Molecular Diagnosis and Monitoring of Viral Infections in the Central Nervous System

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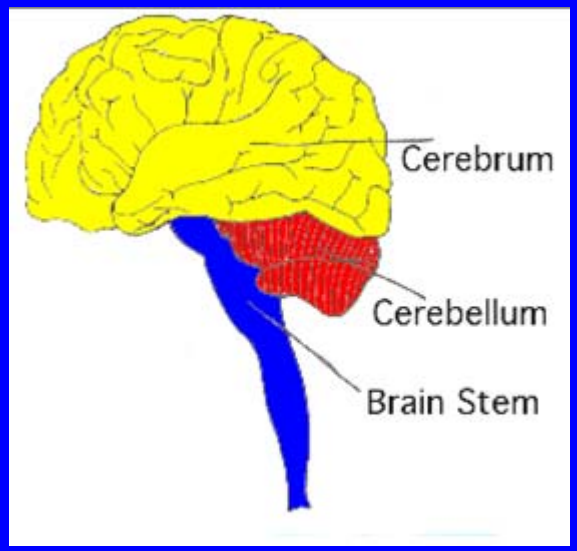
Central Nervous System: Basic Facts



- CNS comprises the brain, the spinal cord, and associates
- Cerebrospinal fluid envelops, carries essential nutrients, cleanses wastes
- Blood brain barrier limits leukocytes, complement and immunoglobulin from peripheral blood into the CNS

Multiple Organisms Can Cause CNS Infections

- Viruses: herpesviruses (e.g., HSV), enteroviruses, arboviruses, polyomaviruses, respiratory viruses (e.g., measles virus), HIV-1, adenoviruses, Nipah/Hendra viruses, parvovirus, rabies virus
- Bacteria: *Neisseria*, *Borrelia*, *Listeria*, *Haemophilus*, *Streptococcus*, *Mycobacterium*, *Mycoplasma*, *Chlamydia*, *Bartonella*, *Ehrlichia*, *Tropheryma*
- Fungi: *Cryptococcus*, *Histoplasma*
- Parasites: *Toxoplasma*

Organism-Specific Diagnosis of CNS Infections Is Important

- Different pathogens can cause similar clinical features
- Several CNS infections have effective, specific anti-microbial therapies
- Enhance the patient care and reduce the cost

Laboratory Techniques for Specific Diagnosis of Viral Infections in the CNS

- Microscopy morphology
- Antigen detection
- Cell culture
- Antibody detection
- PCR-led molecular methods

Outline

- Three clinical case presentations
- Background information reviewing
- Commonly used diagnostic methods
- Take home messages

Molecular Diagnosis of Enterovirus Infections In the Central Nervous System

Case Presentation #1

- A 14 day-old white male presented a day of increased bouts of fussiness with a rectal temperature of 100^{o}F

Case Presentation – cont.

- Per mom, the patient was unable to be consoled and also with a markedly decreased oral intake
- Urine output remained adequate, and stool pattern was unchanged
- The patient was admitted for a septic workup
- He was initially started on meningitic doses of ampicillin, acyclovir, and gentamicin along with maintenance i.v. fluids
- The fever was controlled with Tylenol q.4.h. p.r.n.

Case Presentation – cont.

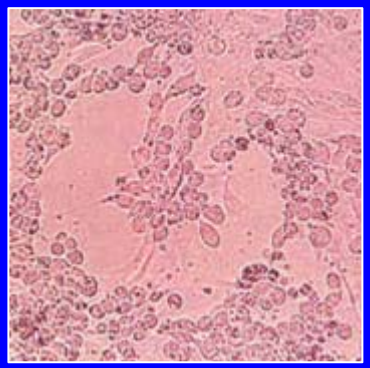
- A cerebrospinal fluid was obtained and was remarkable for a yellow, cloudy appearance
- WBC: 210 with 26% N, 7% L, and 67% M
- RBC: 1,300
- Glucose: 44, total protein: 75

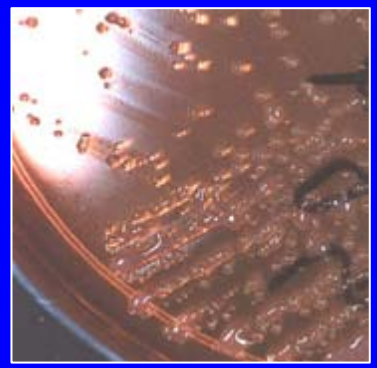
Case Presentation – cont.

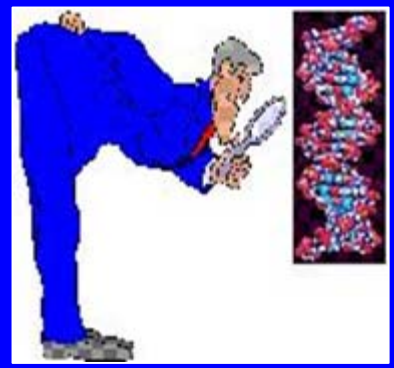
- What can molecular diagnostic microbiologists do?

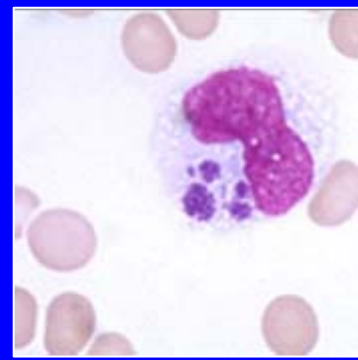
How Do We Recognize Pathogen Existence in Clinical Specimens?

- Bacterial colony
- Viral cytopathic effect
- How to “visualize” DNA?





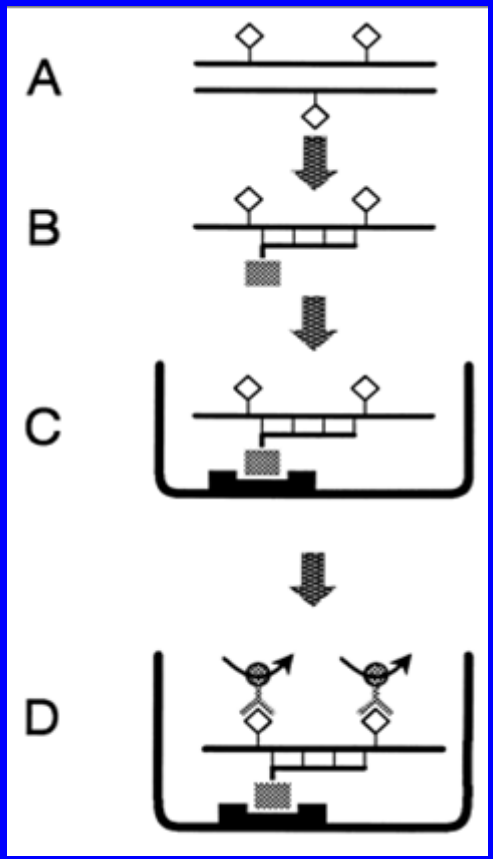


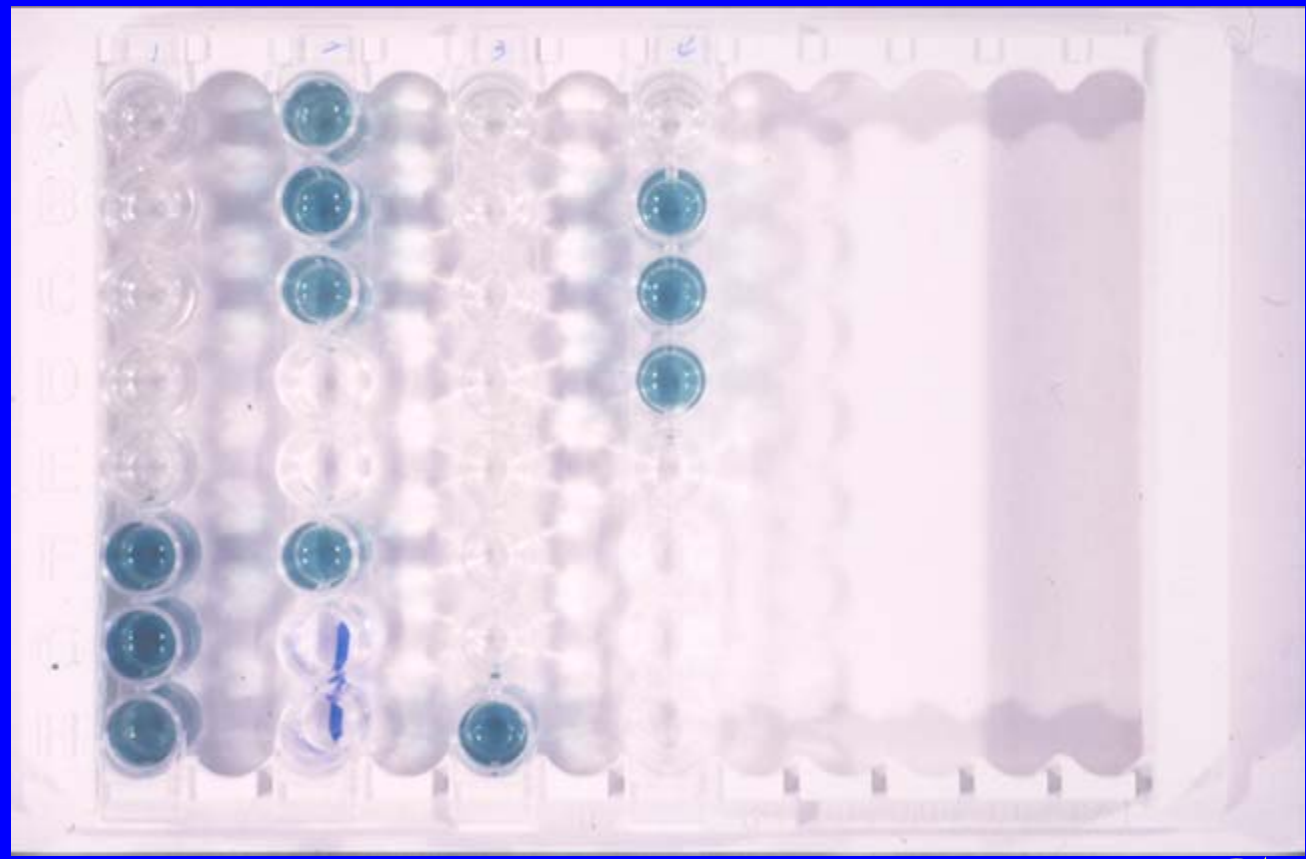


Techniques for Amplification Products Detection and Analysis

- Gel electrophoresis with or without Southern blotting
- Direct amplicon sequencing
- Matrix hybridization
- Simultaneous amplification and detection ("Real-time")
- Colorimetric microtiter plate (PCR-EIA)

A PCR-Positive Equals to a Color Production in Microtiter Plate Wells





Back to Case Presentation

- A negative HSV and a positive enterovirus were reported at his next hospital day
- At his second hospital day, the patient started improving with decreasing fever curve, decreasing fussiness, and increasing oral intake
- Acyclovir was discontinued
- The patient remained on ampicillin and gentamicin
- Bacterial, fungal, and/or viral cultures of blood, nasopharyngeal swab, stool, and CSF remained negative

Case Presentation – cont.

- At his third hospital day, the patient continued to do well with decreasing fever curve, decreasing fussiness, and increasing oral intake
- Bacterial, fungal, and viral cultures of blood, nasopharyngeal swab, stool, and CSF remained negative
- Ampicillin and gentamicin were stopped.
- The patient was deemed ready for discharge with close followup

Case Presentation – End

- The patient kept improving during next two follow-up visits until completely recovered one week after discharge
- Nasopharynx swab grew an enterovirus 8 days after inoculation, further identified as Coxsackie B2 by the Tennessee State Laboratory
- No virus was recovered from CSF

HIV-1 Viral Load Discrepancies Between the Peripheral and Central Nervous Systems

Case Presentation #2

- **A** 42 year-old white male presents with 4 months of forgetfulness and altered mental status

Haas et al, *AIDS Res Hum Retroviruses* **2000**, 16:1491

Case Presentation - Continued

- The patient was diagnosed with HIV infection in September, 2001, with CD4=136 and plasma HIV RNA=60,000 copies/mL
- Altered mental status was noticed on 10/9/01
- Brain MRI shows nonspecific confluent abnormal T2 signal in cerebral white matter c/w HIV encephalopathy
- Started on HAART (ZDV, 3TC, nevirapine) and TMP-SMZ

Case Presentation - Continued

- On 10/28/01, the patient was admitted with seizures, worsening mental status, drug rash
- CSF studies indicated protein=147, glucose=84, and WBC=9. Negative for VDRL, EBV and HSV
- Brain MRI indicated an increased T2 signal in the white matter and both temporal lobes, suspecting viral encephalopathy
- He was discharged on 11/21/01 on HAART (ZDV, 3TC, lopinavir/r)

Case Presentation - Continued

- The patient was re-admitted on 12/14/01 with acutely worse worsening mental status. CD4 = 224
- CSF studies indicated protein=156, glucose=63, WBC=16, Negative for EBV, VZV, JC virus, HSV
- Brain MRI: Many punctate foci of enhancement in the subcortical and periventricular white matter. Worse disease in right temporal lobe and inferior thalamus
- On 12/19/01, his Plasma HIV-1 RNA < 400 copies/mL, CSF HIV-1 RNA = 58,383 copies/mL

Case Presentation – cont.

- What can molecular diagnostic microbiologists do?

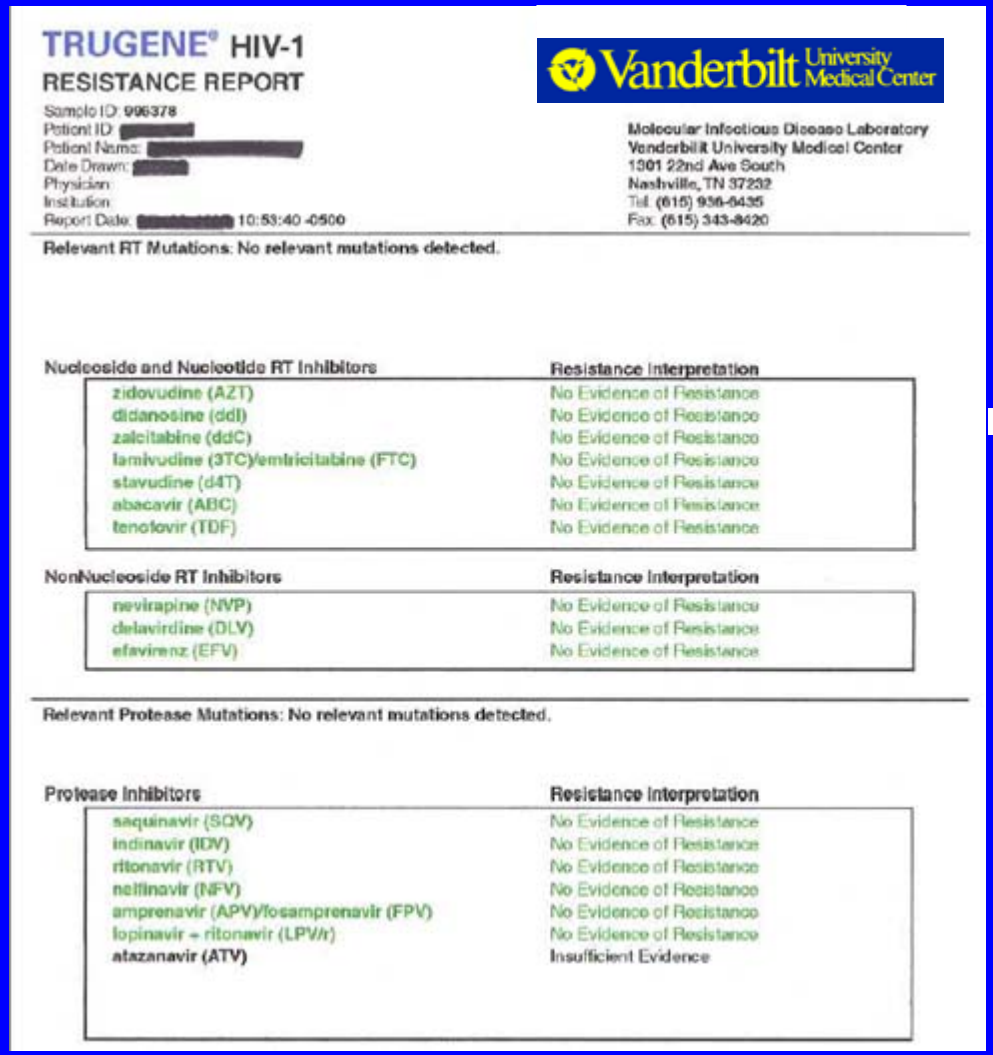
Anti Retroviral Drug Resistance Testing

- Resistance - the ability of HIV to multiply in the presence of antiretroviral drugs
- Phenotypic resistance - the ability of HIV to grow in high drug levels
- Genotypic resistance - viral mutations that cause resistance

Back to Case Presentation

- Total viral RNA was extracted from the CSF specimen and HIV genotyping were performed

Case Presentation - Continued



TRUGENE® HIV-1
RESISTANCE REPORT

Vanderbilt University Medical Center

Sample ID: 996378
Patient ID: [redacted]
Patient Name: [redacted]
Date Drawn: [redacted]
Physician:
Institution:
Report Date: [redacted] 10:53:40 -0500

Molecular Infectious Disease Laboratory
Vanderbilt University Medical Center
1301 22nd Ave South
Nashville, TN 37232
Tel: (615) 936-8435
Fax: (615) 343-8420

Relevant RT Mutations: No relevant mutations detected.

Nucleoside and Nucleotide RT Inhibitors	Resistance Interpretation
zidovudine (AZT)	No Evidence of Resistance
didanosine (ddI)	No Evidence of Resistance
zalcitabine (ddC)	No Evidence of Resistance
lamivudine (3TC)/emtricitabine (FTC)	No Evidence of Resistance
stavudine (d4T)	No Evidence of Resistance
abacavir (ABC)	No Evidence of Resistance
tenofovir (TDF)	No Evidence of Resistance

NonNucleoside RT Inhibitors	Resistance Interpretation
nevirapine (NVP)	No Evidence of Resistance
delavirdine (DLV)	No Evidence of Resistance
efavirenz (EFV)	No Evidence of Resistance

Relevant Protease Mutations: No relevant mutations detected.

Protease Inhibitors	Resistance Interpretation
saquinavir (SQV)	No Evidence of Resistance
indinavir (IDV)	No Evidence of Resistance
ritonavir (RTV)	No Evidence of Resistance
nelfinavir (NFV)	No Evidence of Resistance
amprenavir (APV)/fosamprenavir (FPV)	No Evidence of Resistance
lopinavir + ritonavir (LPV/r)	No Evidence of Resistance
atazanavir (ATV)	Insufficient Evidence

- HIV genotyping shows no evidence of resistance to abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine, delavirdine, efavirenz, nevirapine, amprenavir, indinavir, lopinavir/rit, nelfinavir, ritonavir, and saquinavir

Case Presentation - Ended

- **Clinical Impression:** HIV encephalopathy with viral replication in brain despite control in peripheral lymphoid tissues. Wild-type virus suggests inadequate CNS drug penetration
- **Plan:** Abacavir added to regimen for enhanced potency and favorable CNS penetration
- **Outcome:** Two weeks later, CSF HIV-1 RNA = 1,455, plasma HIV-1 RNA < 400 copies/mL

Molecular Monitoring of Human Cytomegalovirus Infection and Disease in Transplantation

Case Presentation #3

- A 64-year-old male presented with increased lethargy, nausea and vomiting

Miller et al. *Clin. Infect. Dis.* **42**:e26-e29, 2006

Case Presentation – cont.

- The patient was diagnosed with acute myelogenous leukemia in April 2000
- He underwent a mismatched unrelated donor stem cell transplant in March 2001
- The patient was CMV, HSV, and EBV seropositive prior to transplant

Case Presentation – cont.

- He developed GVHD involving the skin and intestines on day 79 and received high dose steroids
- The patient developed CMV reactivation one month post transplant and received multiple courses of both foscarnet and ganciclovir
- CMV pp65 positive cells varied between 0 to 12 per 2×10^5 leukocytes on therapy and and reached as high as 127 per 2×10^5 leukocytes off therapy

Case Presentation – cont.

- The patient was admitted on 9/22/2001, 180 days post transplant, for increasing lethargy, nausea, and vomiting
- FK506 was stopped for possible neurotoxicity without improvement
- A brain MRI revealed no focal lesions
- Lumbar puncture was performed: WBC 26, 94% L, 5% M, 1% N; RBC 11; glucose 72; protein 92
- Routine cultures and cryptococcal antigen were negative

Case Presentation – cont.

- What can molecular diagnostic microbiologists do?

CMV Causes Diseases and Disorders with Broad Spectrum of Clinical Features

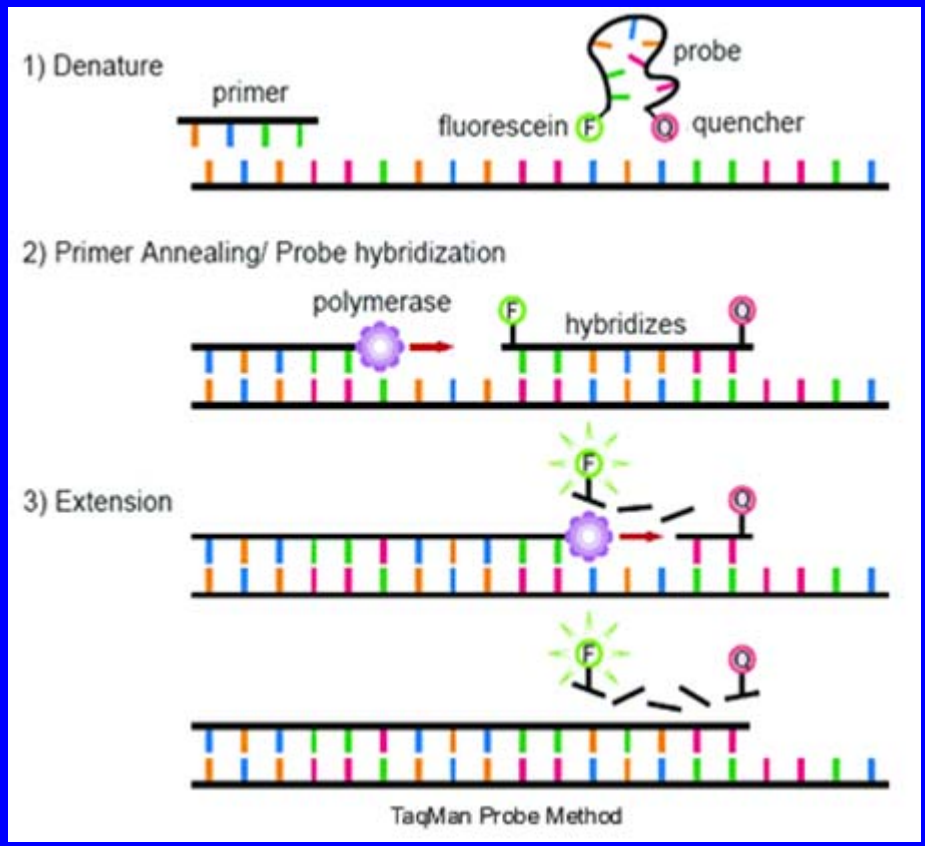
- Congenital and perinatal infection
- Primary infection
- Persistent and reactive infection

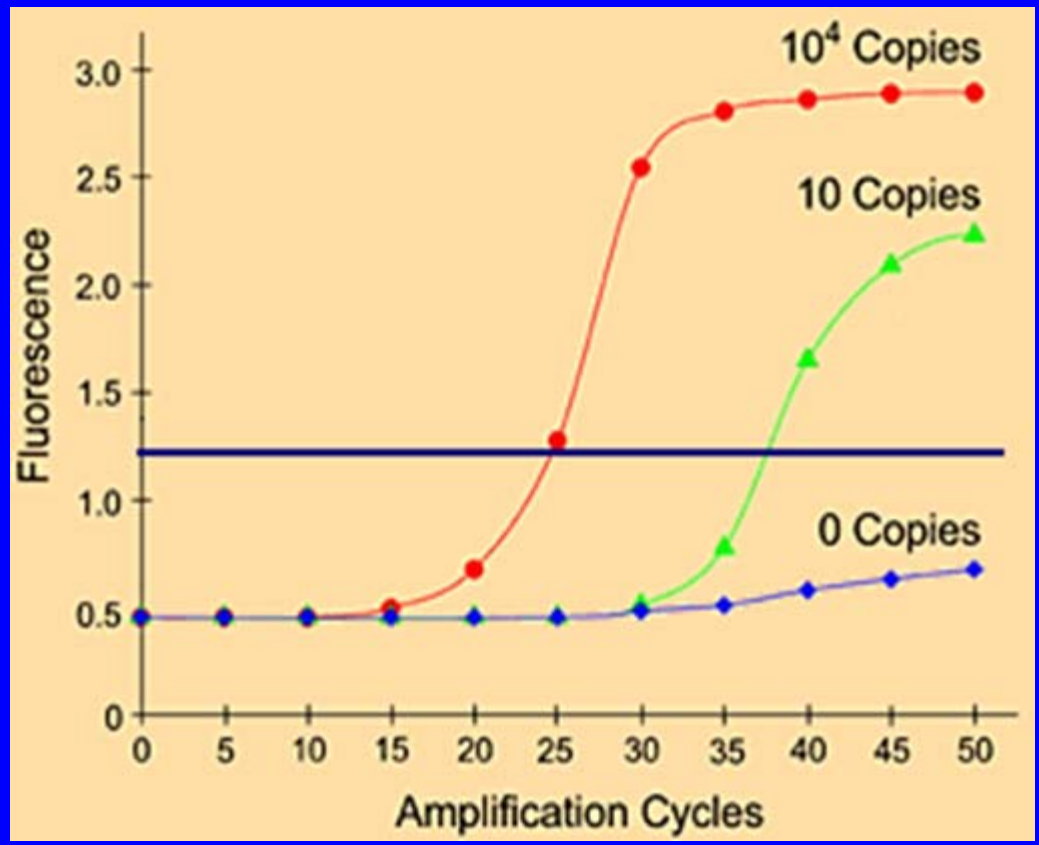
Infection ≠ Disease

Laboratory Methods for the Diagnosis of Cytomegalovirus Infection

Method	Principle	Specimen	TAT time	Clinical utility
Serology	Antibody	Serum	6 hrs	Infection
Culture	Viral grow	Leukocytes	18-48 hrs	Infection
Nuclisens	mRNA	Whole blood	6 hrs	Infection
PCR	DNA	Whole blood	6 hrs	Infection
Antigenemia	pp65 Ag	Leukocytes	6 hrs	Infection/Disease
Digene	DNA	Whole blood	6 hrs	Infection/Disease
Roche Cobas	DNA	Plasma	4 hrs	Infection/Disease
Real-time PCR	DNA	Whole blood	4 hours	Infection/Disease

Fluorescence Resonance Energy Transfer: How TaqMan Real-Time System Works





Back to Case Presentation

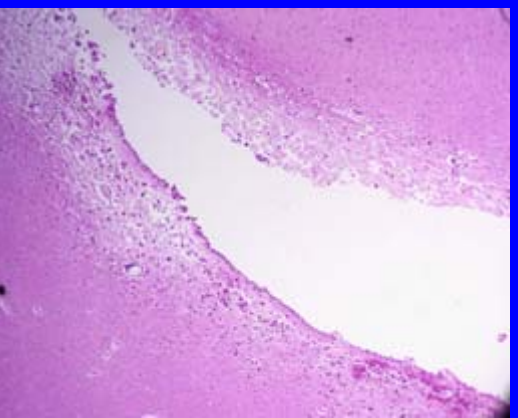
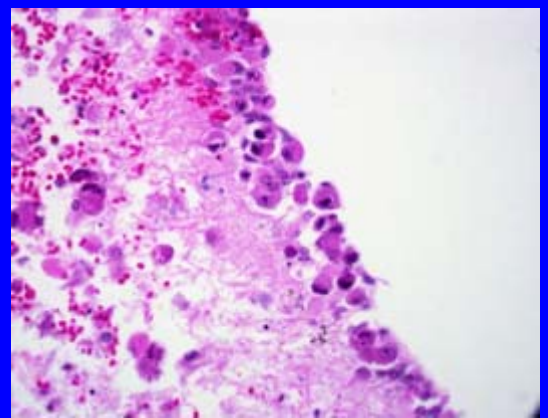
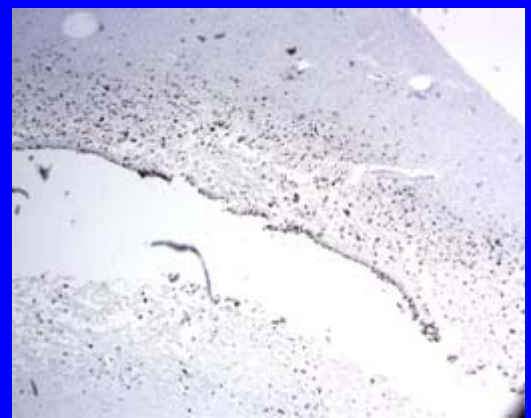
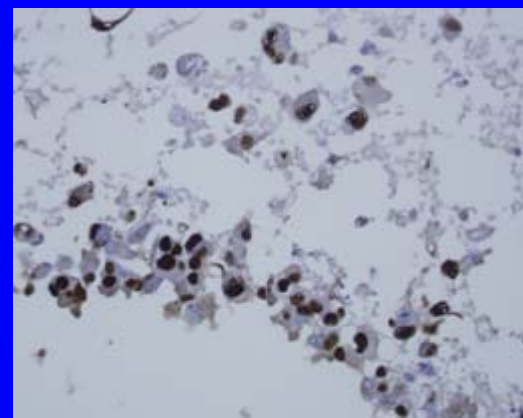
- Qualitative PCR test for HSV, VZV, EBV, HHV-6, and HHV-7 was negative
- The CMV quantification by TaqMan PCR in CSF was performed and revealed $>1\times10^6$ copies/ml
- In comparison, CMV viral loads in blood was 1,412 copies/ml
- No drug resistance-related mutations were identified in the UL97 phosphotransferase gene for CMV from both plasma and CSF

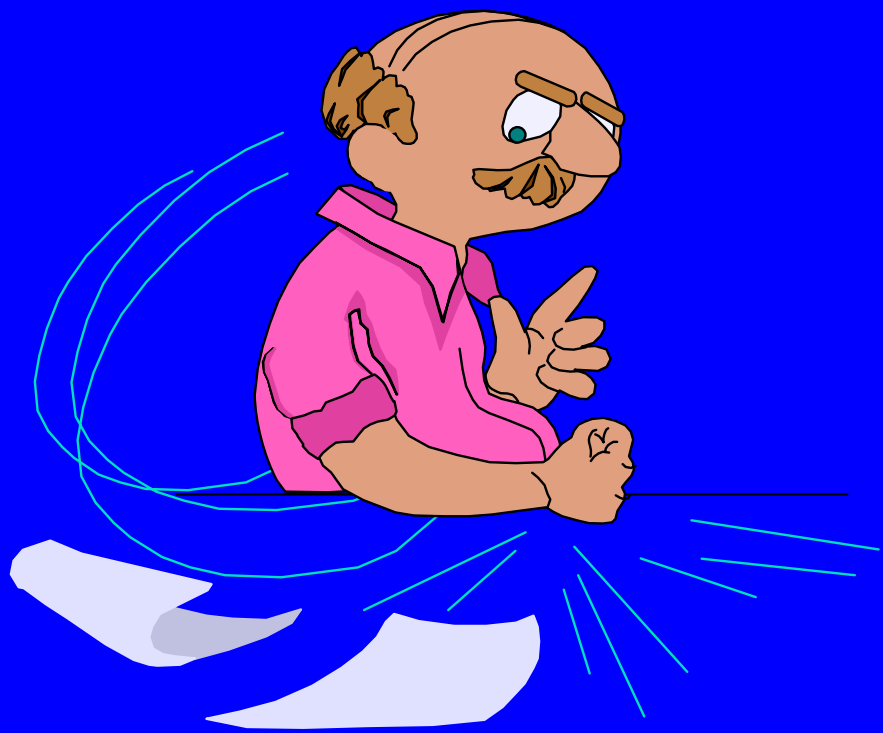
Case Presentation – cont.

- He was treated with increased foscarnet and ganciclovir
- The patient's neurologic status did not improve substantially
- He developed cavitating lesions in the lungs and new cavitating lesions in the brain
- The lung lesion grew *Aspergillus* and the patient died on 10/22/2001

Case Presentation – End

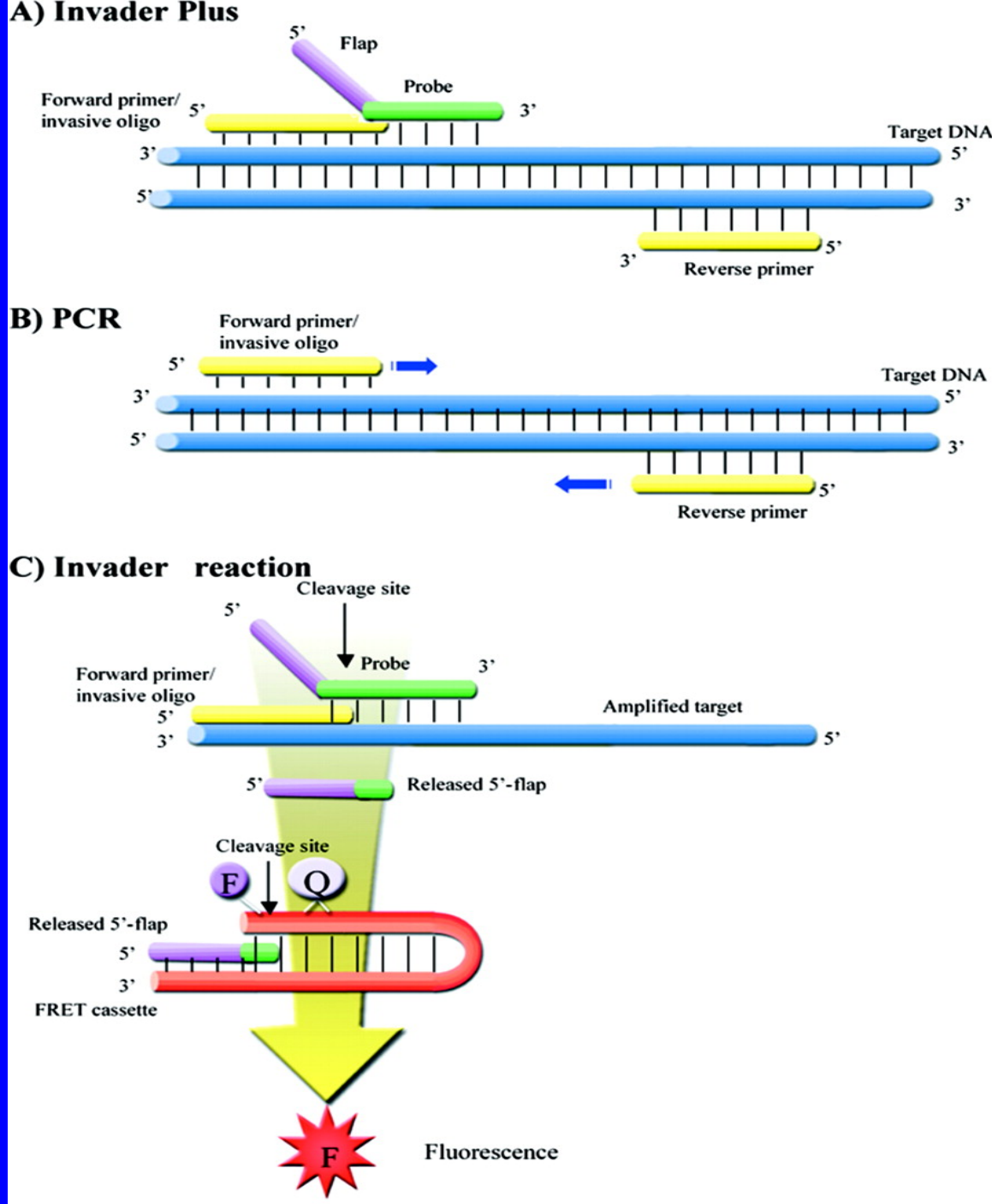
- On autopsy, significant CMV cytopathic change was shown in the ependyma
- An immunohistochemical staining was performed and the ependyma was strikingly positive for CMV
- There are numerous thrombotic infarcts throughout the cortex, perhaps related to *Aspergillus*
- Final diagnosis: (1) CMV encephalitis and (2) disseminated aspergillosis



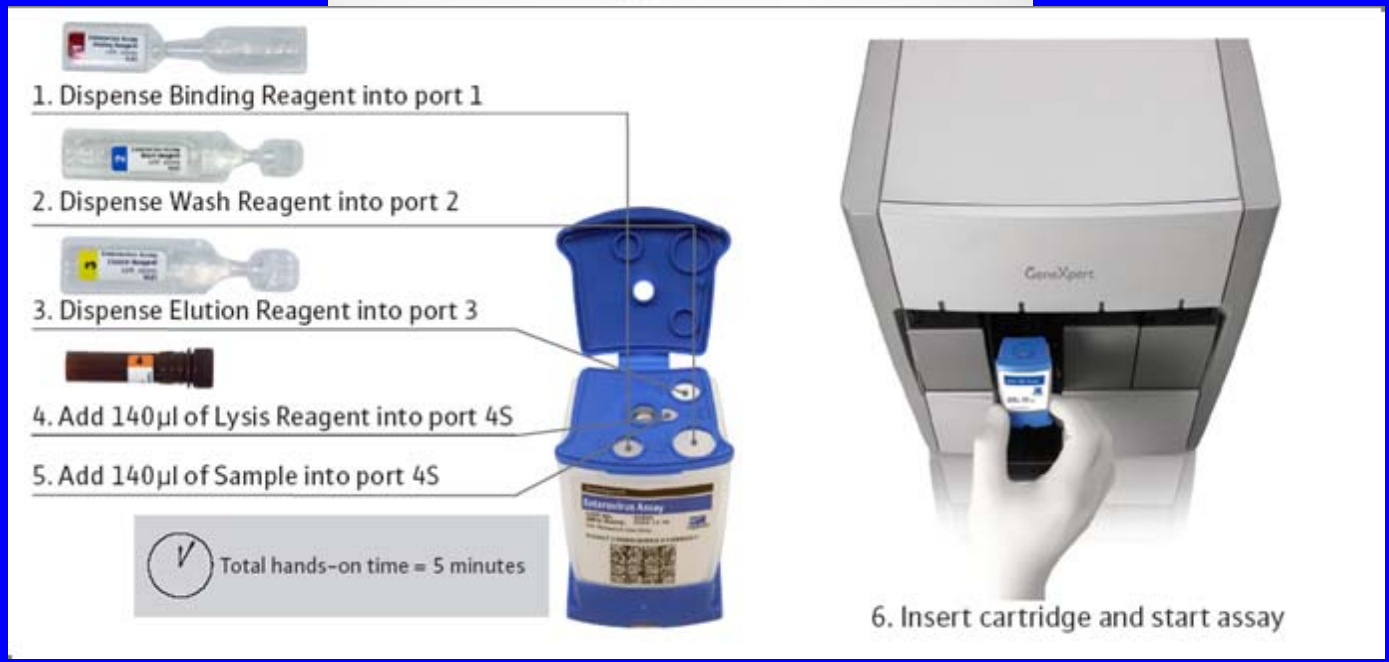
There are take home messages…

Increase Test Sensitivity by Incorporation of an Additional Signal Amplification in Detection Step



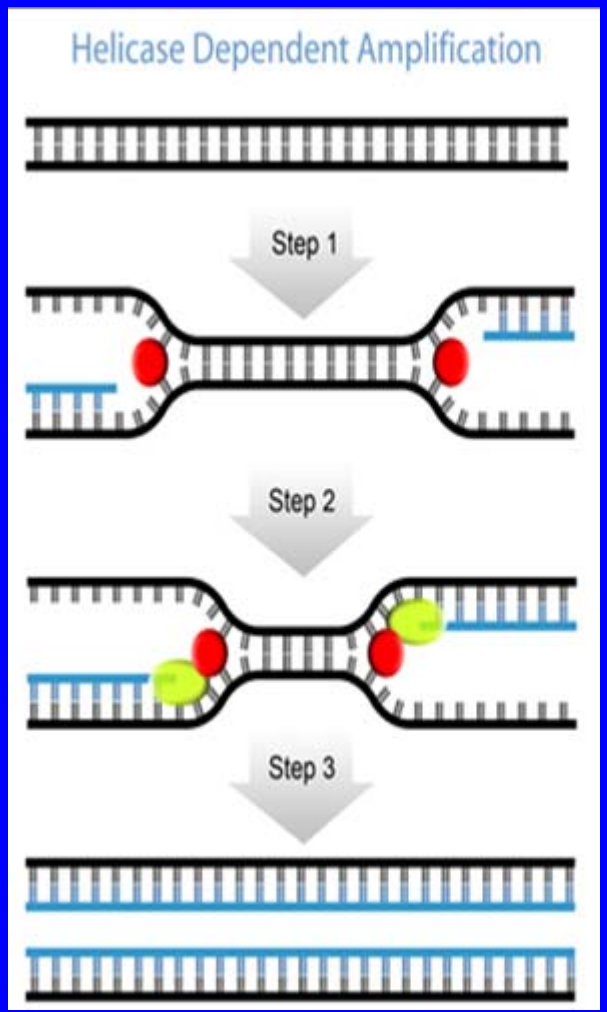
Allawi et al, *J. Clin. Microbiol.* **2006**, 44:3443

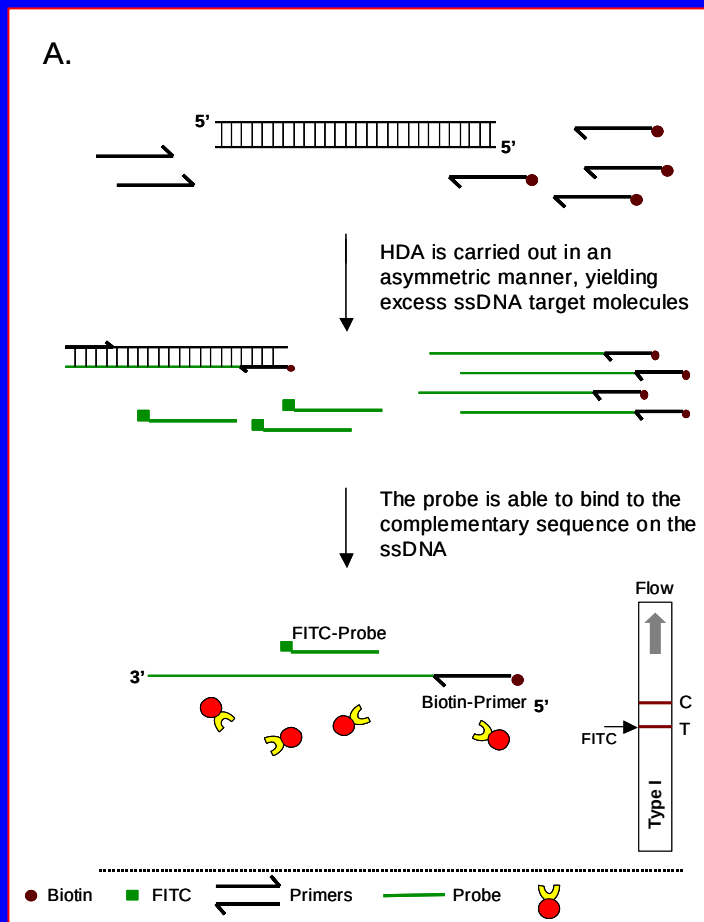
An Integrated Device Combines Nucleic Acid Extraction, PCR Amplification and Detection

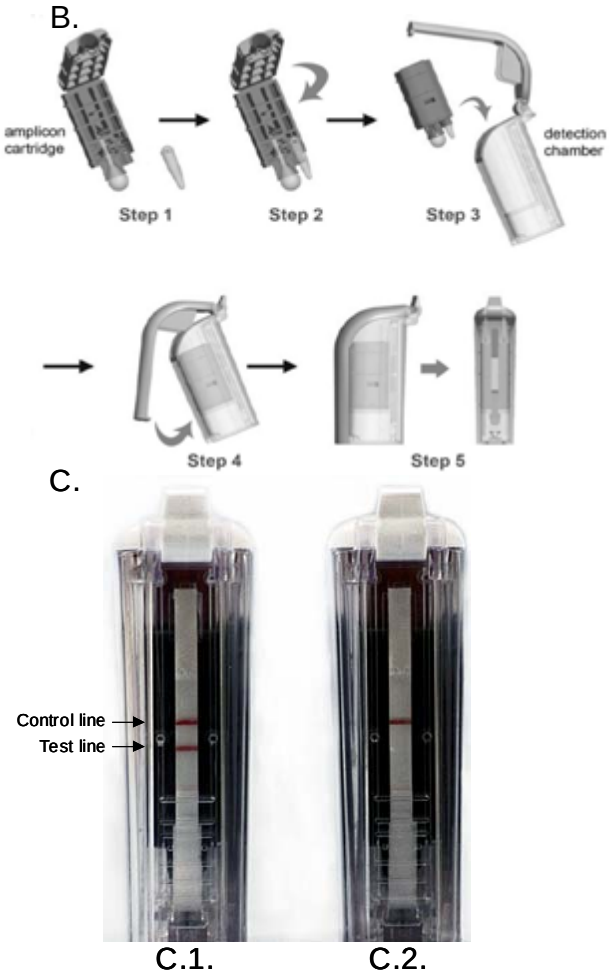


Kost et al, *J. Clin. Microbiol.* **2007**, 45:1081

Rapid and Simple Detection By Isothermal Amplification and Lateral Flow Detection

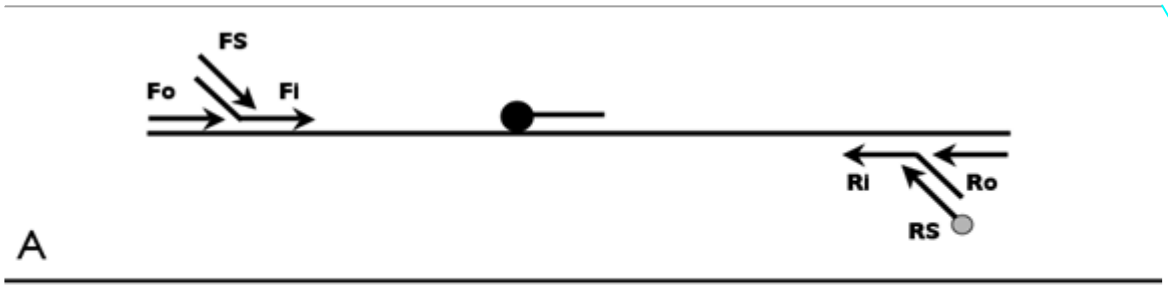






Goldmeyer et al, *J. Clin. Microbiol.* **2008**, 46:1534

Simultaneous Detection and Identification of A Panel of Pathogens Causing CNS Infections



A

i. Enrichment Stage ii. Tagging Stage iii. Amplification Stage

B

Han, *J. Clin. Microbiol.* **2006**, 44:4157

VANDERBILT 范德堡

“饭得饱”

University Ranking: 19 (2008)
School Ranking: 16 (2008)
Hospital Ranking: 15 (2008)

“Harvard in South”