

Modern Biotechnology in the Clinical Microbiology Lab

Faster Methods for Getting Actionable Intelligence to Physicians

Robert J. Cybulski Jr., PhD

Microbiology Fellow

UWMC Department of Laboratory Medicine

cybulski@uw.edu

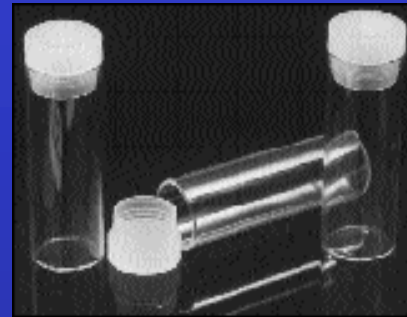
Clinical Scenario #2

29 year old active duty Naval officer currently in medical school, recently returned from a overseas rotation in Kenya, is admitted with new onset of fever and headache. His son goes to daycare and comes home frequently with upper respiratory infection symptoms, but last week had a bad cold along with diarrhea. Patient is mildly confused and has a stiff neck. Lumbar puncture is performed and labs show signs of infection that are inconclusive in differentiating between a bacterial, viral or fungal cause. Patient is started empirically on vancomycin, ceftriaxone, and dexamethasone...

Viral Culture and Identification Timeline



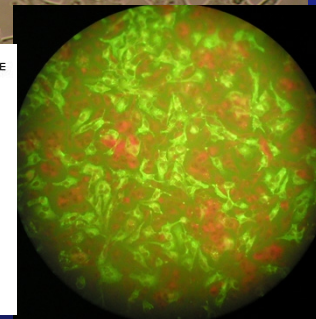
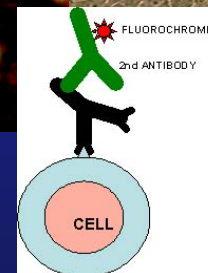
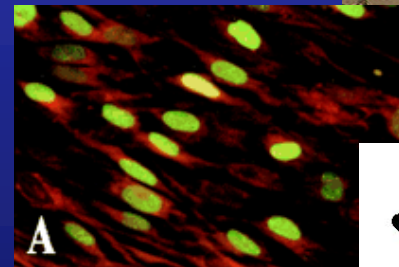
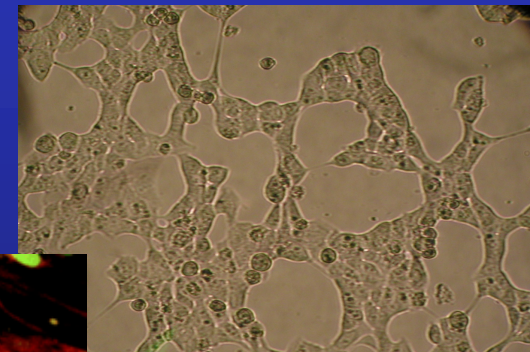
Next day
(M-F)

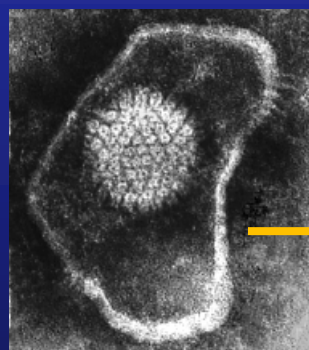
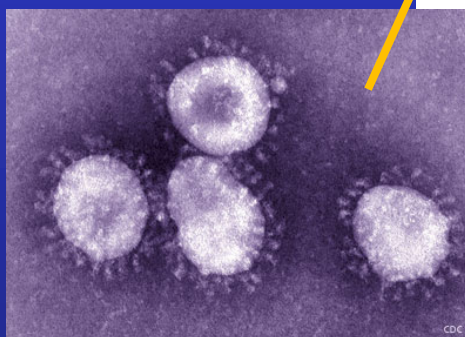
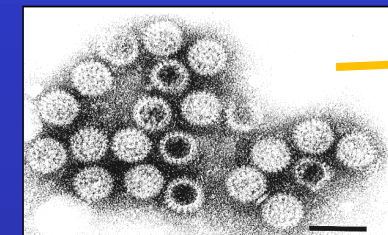
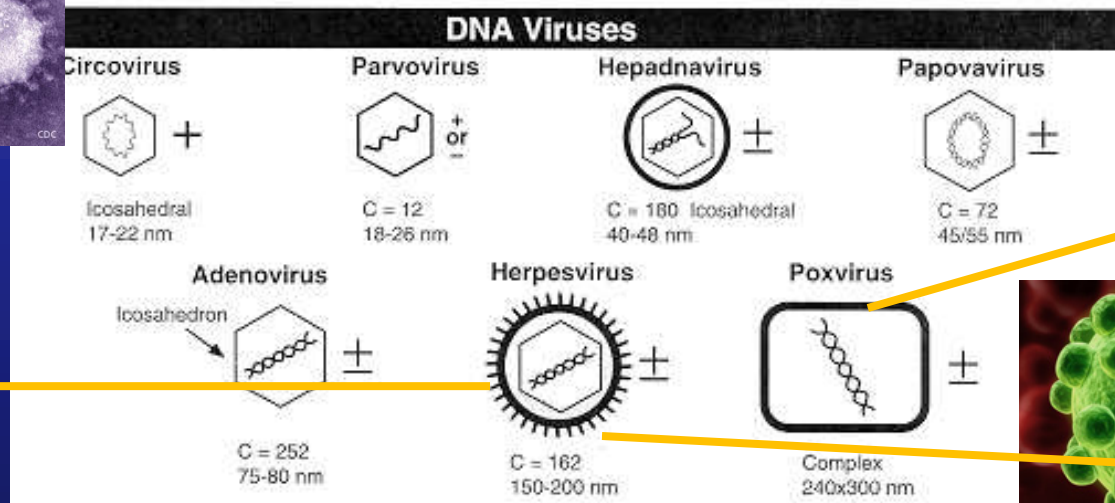
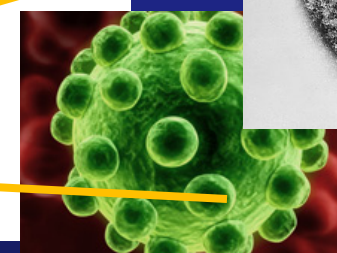
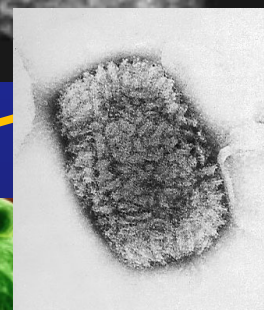
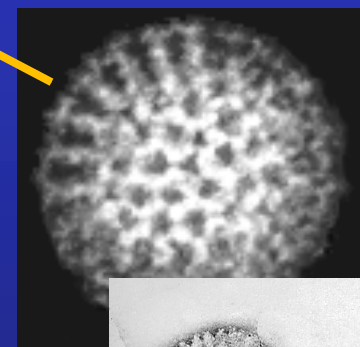
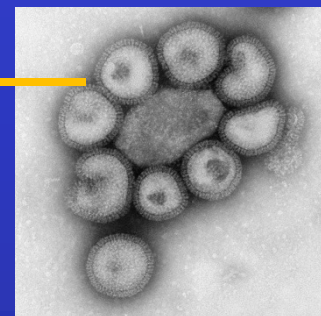
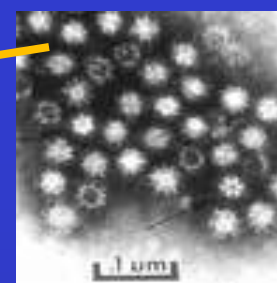
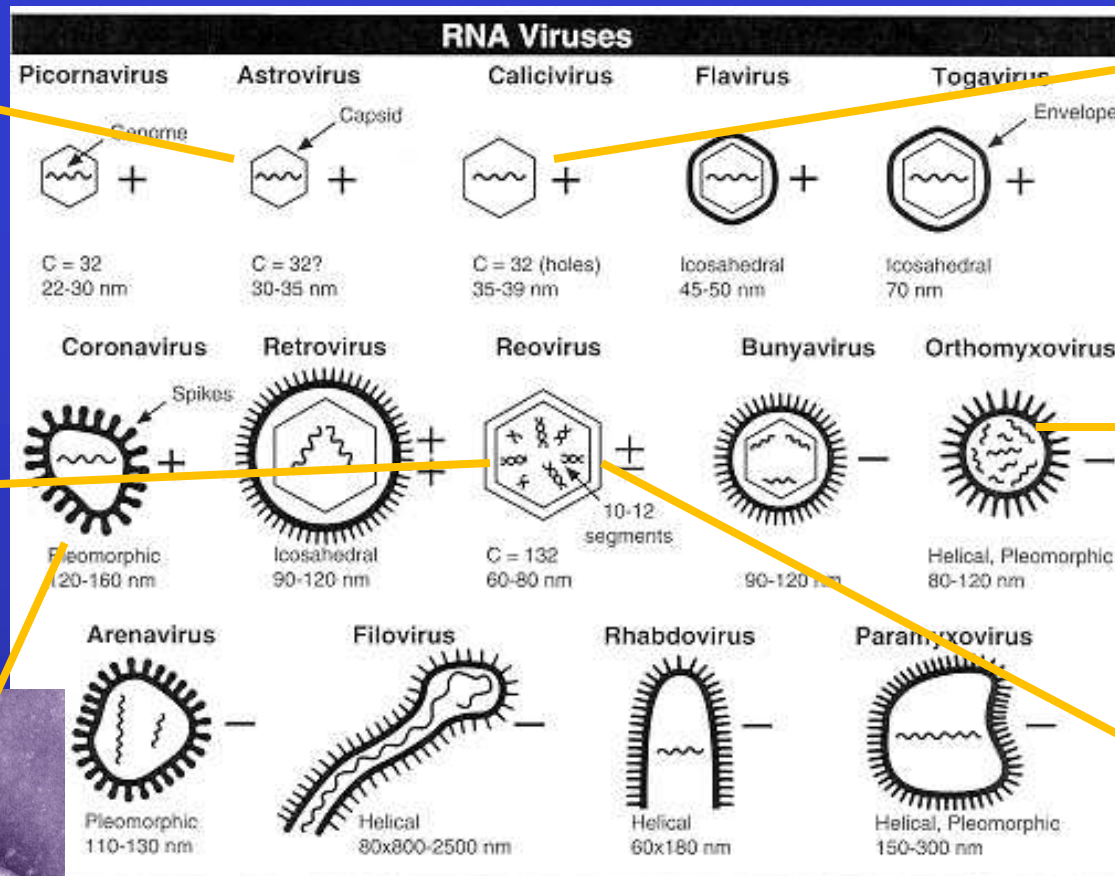
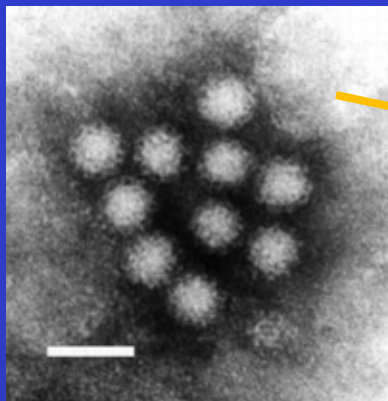


1-5 days

Total time to ID: 1-6 days

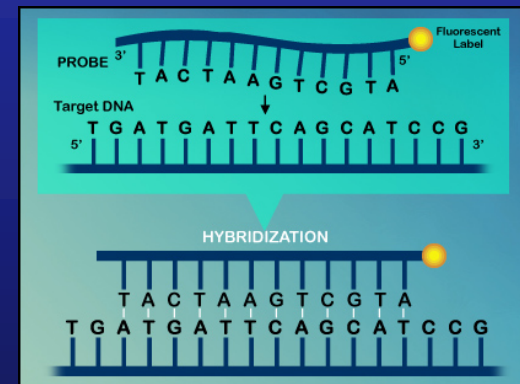
- Alternative: Direct Detection
 - Rapid Antigen tests (Flu, RSV)
 - Nucleic acid via PCR/RT-PCR





Nucleic Acid-based Diagnosis of Infectious Diseases

- Made possible by 3 key principles:
 - **All** (?) infectious agents contain nucleic acids:
 - DNA, RNA or both
 - **Unique** nucleic acid sequences can be identified that are specific for an organism (and only that organism)
 - Probes can be designed to bind to these organism-specific sequences in a process known as **hybridization**

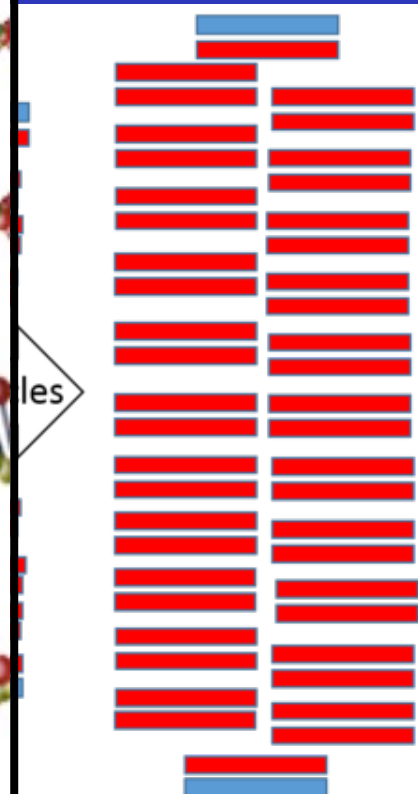
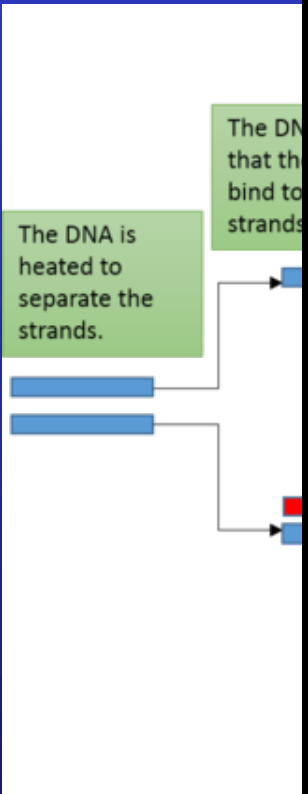
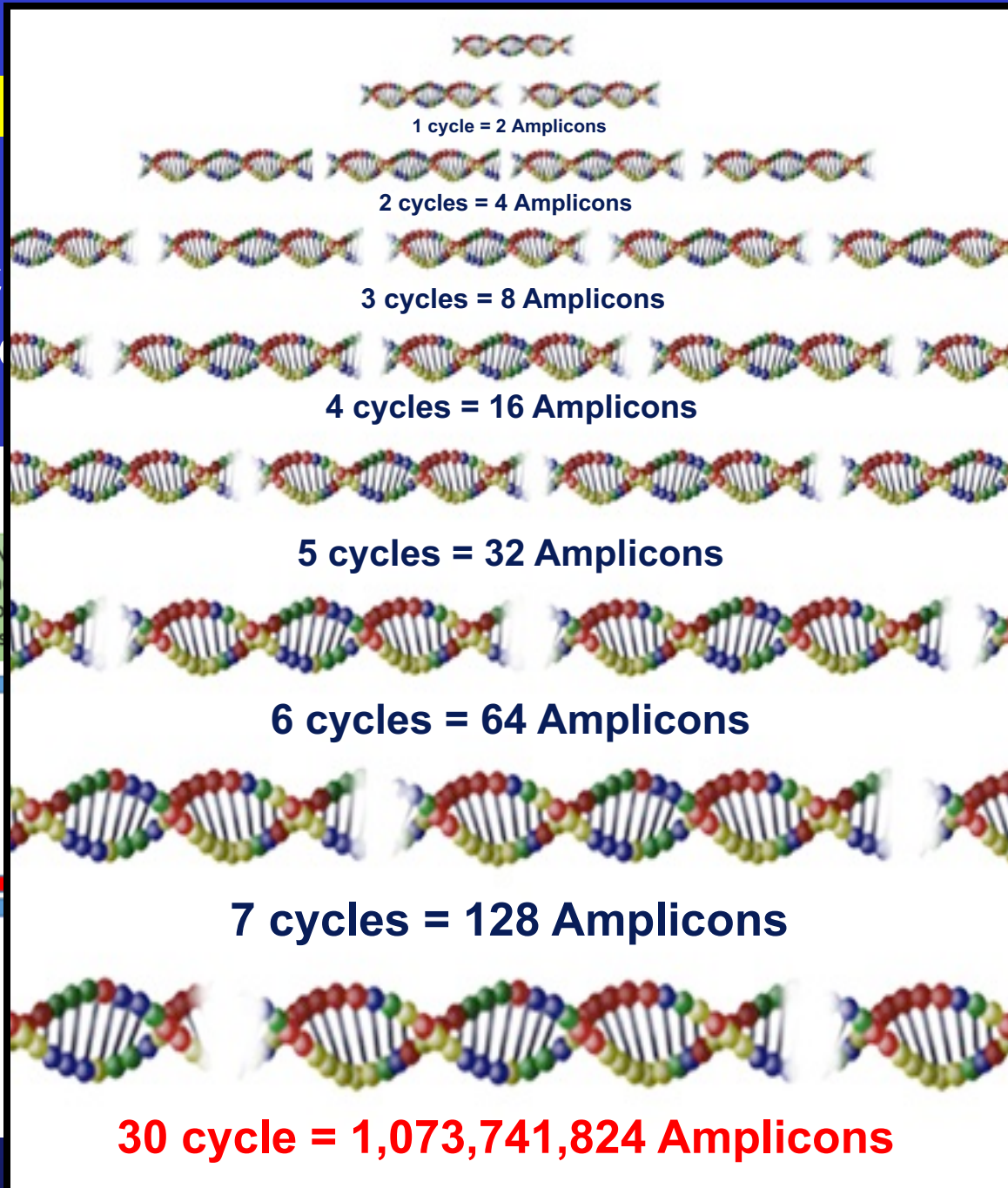


Pol

(PCR)

- PCR
acid

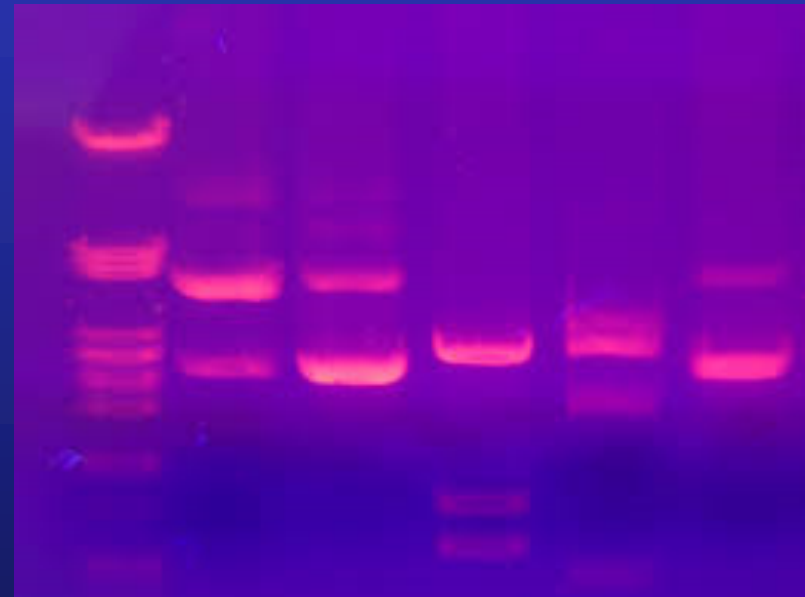
fy nucleic
(es)



Polymerase Chain Reaction (PCR)

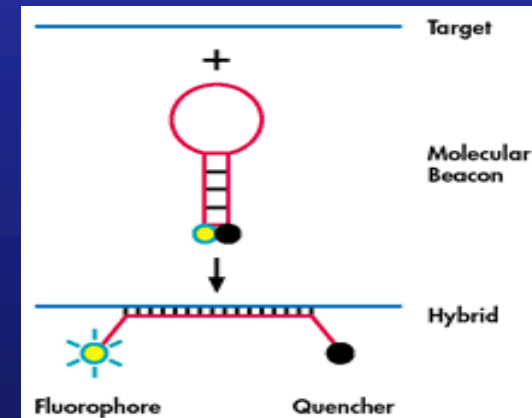
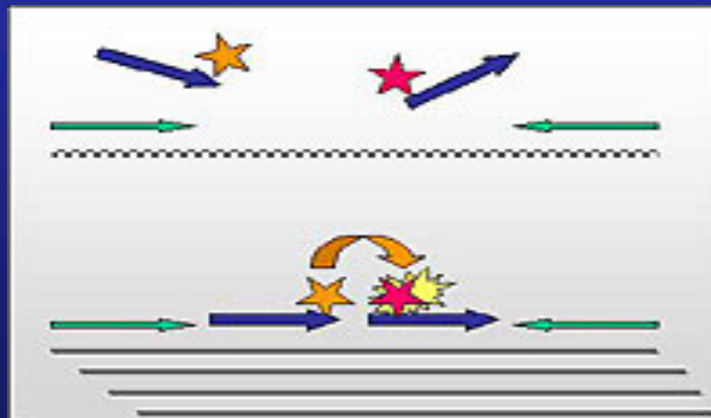
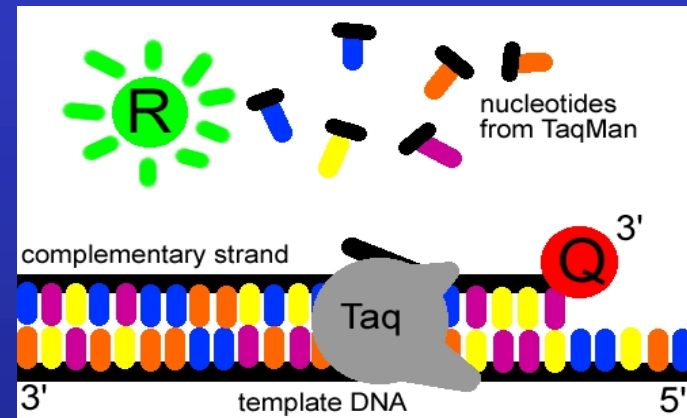
Gel Electrophoresis

- Uses an electric field to separate nucleic acids.
- DNA moves toward the cathode
- Molecules of the gel polymer impede the fragments of nucleic acid. Larger pieces are impeded more than smaller pieces
- Smaller pieces move faster.



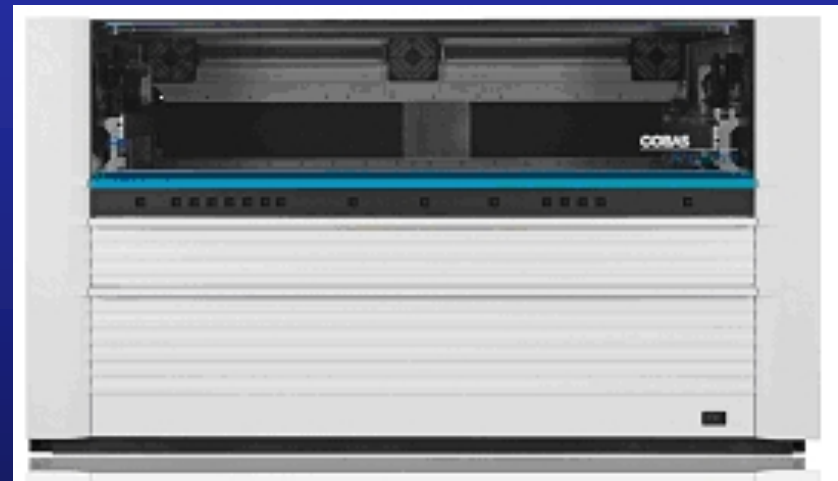
Real-time PCR (RT-PCR)

- Detection of product **in solution in real time**
- Amplification of target with target-specific primers, in the presence of signal probes
- Examples
 - FRET
 - TaqMan
 - Molecular Beacon
 - (SYBR Green)



Roche COBAS® TaqMan™48 and Ampliprep Systems

- TaqMan Analyzer
 - Real-time automated TaqMan™ PCR system
 - Two independent 24-well thermal cyclers
 - Assay time 90-120 minutes
 - No open reagents/fluids
- Ampliprep
 - Automatic extraction
 - Pre-packaged, self-sealing reagent cassettes
 - Up to 72 specimens at a time running up to 4 protocols simultaneously
- Used for HBV, HCV, CMV testing
- **Monday-Friday, 0700-1530 ONLY**

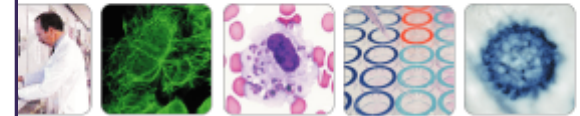


UW Virology

Extended Respiratory Virus PCR Panel [Code: REVSQT]

Lab Name	Resp Virus Semi-Quant PCR		
Synonyms	MPV, PIV1-4, RSV, FluA, FluB, coronaviruses, rhinoviruses, adenoviruses, and human bocavirus, Respiratory Virus PCR, Influenza A and B by PCR		
Specimen Type	BAL, nasal wash, NP or nasal swab, tracheal aspirate		
ORCA Name	Extended (RARE) Respiratory Virus Panel		
General Information	Includes: MPV, PIV1-4, RSV, FluA, FluB, Coronavirus, Rhinovirus, Adenovirus and Bocavirus.		
Components	Component Name	Lab Code	Component Type
	Resp Virus PCR Spec	RSQSD	Test
	Metapneumovirus PCR Qual Rslt	REVMVPV	Test
	Metapneumovirus Ct Value	CTMPV	Test
	Parainfluenza 1 PCR Qual Rslt	REVP1	Test
	Parainfluenza 1 Ct Value	CTP1	Test
	Parainfluenza 2 PCR Qual Rslt	REVP2	Test
	Parainfluenza 2 Ct Value	CTP2	Test
	Parainfluenza 3 PCR Qual Rslt	REVP3	Test
	Parainfluenza 3 Ct Value	CTP3	Test
	Parainfluenza 4 PCR Qual Rslt	REVP4	Test
	Parainfluenza 4 Ct Value	CTP4	Test
	RSV PCR Qual Result	REVRSV	Test
	RSV Ct Value	CTRSV	Test
	Influenza A PCR Qual Result	REVFLA	Test
	Influenza A Ct Value	CTFLA	Test
	Influenza B PCR Qual Result	REVFLB	Test
	Influenza B Ct Value	CTFLB	Test
	Coronavirus PCR Qual Result	REVCV	Test
	Coronavirus Ct Value	CTCV	Test
	Rhinovirus PCR Qual Result	REVRHN	Test
	Rhinovirus Ct Value	CTRHN	Test
	Adenovirus PCR Qual Result	REVADV	Test
	Adenovirus Ct Value	CTADV	Test
	Bocavirus PCR Qual Result	REVBOC	Test
	Bocavirus Ct Value	CTBOC	Test
	Resp Virus Semi-Quant Interp	RSQINT	Test

Department of Laboratory Medicine



[Notes ↓](#)

Marrow

Swabs, Tissues

Marrow, PBMC,
Mononuclear Cells, Swab

Marrow, PBMC, Swab

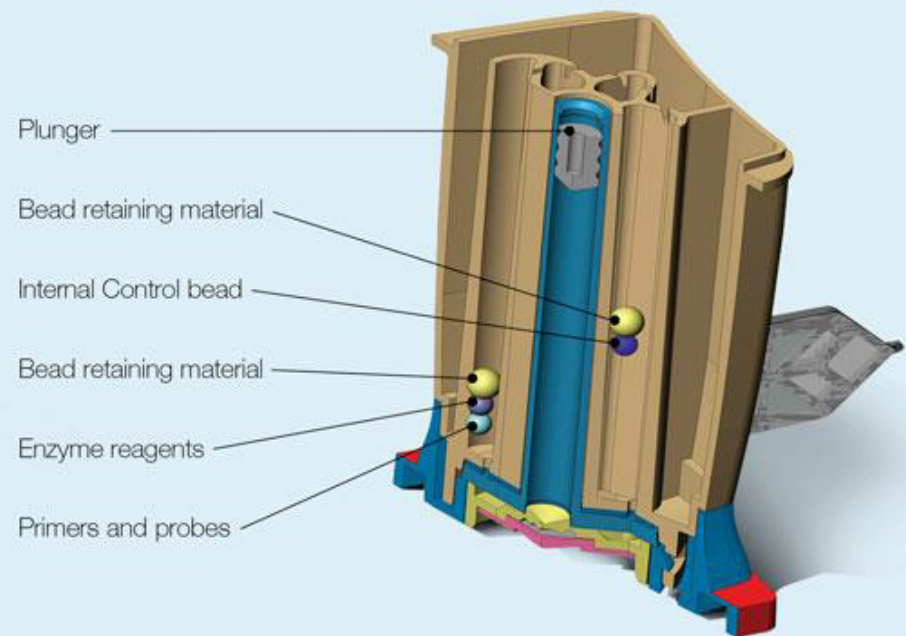
Spinal Fluid

Swabs, Tissues

Biopsies, autopsies,
()

Cepheid GeneXpert®

- Automates and integrates into a single disposable cartridge:
 - Sample purification
 - Nucleic acid amplification using RT-PCR
 - Detection of the target sequence
- **Prep in minutes, 60-75 minute turnaround time, 24/7**



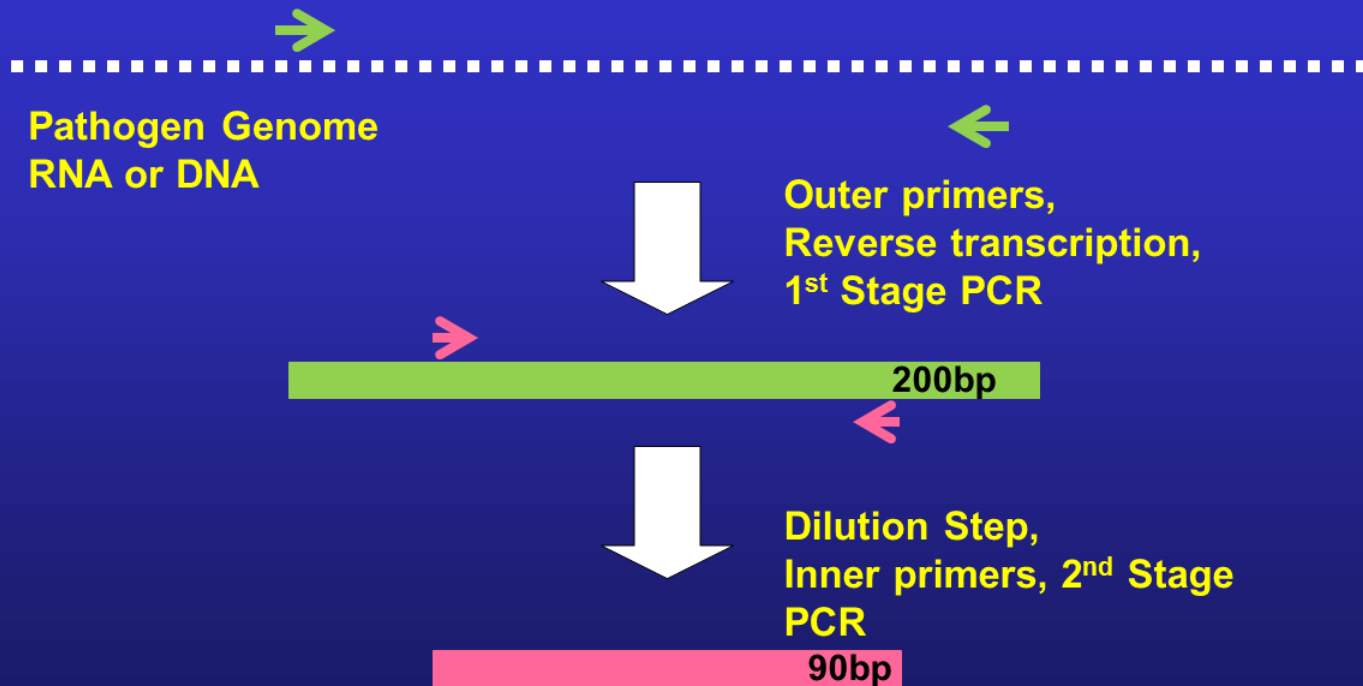
Cepheid GeneXpert®

- Currently used for detecting:
 - *Clostridium difficile* (stool)
 - Enterovirus (CSF)
 - Group B *Streptococcus* (Vaginal Swab)
 - Influenza A/B (UTM)
 - MRSA (Nares swab)
 - MRSA (Blood culture)
 - *C. trachomatis*/*N. gonorrhoeae* (Swabs)
 - *Mycobacterium tuberculosis* (Sputum/BAL)

BioFire FilmArray®

Nested Multiplex PCR (nmPCR)

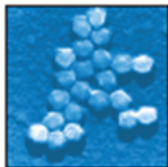
- High-sensitivity assay for large panel of biological agents



BioFire FilmArray®

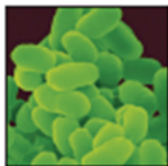
- FDA-approved
 - Respiratory Panel – 20 pathogens
 - GI Panel – 22 pathogens
 - Blood Panel – 24 pathogens and 3 antibiotic resistance genes
 - Meningitis/Encephalitis – 16 pathogens

1 Test. 20 Respiratory Pathogens. All in about an hour.



Viruses

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



Bacteria

- *Bordetella pertussis*
- *Chlamydomphila pneumoniae*
- *Mycoplasma pneumoniae*



FDA-Approved Multiplex systems

- Biofire Film Array ®
- Verigene Nanosphere ®
- Luminex (multiple)
- GenMark ePlex ®



Clinical Scenario #2

29 year old active duty Naval officer currently in medical school is admitted with new onset of fever and headache. His son goes to daycare and comes home frequently with upper respiratory infection symptoms, but last week had a bad cold along with diarrhea. Patient is mildly confused and has a stiff neck. Lumbar puncture is performed and labs show signs of infection that are inconclusive in differentiating between a bacterial, viral or fungal cause. Patient is started empirically on vancomycin, ceftriaxone, and dexamethasone... Multiplex PCR is sent and returns in an hour with Enterovirus positive and all other bacterial pathogens negative. Antibiotics are stopped and patient is given supportive care and has complete recovery. Lab is able to grow Enterovirus from the CSF as well.