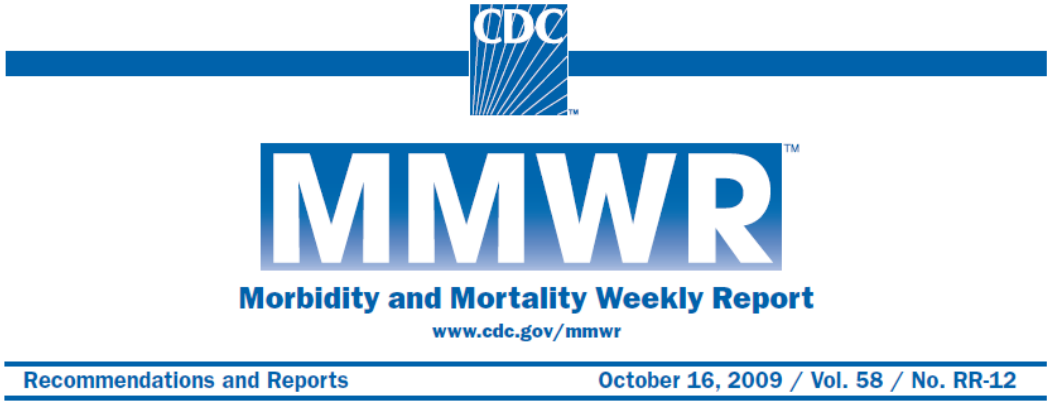
- Common serotype: O157-H7
- >100 serotypes cause illness

Stool culture is selective for O157

- Infections attributed to non-O157 STEC may be more prevalent than those of O157

(Johnson *et al.* 2006. Clin Infect Dis 43:1587-95; CDC. 2013. MMWR. 62:283-7)

Shiga Toxin-Producing *E. coli*



CDC

MMWR

Morbidity and Mortality Weekly Report

www.cdc.gov/mmwr

Recommendations and Reports — October 16, 2009 / Vol. 58 / No. RR-12

Recommendation for Identification of STEC by Clinical Laboratories

All stools submitted for testing from patients with acute community-acquired diarrhea (i.e., for detection of the enteric pathogens *Salmonella, Shigella,* and *Campylobacter*) should be cultured for O157 STEC on selective and differential agar. These stools should be simultaneously assayed for non-O157 STEC with a test that detects the Shiga toxins or the genes encoding these toxins. All O157 STEC isolates should be

= use culture and PCR for detection

Shiga Toxin-Producing *E. coli*

Expected positivity by PCR: 1–2% in North America

(Buchan *et al.* 2013. J Clin Microbiol 51:4001-7; Chui *et al.* 2011. J Clin Microbiol 49:4307-10; Lefterova *et al.* 2013. J Clin Microbiol 51:3000-5)

Culture and PCR for detection:

Associated cost in personnel, equipment, and reagents is a barrier for implementation

(Stigi *et al*. 2012. Emerg Infect Dis 18:477-9; Kiska *et al.* 2011. J Clin Microbiol 14:2394-7)

Pooled NAT in detection of STEC

Pools of 10 enriched broths (n=30 pools, 1+ve/pool)

- Sensitivity of pooled PCR: 100%
- ΔCt: 3.9 for *stx*$_1$, 4.3 for *stx*$_2$: change in limit of detection
  - LOD from ~8 × 10^2 to ~2 × 10^4 CFU/ml
  - Low positive specimens (>Ct 35) are <5% of positives

Cost and workflow analysis:

Cost-savings of 53-81% and time-savings of 1-3 hours for 90 specimens

➢ **Suitable for large-scale testing**

Pathogen Panel NAT

- Useful in patients with nonspecific symptoms that can be caused by various pathogens

Respiratory

Influenza A
Influenza A H1
Influenza A H3
Influenza A 2009 H1N1
Influenza B
Respiratory Syncytial V A
Respiratory Syncytial V B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Human Bocavirus
Human Metapnuemovirus
Rhinovirus/Enterovirus
Adenovirus
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Coronavirus 229E
C. Pneumoniae
L. Pneumophila
M. Pneumonaie
B. pertussis

Gastrointestinal

Campylobacter (jejuni, coli and upsaliensis)
Clostridium difficile
Plesiomonas shigelloides
Salmonella
Yersinia enterocolitica
Vibrio (parahaemolyticus, vulnificus and cholerae)
Enteroaggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC)
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
Shigella/Enteroinvasive *E. coli* (EIEC)
Cryptosporidium
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia
Adenovirus F40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus (I, II, IV and V)

Bloodstream

Enterococcus
Listeria monocytogenes
Staphylococcus aureus
Streptococcus (agalactiae, pneumoniae, pyogenes)
Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Enterobacter cloacae complex
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus
Serratia marcescens
Candida (albicans, glabrata, krusei Parapsilosis, tropicalis)
mecA - methicillin resistance
vanA/B - vancomycin resistance
KPC - carbapenem resistance

CNS

Escherichia coli K1
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitidis
Streptococcus agalactiae
Streptococcus pneumoniae
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)
Cryptococcus gattii
Cryptococcus neoformans

*in development FilmArray

Pathogen Panel NAT

- Useful in patients with nonspecific symptoms that can be caused by various pathogens
  - Can impact patient care
  - Simplify physician ordering
  - Limited understanding of co-infection
  - May not detect emerging pathogens
  - High capitol and per test cost

Respiratory Pathogen Panel NAT

Lab considerations: One size does not fit all

Tests and algorithms consider patient population (cost-effectiveness, clinical benefits, pretest probability)

Test	Complexity	Labor	TAT*	Throughput	Cost
Filmarray	Low	Low	1 hr	Low	High
Luminex	Mod	Mod	6-8 hr	High	Mod
In-house	High	High	4 hr	High	Low

*TAT affected by specimen batching

Respiratory Panel NAT Impacts

- Inpatients: enhanced & rapid detection associated with reduced hospital stays/costs and antibiotic use

(Rogers *et al.* 2015. Arch Pathol Lab Med 139:636-41; Gelfer *et al.* 2015. Diagn Microbiol Infect Dis Epub; Mahony *et al*. 2009. J Clin Microbiol 47:2812-7)

- Outpatients: initial reduction in antibiotic prescription rate is not sustained on follow-up

(Brittain-Long *et al*. 2011. BMC Med 9:44)

- Children & immunocompromised patients: co-infection associated with increased disease severity?

(Vallieres & Renaud. 2013. Diagn Microbiol Infect Dis. 76(3):255-61; Asner *et al*. 2015. Clin Microbial Infect 21:264.e1-6)

Respiratory Pathogen Panel NAT

BCCDC PHL: initial screen with Flu A/B & RSV PCR

- If negative, perform Luminex panel PCR
- If positive & patient meets criteria*, perform Luminex pathogen panel PCR

- Luminex panel PCR performed twice/week

In-house PCR cost: ~$8/test
Luminex panel cost: ~$50/test

*Criteria: <5 years old, BMT, ICU, outbreak case, surveillance…
Luminex panel currently used detects virus pathogens

Respiratory Pathogen Panel NAT

At BCCDC PHL:

Year	No. Flu/RSV PCR	No. Luminex Panel	Total No.	Cost
2010/11	5590	5528	11,118	$276,400
2011/12	5216	5196	10,412	$259,800
2012/13	8802	4108	12,910	$205,400
2013/14	10807	2899	13,706	$144,950
2014/15	13338	3828	17,166	$194,400

Raw data collected by LUMNX code and resulting, may not include surveillance specimens
Cost is an approximation

Respiratory Pathogen Panel NAT

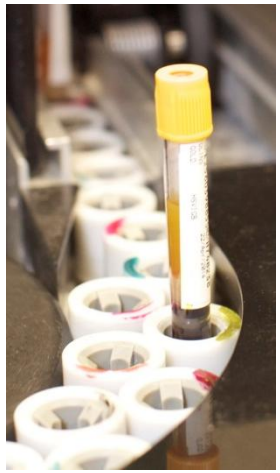
Influenza A
Influenza A H1
Influenza A H3
Influenza A 2009 HIN1
Influenza B
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Human Bocavirus
Human Metapneumovirus
Rhinovirus/Enterovirus
Adenovirus
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Coronavirus 229E

BCCDC PHL: **bacterial targets** to complicate algorithm in the future? 2015 validation

Chlamydophila pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

Automation of NAT

- Useful in high-volume scenarios & for point-of-care testing
  - Decrease hands-on time
  - Can provide more consistency
  - Require large capital investment



Automation of NAT

For high-throughput

- *N. gonorrhoeae & C. trachomatis*
- HIV, HCV, HPV, CMV/EBV
- Automation of NAT steps (liquid handlers)

For point-of-care

- *C. difficile*, *M. tuberculosis,* resistance genes

Some performed outside of acute-care setting & require specimen batching

NAT Limitations

Laboratory & Clinical Considerations

- Capital cost & cost per test
- Turn-around-time if batching
- Requirement of technical expertise
- Target specificity limits broad detection
- Increased sensitivity effects on interpretation

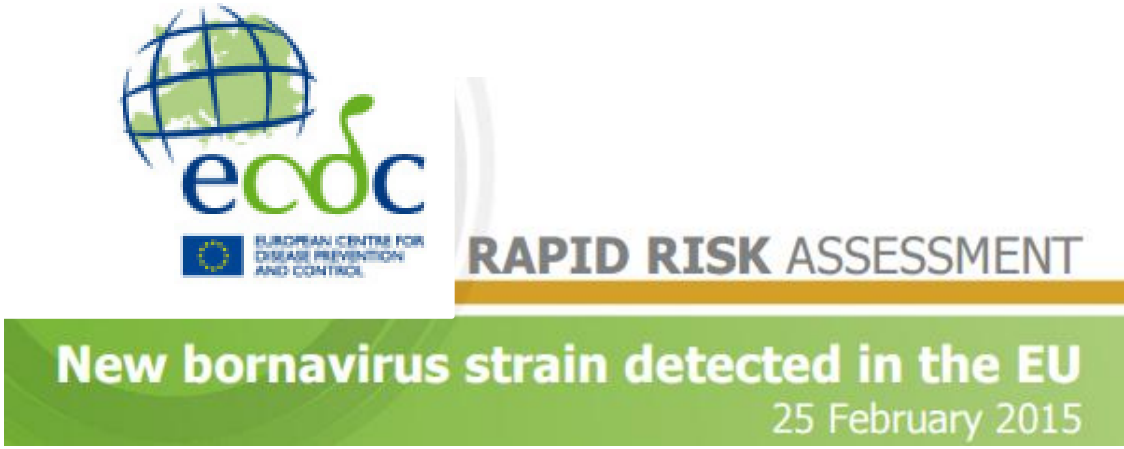
The Technological GAP

Novel NAT techniques consider:

- Evolving & emerging pathogens
- Mechanisms of resistance
- Molecular epidemiology (outbreaks)

NAT: The Future

Patient-centric Impact of NAT



Metagenomic analysis found an association between a novel bornavirus & the three fatal human CNS infections

Hoffman *et al.* 2015. N Engl J Med 9:154-62.

Public Health Impact of NAT

Gardy JL, Johnston JC, Ho Sui SJ, Cook VJ, Shah L, Brodkin E, Rempel S, Moore R, Zhao Y, Holt R, Varhol R, Birol I, Lem M, Sharma MK, Elwood K, Jones SJ, Brinkman FS, Brunham RC, Tang P. **2011**. N Engl J Med. 364:730-9.

Whole-genome sequencing and social-network analysis of a tuberculosis outbreak

Prystajecky N, Tsui CK, Hsiao WW, Uyaguari-Diaz MI, Ho J, Tang P, Isaac-Renton J. **2015.** Appl Environ Microbiol. 81:4827-34.

Giardia spp. Are Commonly Found in Mixed Assemblages in Surface Water, as Revealed by Molecular and Whole-Genome Characterization

Gardy JL, Naus M, Amlani A, Chung W, Kim H, Tan M, Severini A, Krajden M, Puddicombe D, Sahni V, Hayden AS, Gustafson R, Henry B, Tang P. **2015**. J Infect Dis. pii: jiv271.

Whole-Genome Sequencing of Measles Virus Genotypes H1 and D8 During Outbreaks of Infection Following the 2010 Olympic Winter Games Reveals Viral Transmission Routes

Addressing Current NAT Limitations

- Molecular methods will continue to be a leading area of growth in lab medicine
  - Novel technologies & applications
  - Less complex & costly = more accessible

- Clinical & financial outcome data will be needed to justify use of testing

Acknowledgements

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