



以分子診斷策略探索不明原因之 中樞神經系統感染

09/28/2013 杭州

許建清

台灣 奇美醫院



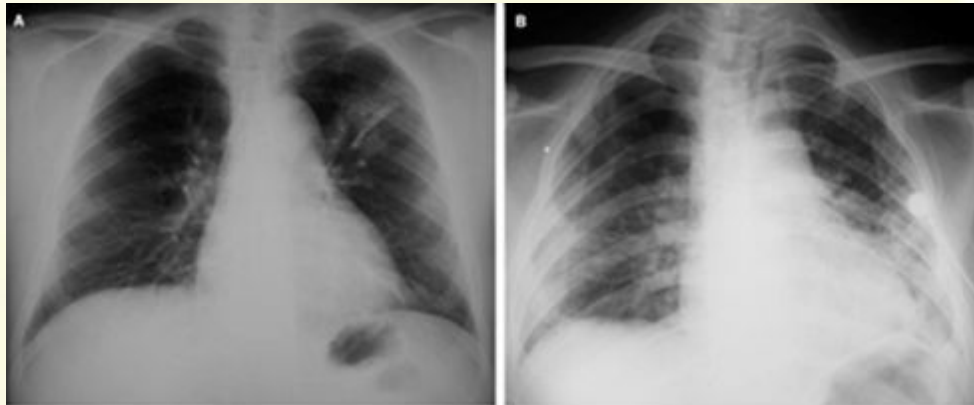
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關懷、專業、效率



急性感染症症候群

- 呼吸道徵候群 (Respiratory syndromes)





急性感染症症候群

- 呼吸道徵候群 (Respiratory syndromes)
- 腹瀉症候群 (Diarrheal syndromes)
- 中樞神經系統感染症候群 (CNS infections syndromes)
- 出血熱症候群 (Hemorrhagic fever syndromes)



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台灣傳染疾病監測系統

- 法定傳染病監測通報系統
- 學校傳染病監測通報系統
- 人口密集機構傳染病監測通報系統
- 院內感染監測通報系統
- 症狀監測通報系統



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傳染病統計資料查詢系統

Notifiable Infectious Diseases Statistics System



◎ 目前位置：首頁

請選擇疾病

請輸入疾病名稱

搜尋

疾病現況資料查詢

● 依傳染病防治法分類

第一類法定傳染病

第二類法定傳染病

第三類法定傳染病

第四類法定傳染病

第五類法定傳染病

● 依傳染途徑分類

腸道傳染病

急性病毒性肝炎

呼吸道傳染病

蟲媒傳染病

性傳染病

其他傳染病

● 急診傳染病監測統計

地區別就診比率監測趨勢圖

就診比率地理分佈

年齡別監測趨勢分析

手足口病與疱疹性咽峽炎就診比率比較圖

確定病例地理分佈

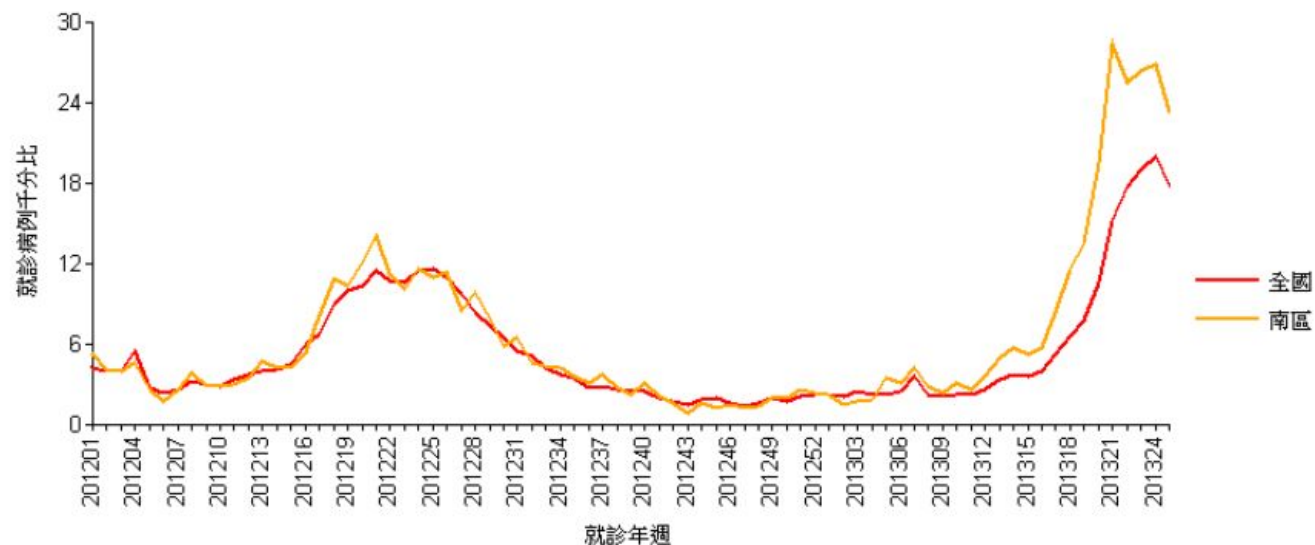
地區別就診比率監測趨勢圖

資料期間	2012 年 01 週 ~ 2013 年 25 週	疾病別	腸病毒 腸病毒	查詢
地區別	<input checked="" type="checkbox"/> 全國 <input type="checkbox"/> 台北區 <input type="checkbox"/> 北區 <input type="checkbox"/> 中區 <input checked="" type="checkbox"/> 南區 <input type="checkbox"/> 高屏區 <input type="checkbox"/> 東區 全選 / 取消全選			

趨勢圖

報表

2012年01週-2013年25週急診腸病毒地區別每週就診千分比監測趨勢圖



資料來源：疾病管制局 Taiwan CDC 2013/07/05

註一：資料更新時間為 2013/07/02 08:05:48，最新呈現資料為【2013/26】週，本週為【2013/27】週，每週二上午進行資料更新。

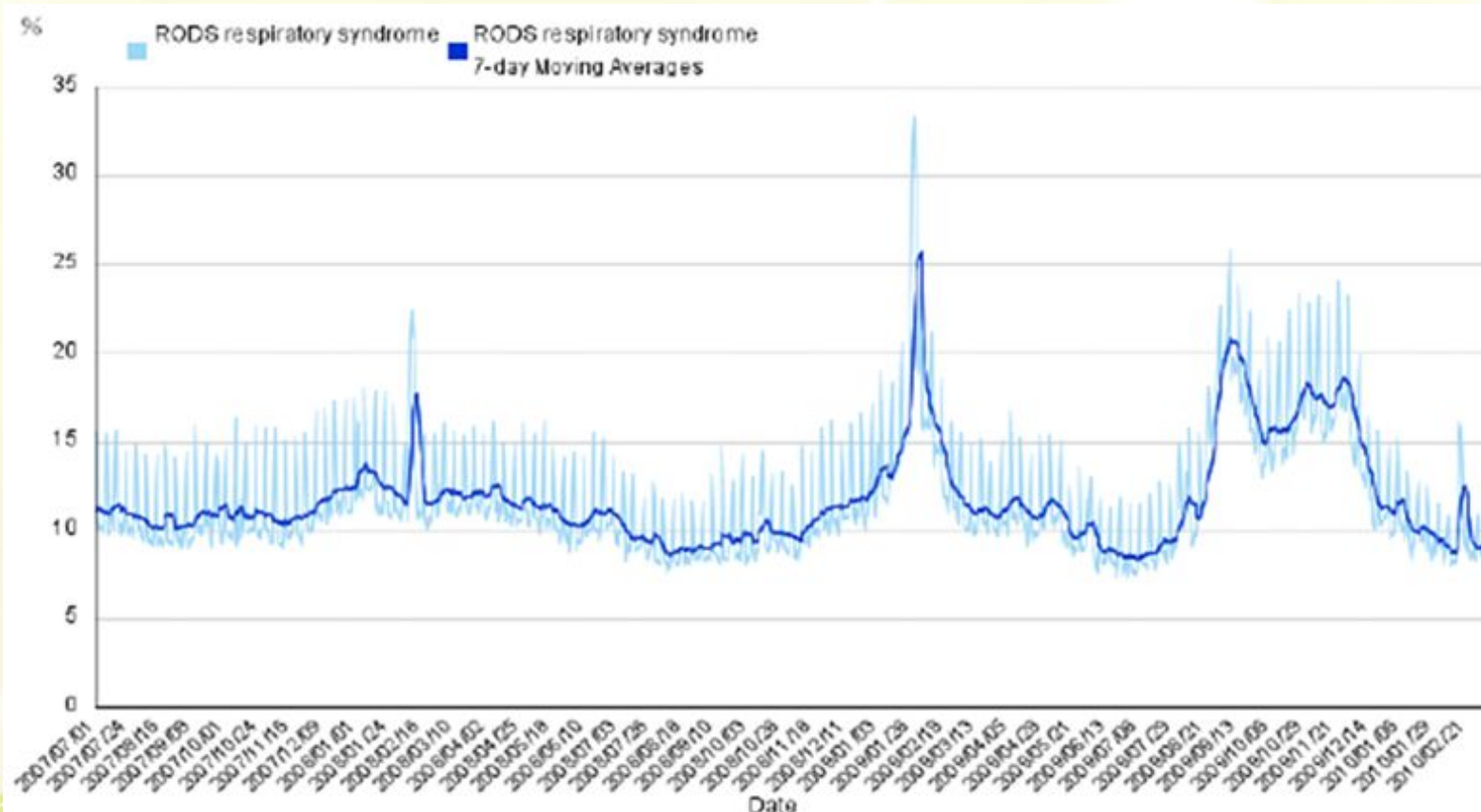
註二：本查詢結果為系統自動產生，數據隨時可能因未來修正而變動。

註三：分區依據為就診醫院所在地。



急性感染症症候群之監測

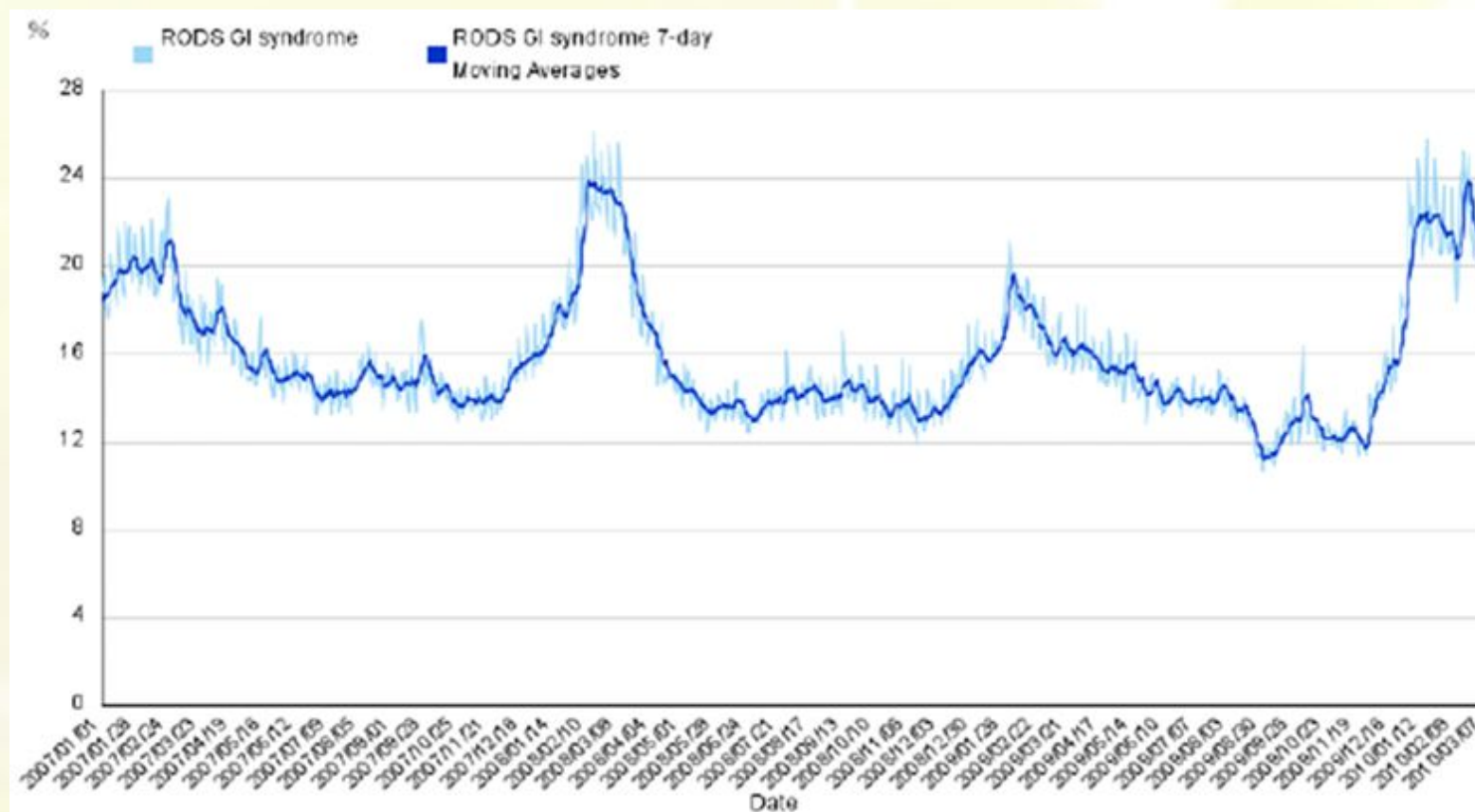
- 急診呼吸道症候群





急性感染症症候群之監測

- 急診腸胃道症候群



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Epidemiology of CNS infections in Taiwan, 1996-2008

- The incidence of meningitis: 12.6 cases/100,000 population
- The incidence of encephalitis: 3.3 cases/100,000 population



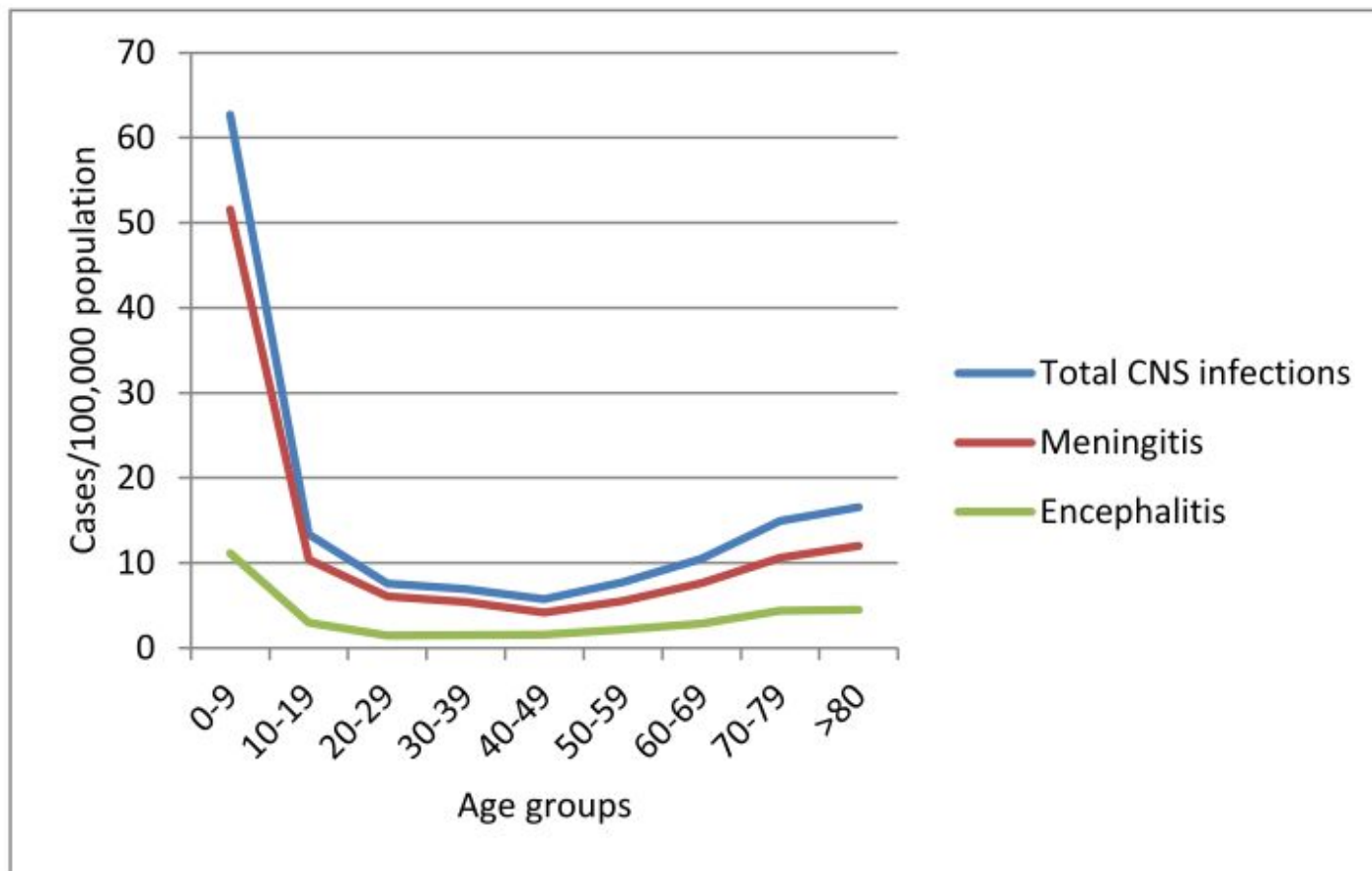
Meningitis cases

Encephalitis cases





The Incidence of Meningitis and Encephalitis in Taiwan

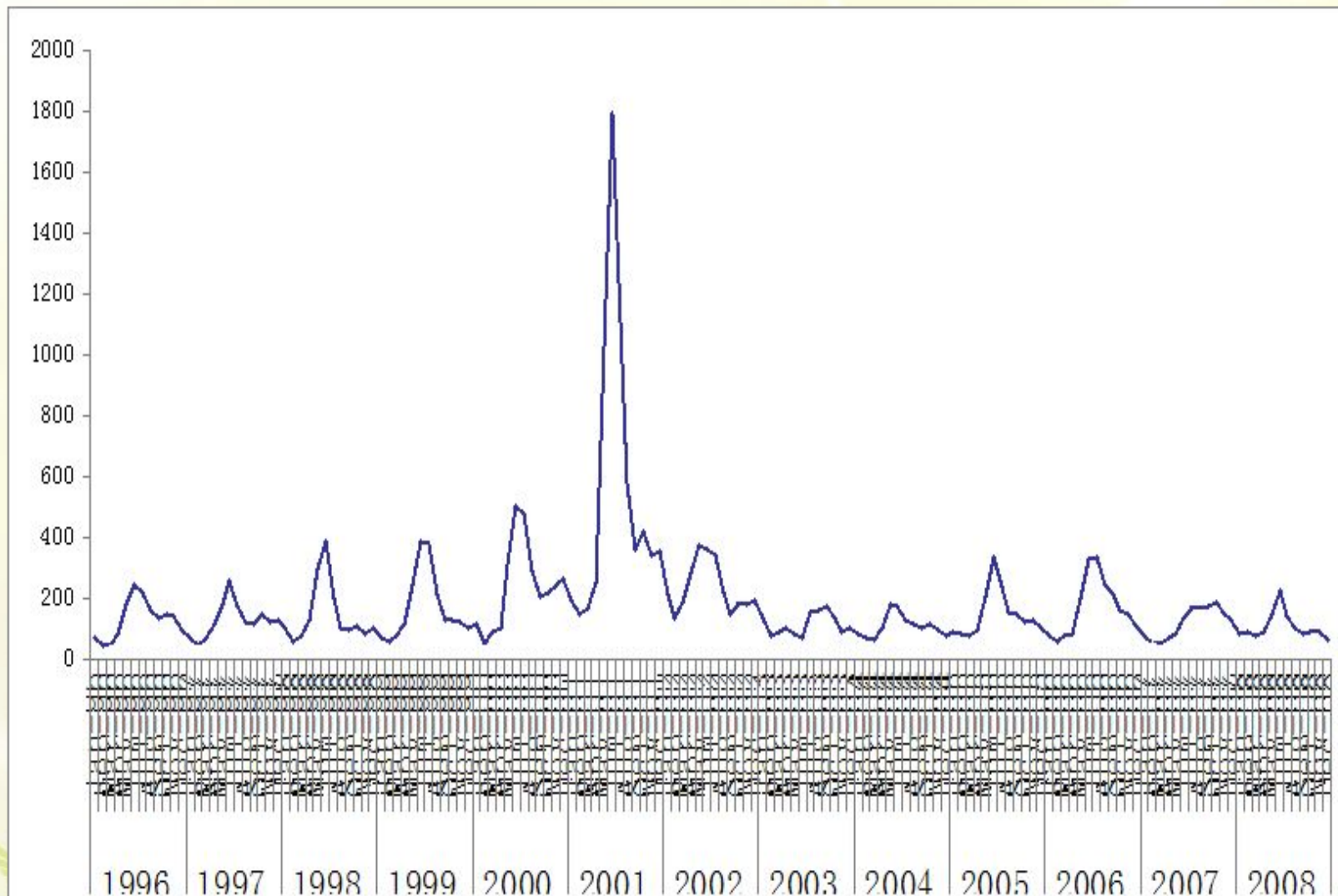


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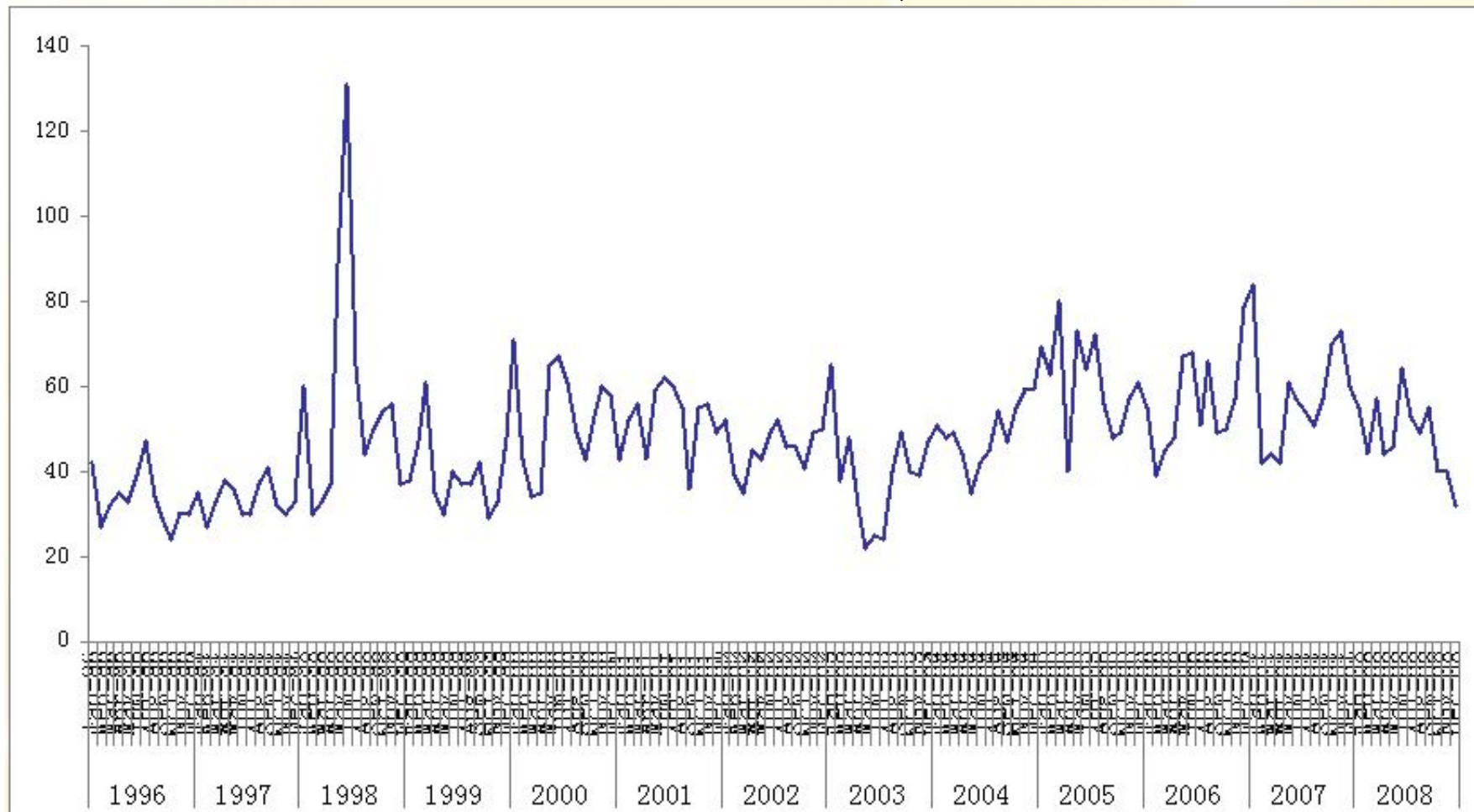


The Trend of Meningitis of Unknown Cause in Taiwan, 1996-2008





The Trend of Encephalitis of Unknown Cause in Taiwan, 1996-2008



↑
EV 71

↑
EV 71

↑
EV 71

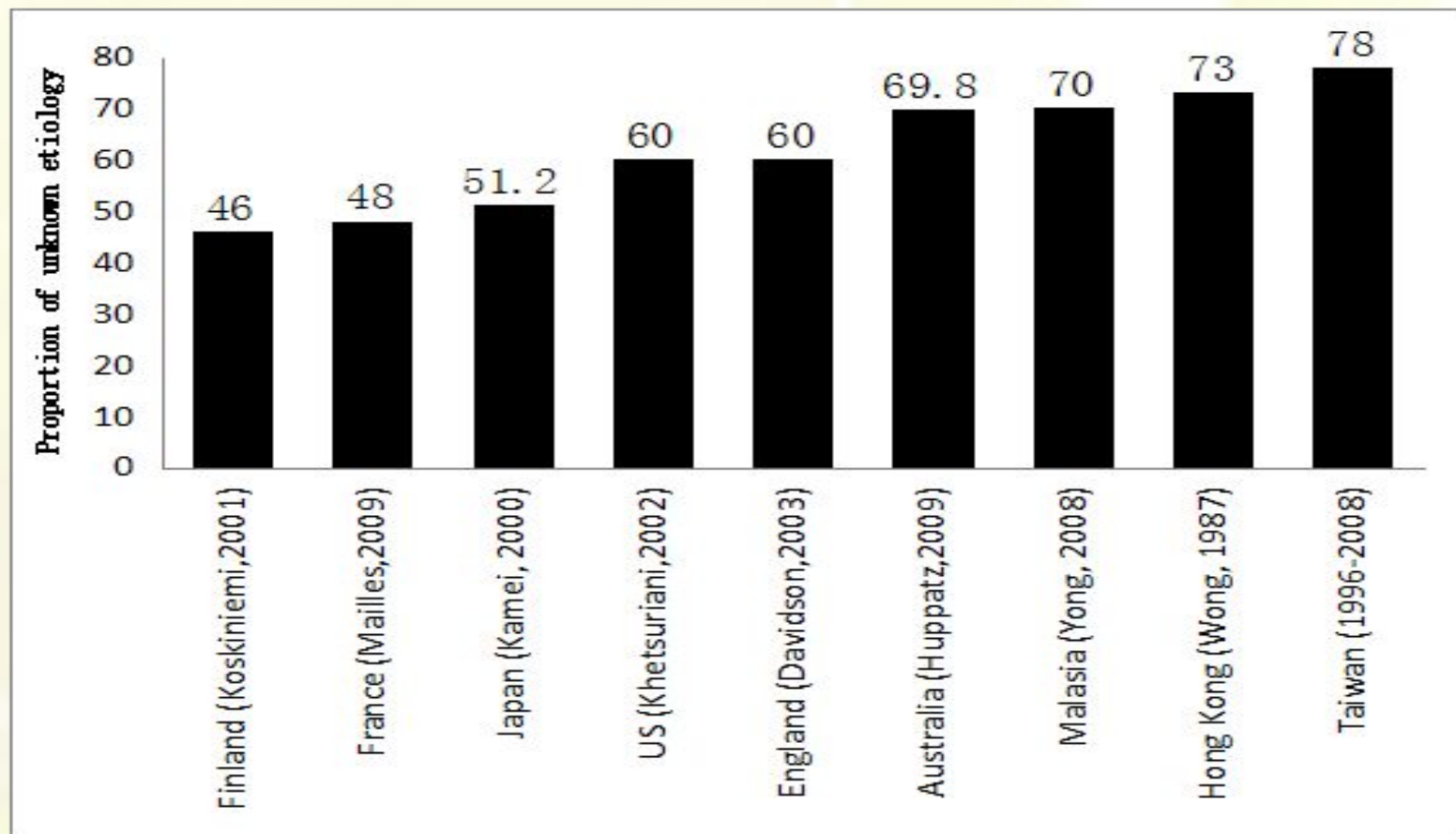
↑
Coxsackievirus B3
發燒、鼻塞、紅疹

↑
EV 71





The majority of encephalitis cases are unexplained across countries





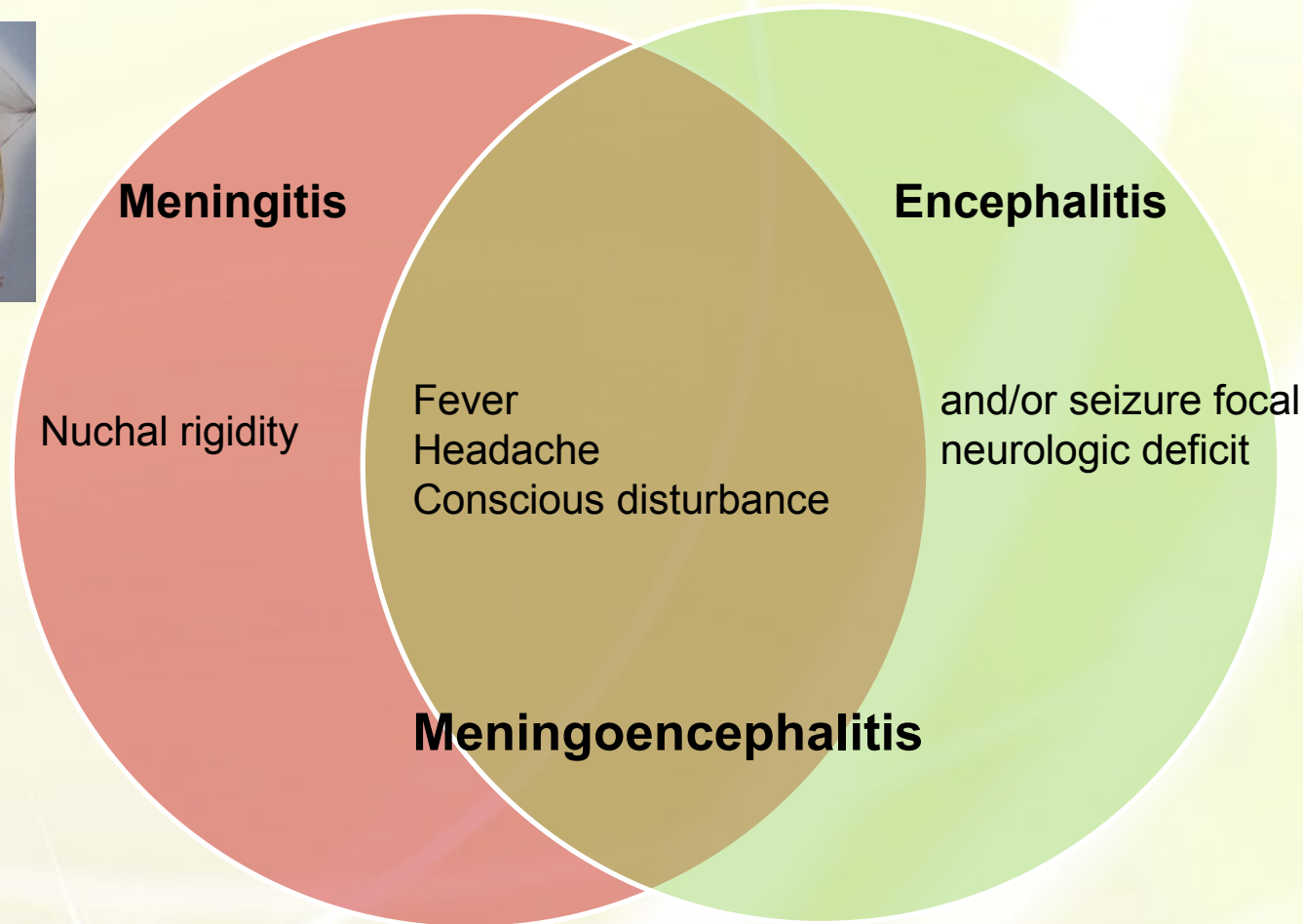
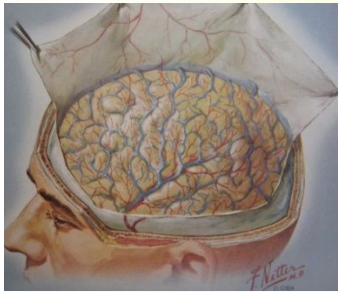
Pathogens Causing CNS Infections

Pathogens		
Viruses	Bacteria	Fungi
Enteroviruses	<i>Streptococcus pneumoniae</i>	Cryptococcus
Herpes simplex virus (HSV)	<i>Neisseria meningitidis</i>	Aspergillosis
Varicella-zoster virus (VZV)	<i>Hemophilus influenza</i>	Candidiasis
Epstein-Barr virus (EBV)	<i>Listeria monocytogenes</i>	Coccidiomycosis
Cytomegalovirus (CMV)	Chlamydia spp.	Histoplasmosis
Human Herpes virus 6 and 7 (HHV 6/7)	<i>Mycobacterium tuberculosis</i>	North American blastomycosis
Mumps virus	<i>Mycoplasma pneumonia</i>	Parasites
Measles virus	<i>Borrelia burgdorferi</i>	<i>Toxoplasma gondii</i>
Rubella	Leptospirosis	Cerebral malaria
Adenovirus	Legionella	<i>Echinococcus granulosus</i>
West Nile virus	<i>Tropheryma whippelli</i>	Schistosomiasis
Nipah virus	<i>Nocardia actinomyces</i>	Human African trypanosomiasis
Rabies virus	<i>Treponema pallidum</i>	
Lymphocytic choriomeningitis virus (LCMV)	<i>Salmonella typhi</i>	
Tick-borne encephalitis virus	Rickettsia	
Japanese encephalitis virus	<i>Rickettsia rickettsia</i>	
St. Louis encephalitis virus	<i>Rickettsia typhi</i>	
Murray Valley encephalitis virus	<i>Rickettsia prowazeki</i>	
Human immunodeficiency virus (HIV)	<i>Coxiella burnetti</i>	
Influenza virus A and B	Ehrlichiosis	





Clinical Presentations of CNS Infections Are Non-specific



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Laboratory Assisted Syndromic Surveillance

- Diagnostic Strategies
 - Culture
 - Microscopy
 - Serology
 - ELISA, immunofluorescence, neutralization, Western blot
 - Molecular (nucleic acid)
 - PCR, DNA sequencing, nucleic acid arrays





Center for Infection and Immunity Columbia University



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Microbes Hunter in 21st Century



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Molecular Investigations of CNS Infections of Unknown Cause

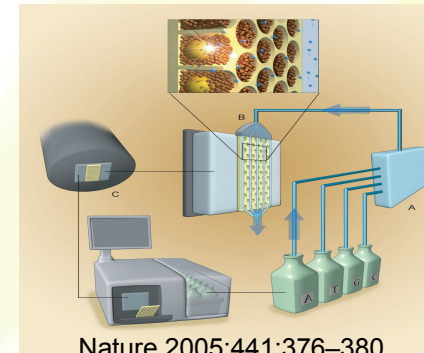
- A staged sequence-based approach



MassTag PCR



Greenchips DNA microarray



High-throughput sequencing



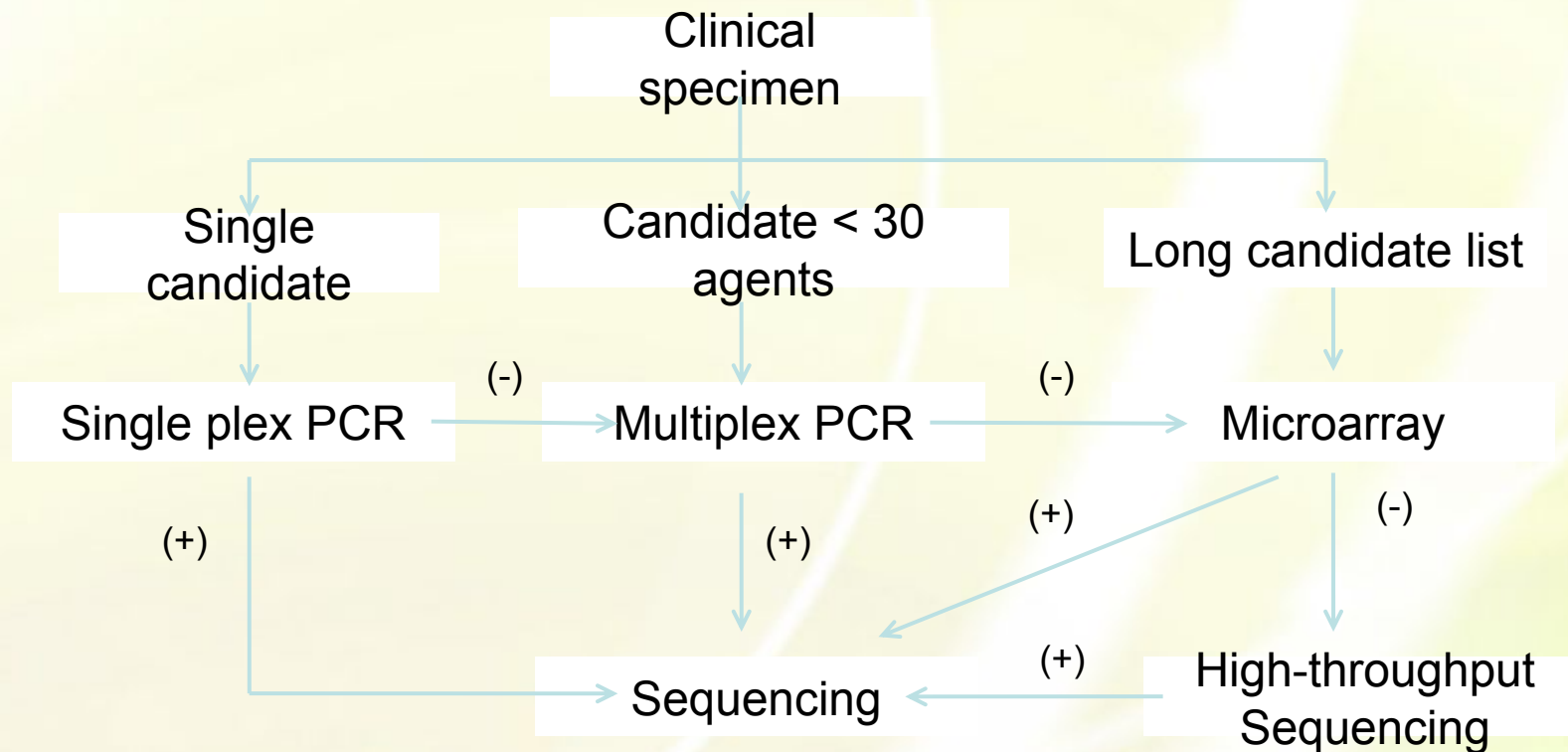
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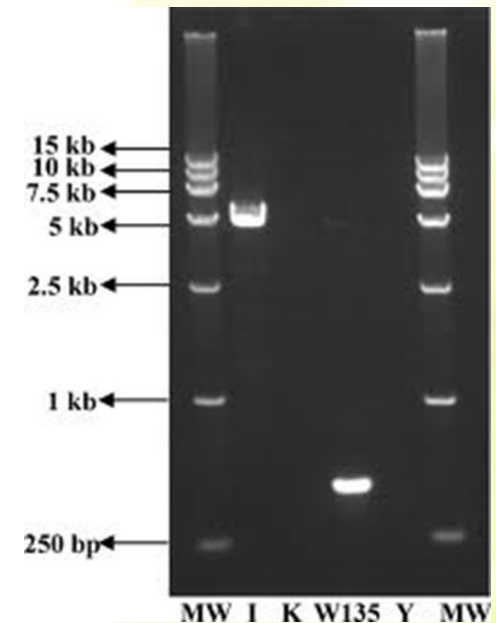
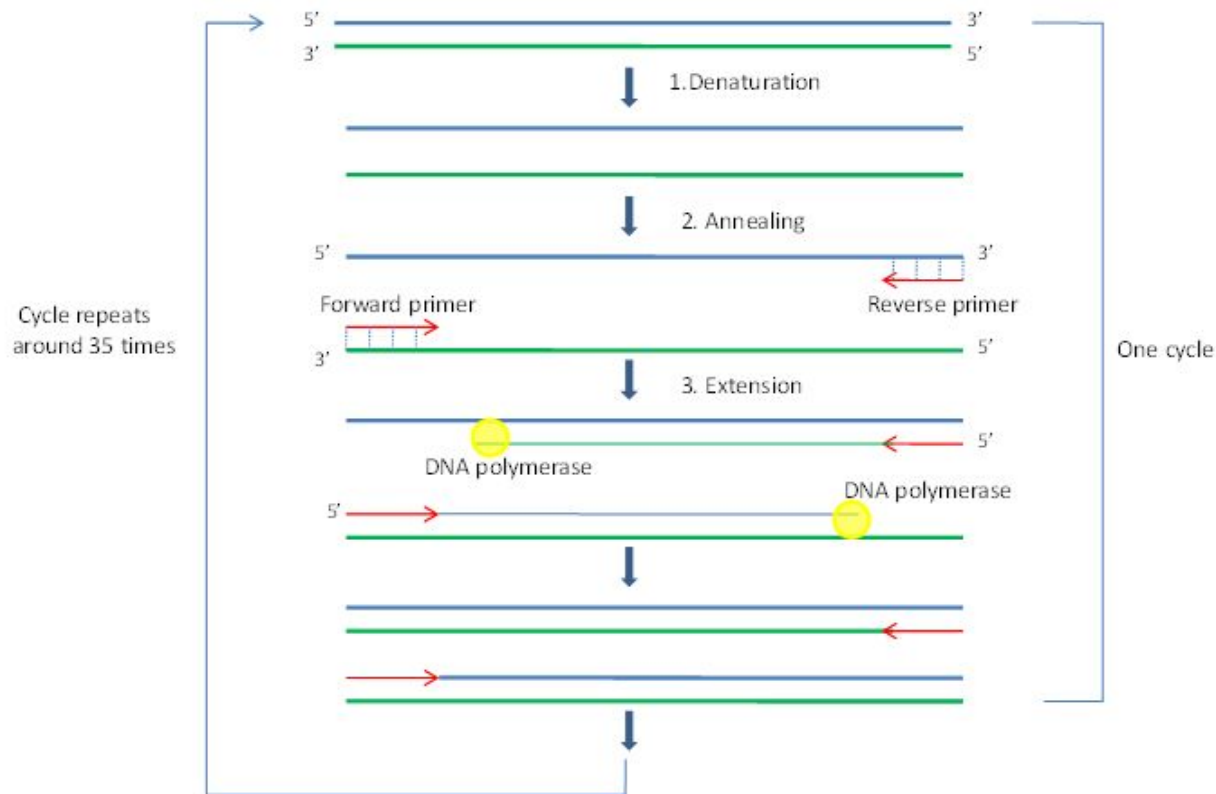


A Staged Molecular Approach for Diagnosis of CNS Infections



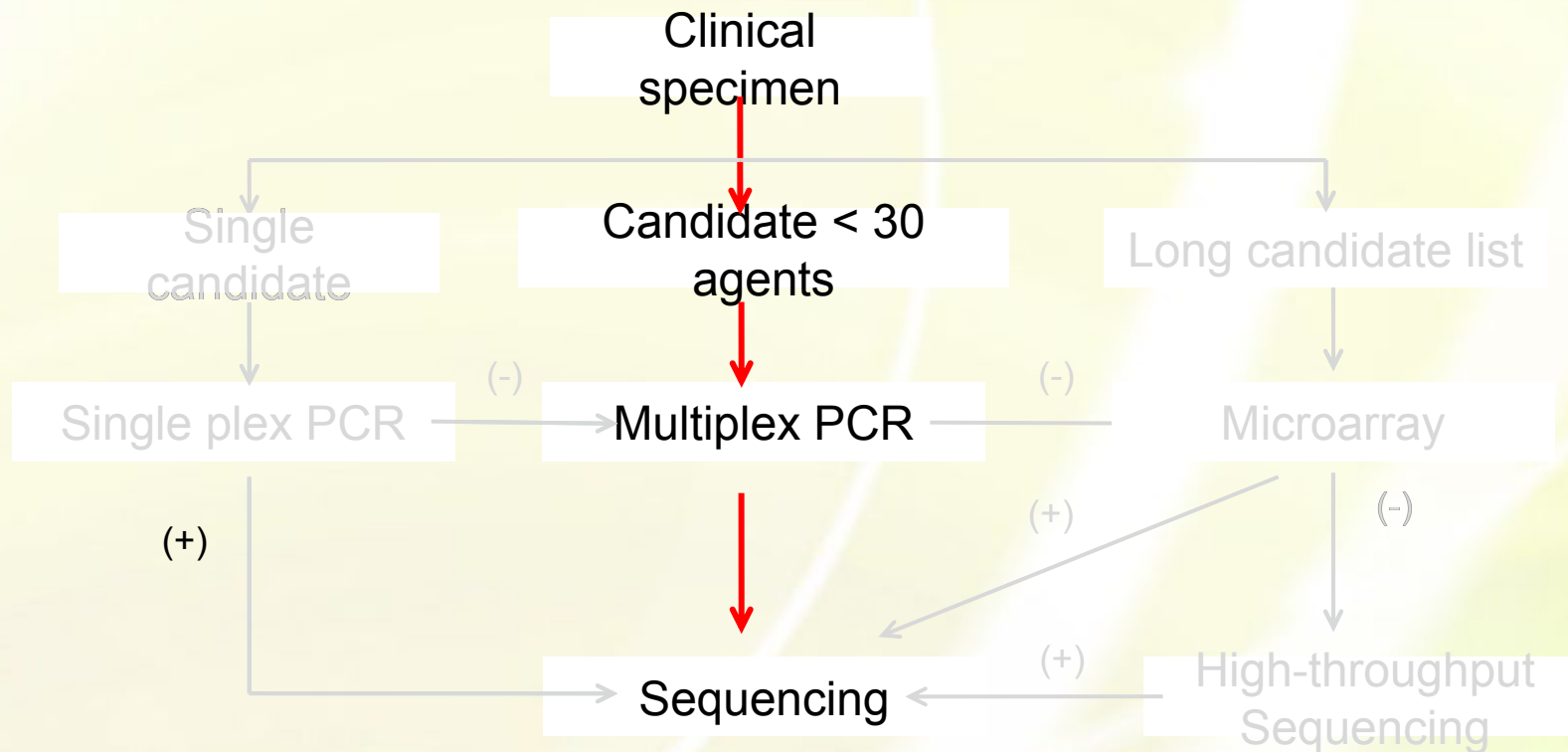


Polymerase Chain Reaction





A Staged Molecular Approach for Diagnosis of CNS Infections

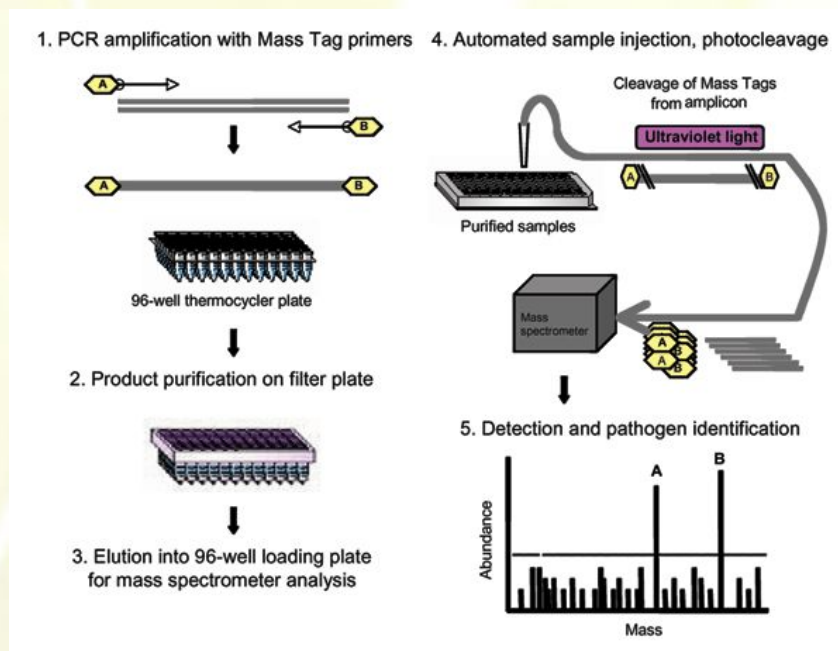
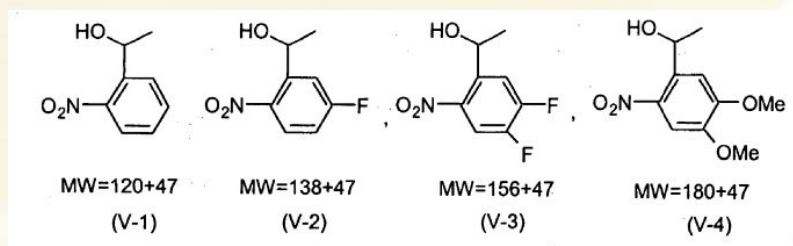
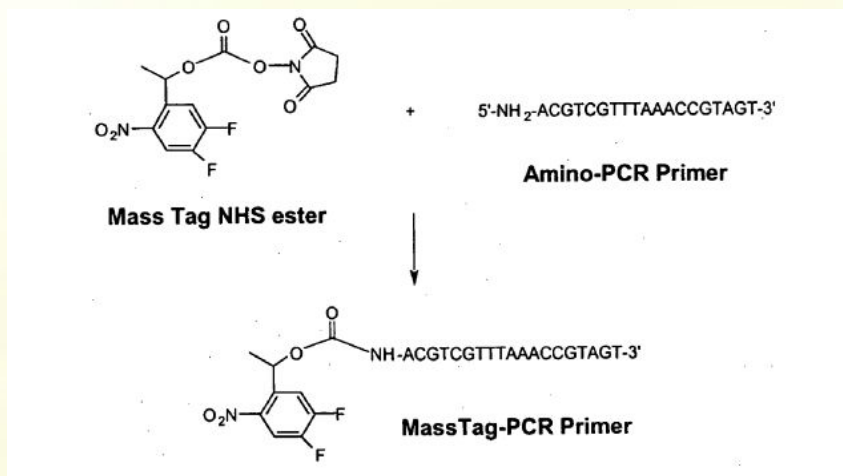


MassTag PCR Panel for Encephalitis/Meningitis

RNA pathogens (11 plex)	Mass code	DNA pathogens (15 plex)	Mass code
Japanese Encephalitis virus	582/698	Adenovirus	503/630
Enterovirus	702/495	Cytomegalovirus	626/610
Nipah/Hendra virus	519/566	Epstein Barr virus	570/463
Parechovirus	606/357	Varicella Zoster virus	471/515
La Crosse virus	590/686	Herpes Simplex virus 1	666/706
Lymphocytic Choriomeningitis	614/654	Herpes Simplex virus 2	527/642
St. Louis Encephalitis virus	658/423	Hemophilus influenzae	734/726
Mumps	586/704	Streptococcus pneumoniae	714/694
West Nile virus	539/499	Neisseria meningitidis	439/730
Rabies virus	558/475	Cryptococcus neoformans	638/650
Influenza A virus	690/618	Leptospira interrogans	634/383
		Mycobacterium tuberculosis	395/475
		Candida albicans	602/670
		Toxoplasma gondii	548/562
		Human Herpes Virus 6	718/357



Multiplex Mass-Tag PCR



Emerg Infect Dis. 2005 Feb;11(2):310-3.

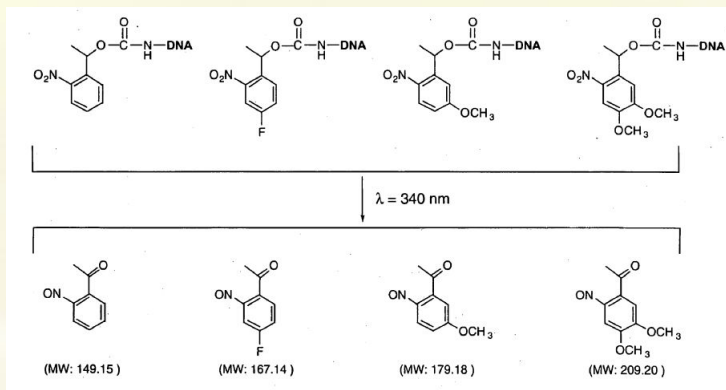


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Tag Read by Mass Spectrometry



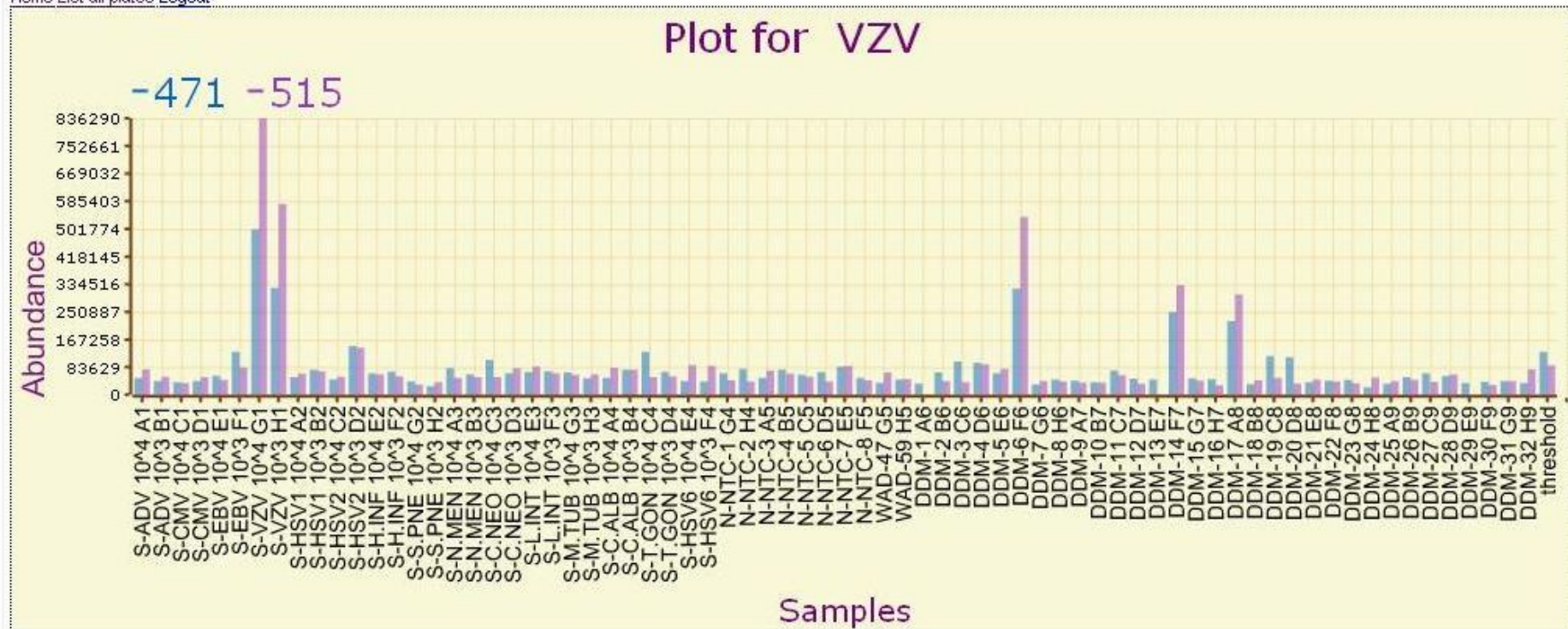
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Signal Plots by Agent for Plate #90597

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[ADV](#) || [C-ALB](#) || [C-NEO](#) || [CMV](#) || [EBV](#) || [H-INF](#) || [HSV1](#) || [HSV2](#) || [L-INT](#) || [M-TUB](#) || [N-MEN](#) || [S-PNE](#) || [T-GON](#) || [VZV](#)



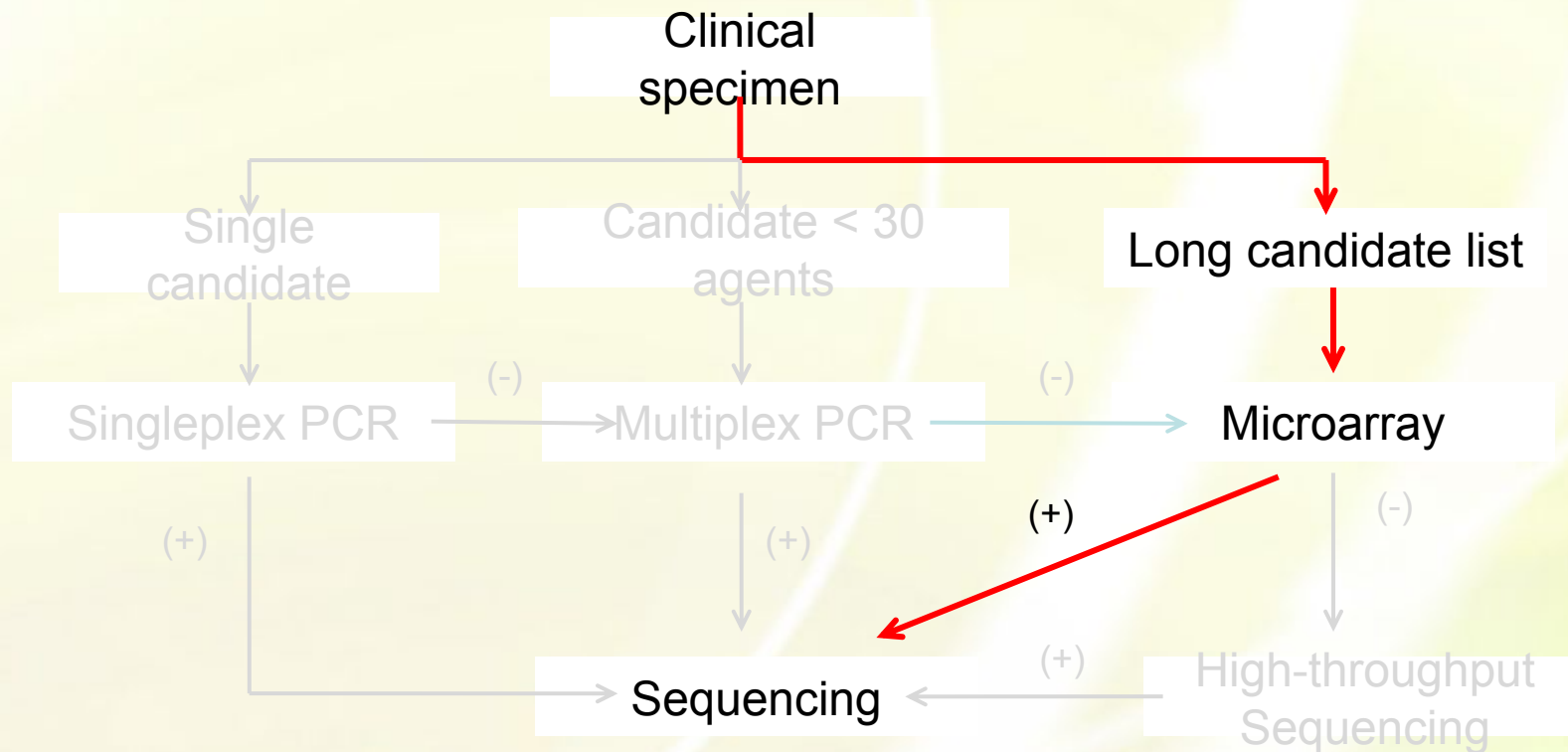
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A Staged Molecular Approach for Diagnosis of CNS Infections



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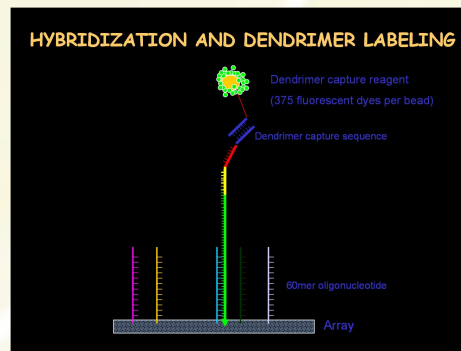
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GreeneChips PanViral Microarray

- Over 66,000 genetic probes on a glass slide
 - A comprehensive diagnostic tool
 - Screen for all known viruses



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Universal Amplification: cDNA Synthesis



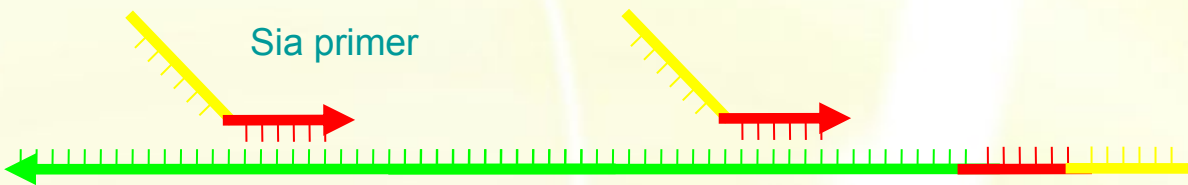
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Random PCR Amplification

Low stringency cycles
(8 cycles at 25 C)

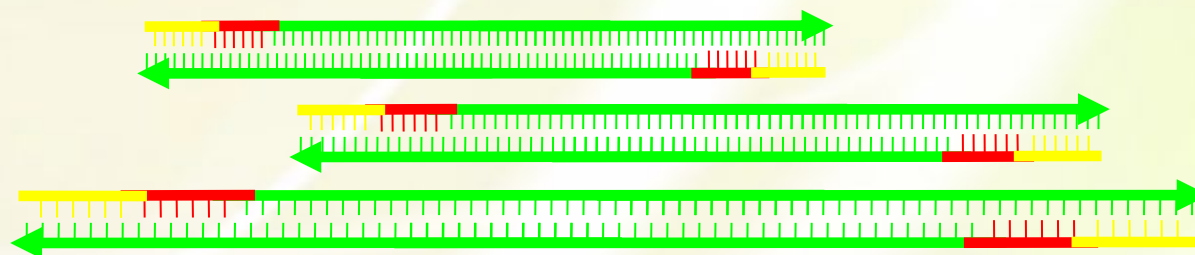
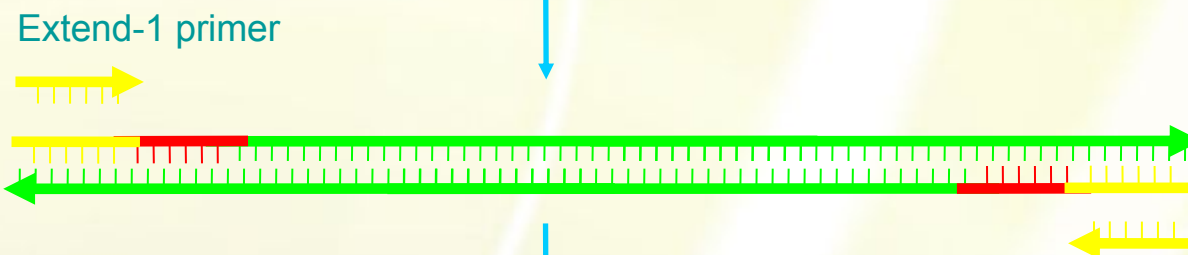
Sia primer



High stringency cycles
(40 cycles at 55 C)

Extend-1 primer

Extend-1 primer



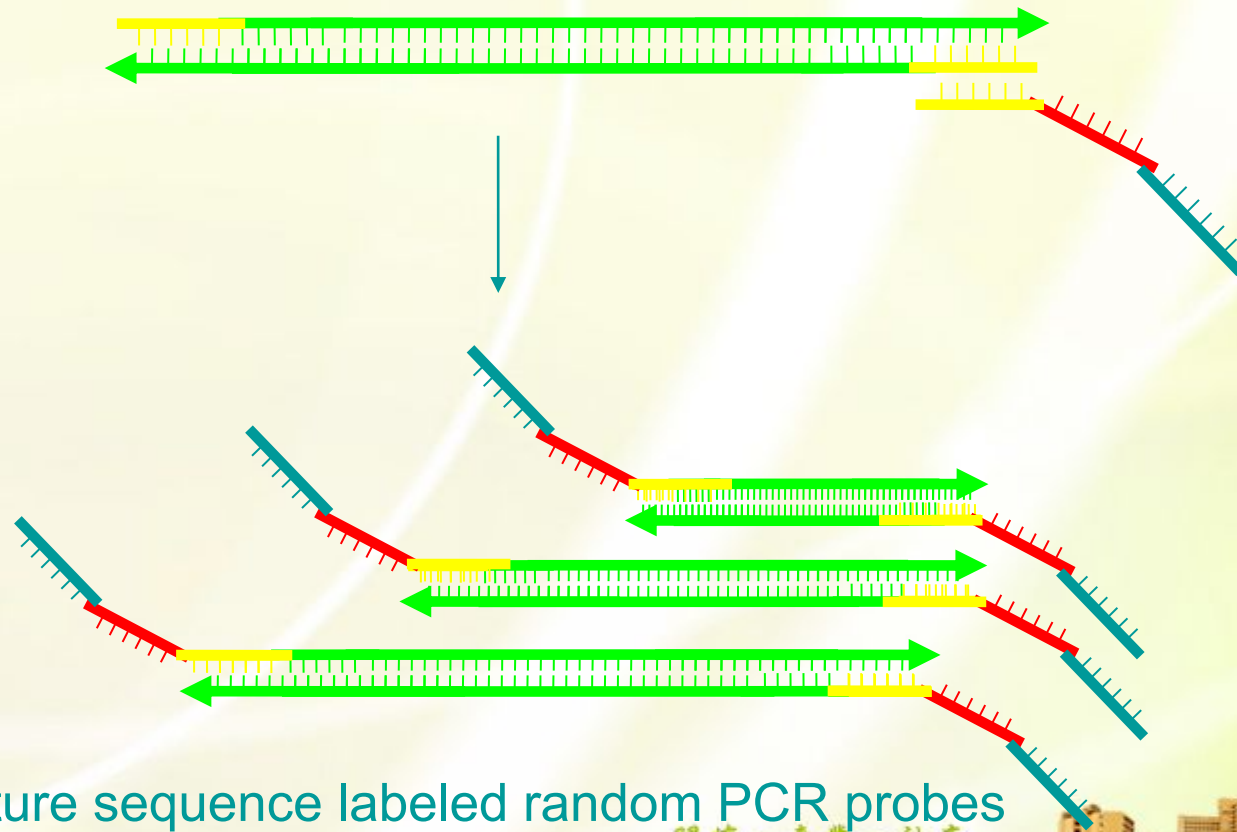
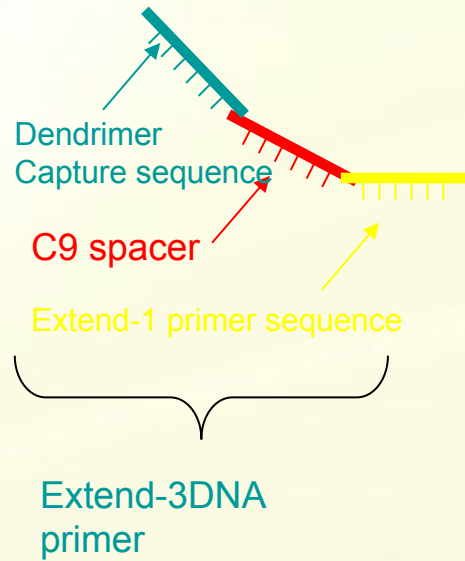
Unlabeled random PCR products



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Labeling PCR

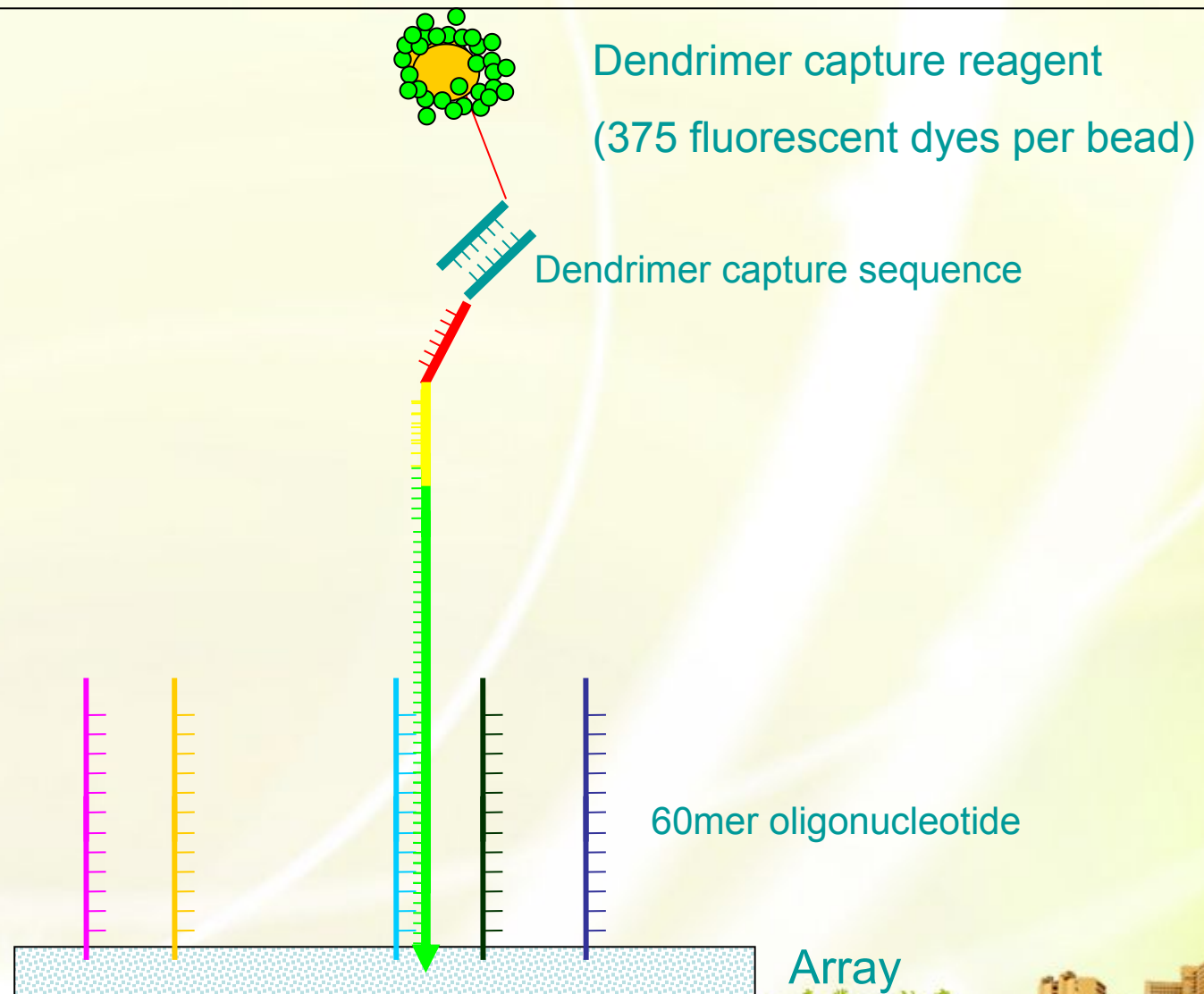


Capture sequence labeled random PCR probes

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Hybridization and Dendrimer Labeling





GreenePortal

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Wikis - Collaborate on projects

- [Pathogen Discovery](#)
- [Mass Tag](#)
- [Filovirus Immunosuppression](#)



GreeneLAMP - Software for GreeneChip analysis

- [GreeneLAMP Report Browser](#)
- [... Reports in labuser Account](#)
- [Upload array data for analysis](#)
- [GreeneLAMP Help Wiki](#)
- [GreeneLAMP specifications document \(Word\)](#)
- [Hybridization submission form \(updated 11/02/07\)](#)



High Throughput Sequencing - Pathogen Discovery using 454 Sequencing

- [High Throughput Sequencing Results](#)
- [High Throughput Sequencing Results \(beta\)](#)
- [Upload sequence data for analysis](#)
- [Explanation of HTS Data Analysis and Report Format \(Word\)](#)
- [CD-HIT - Huang & Madan \(PDF\)](#)
- [CAP3 - Li et al. \(TXT\)](#)



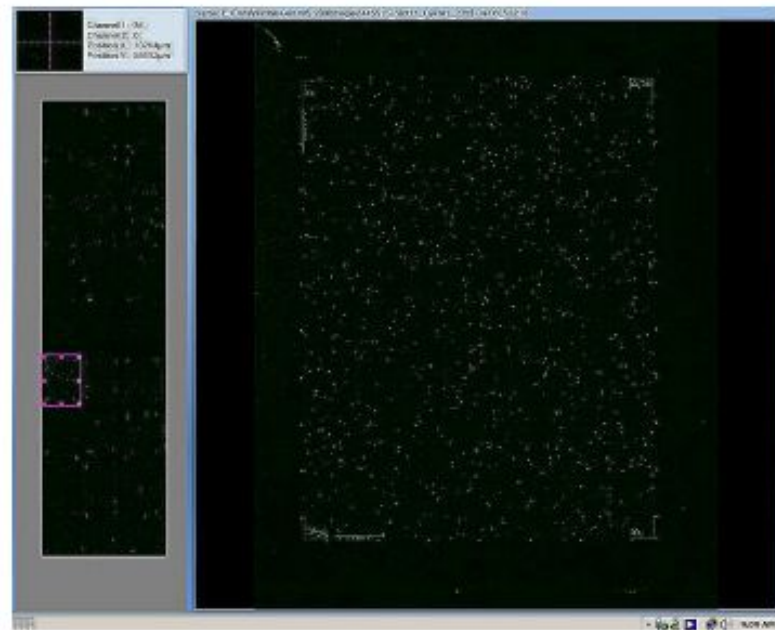
GreeneTools - Tools for Sequence Analysis

- [SCPrimer](#)
- [Classification of PCR Products by Needleman Wunsch Alignment](#)
- [Local BLAST \(on Tako\)](#)
- [Sequence Mask and BLAST \(Instructions\)](#)
- [Tools from Dekka](#)
- [BlastGrabber](#)
- [Perl scripts](#)
- [Greene_chip Analyzer 2005](#)



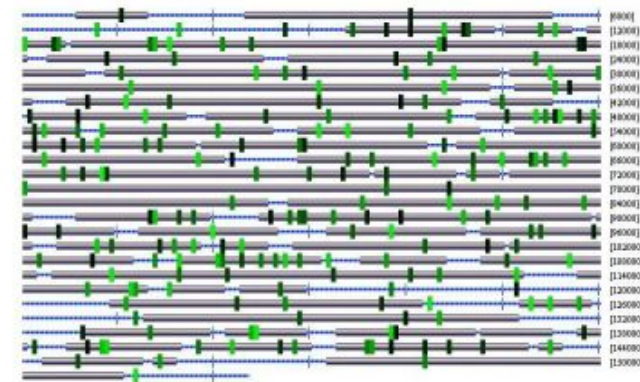


Scan and Analysis of Microarray



Accession	taxid	Description	P_value	q	Probe_count/Total
NC_006560	10317	Cercopithecine herpesvirus 2, complete genome.	3.74572978980651e-11		192/1294
NC_004812	10325	Cercopithecine herpesvirus 1, complete genome.	6.84626259287499e-10		190/1315
NC_007653	340907	Cercopithecine herpesvirus 16, complete genome.	5.20677520195196e-08		191/1289
NC_006151	10345	Suid herpesvirus 1, complete genome.	8.73166797798223e-07		191/996
NC_001806	10298	Human herpesvirus 1, complete genome.	9.73997263029271e-06		213/1614
NC_001798	10310	Human herpesvirus 2, complete genome.	1.29079422744542e-05		221/1577
NC_001847	10320	Bovine herpesvirus 1, complete genome.	3.7091340240379e-05		182/1029
NC_002794	10397	Tupaia herpesvirus, complete genome.	0.0010849560689833		149/707

Probe plot for : NC_001806 Human herpesvirus 1, complete genome.

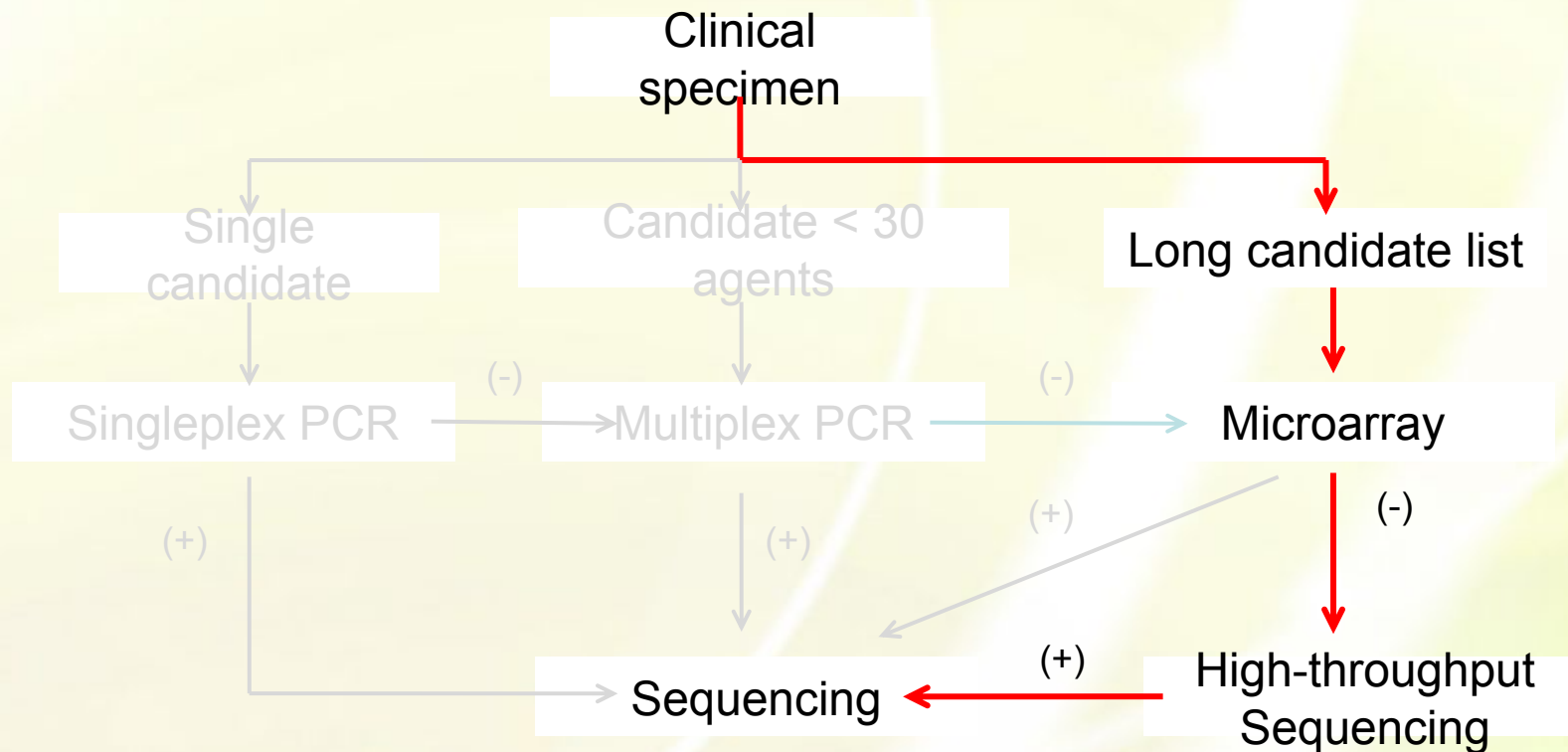


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A Staged Molecular Approach for Diagnosis of CNS Infections



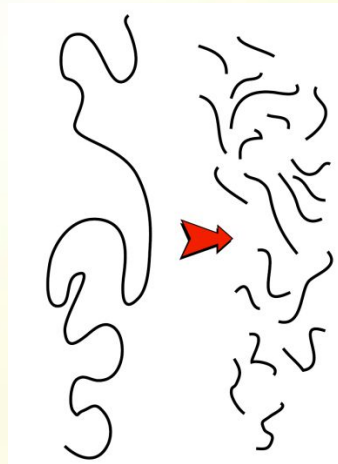
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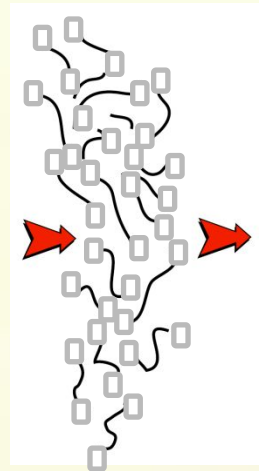


Pyrosequencing with 454 platform

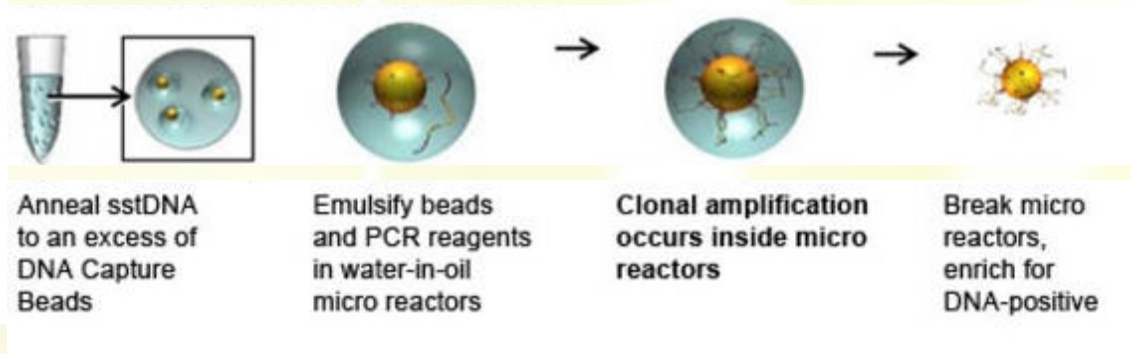


Transcriptome
cDNA

Shearin
g



Adaptor
Ligation



Emulsion PCR



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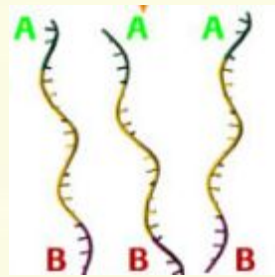
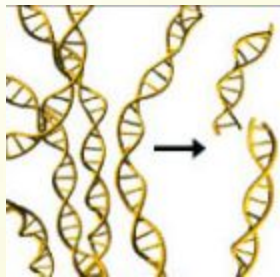
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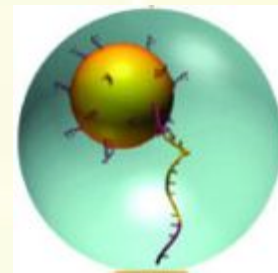


High Throughput Pyrosequencing

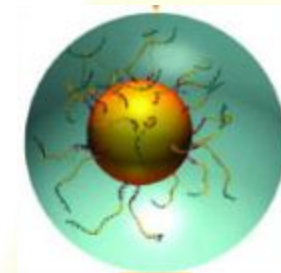
Library preparation



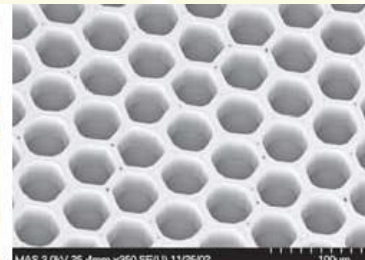
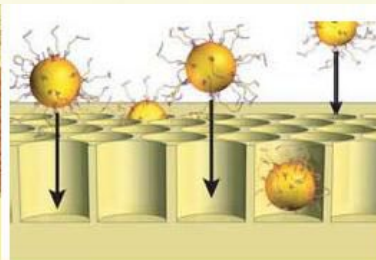
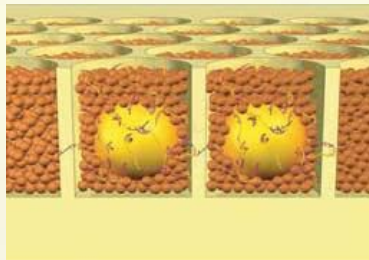
One fragment
=one bead



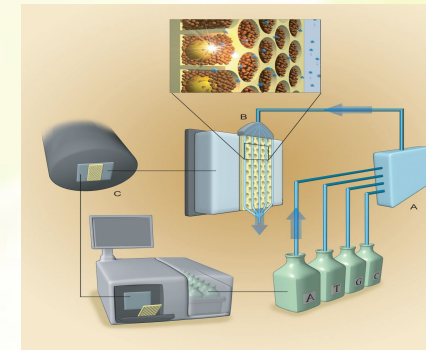
Emulsion PCR
amplification



One bead=one read



Fused optical fibers with etched wells
Dimensions: 70X75 mm
Number of wells: 1.6 million
Well diameter: 40 microns



Source: 454 Life Sciences

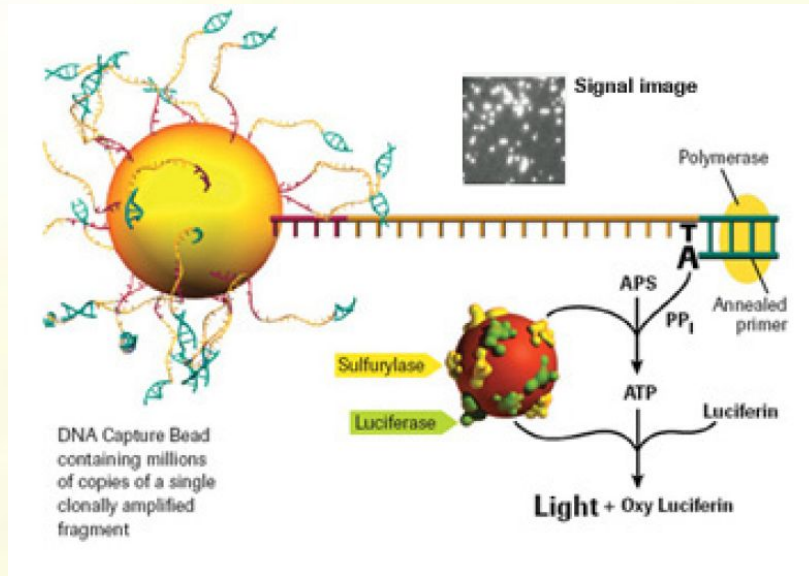


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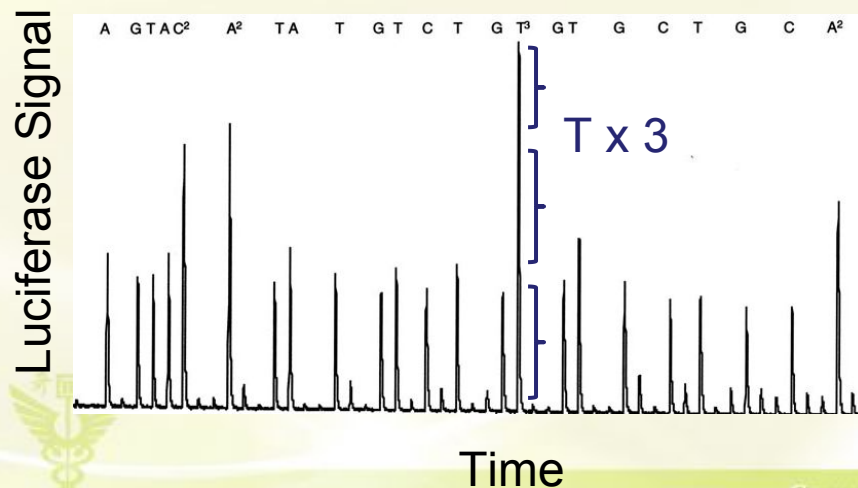
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Sequencing on Synthesis

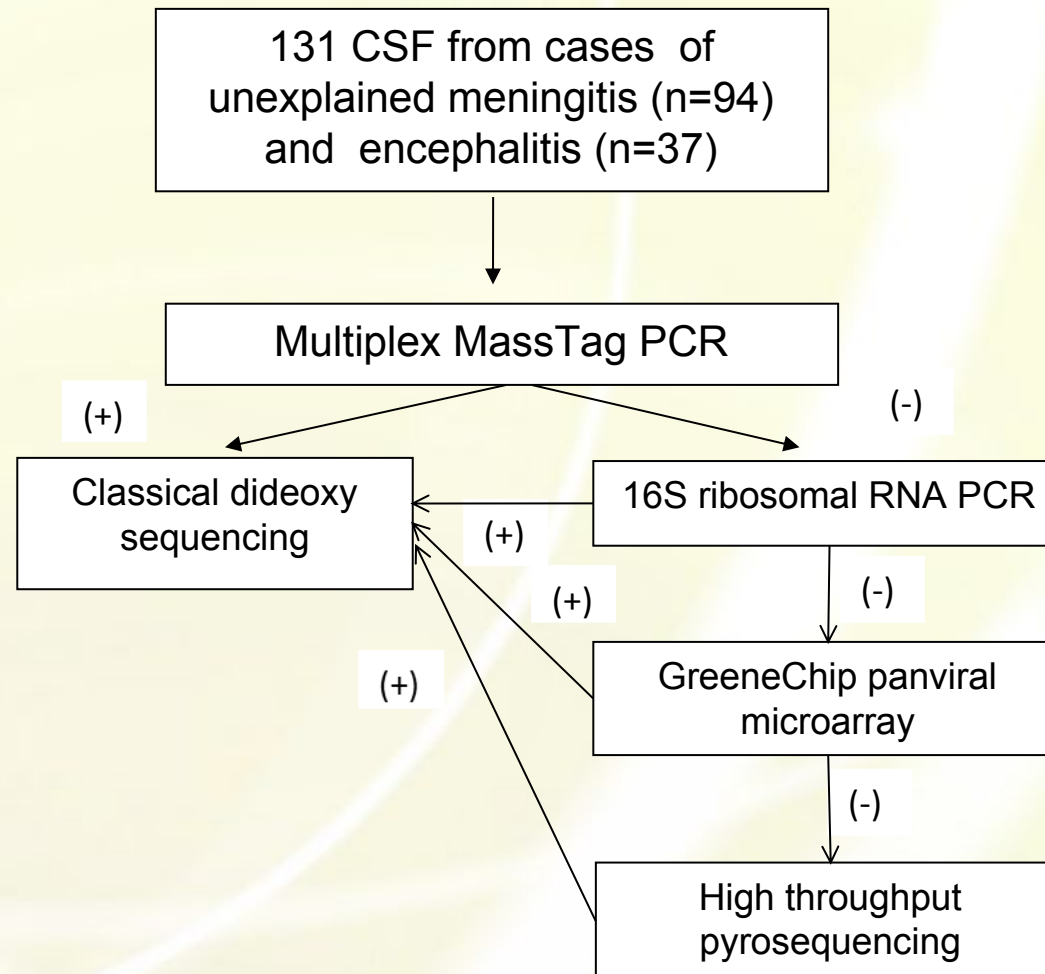


- Light production for each base incorporated
- Data captured as movie of flashing lights
- Known nucleotide addition pattern used to infer nucleic acid sequence





Pathogen Discovery in Unexplained Meningitis and Encephalitis in Taiwan





RESEARCH

Use of Staged Molecular Analysis to Determine Causes of Unexplained Central Nervous System Infections

Chien-Chin Hsu, Rafal Tokarz, Thomas Briese, Hung-Chin Tsai, Phenix-Lan Qian, and W. Ian Lipkin

No agent is implicated in most central nervous system (CNS) infections. To investigate cerebrospinal fluid samples from patients with CNS infections of unknown cause in 1 hospital in Taiwan, we used a staged molecular approach, incorporating techniques including multiplex MassTag PCR, 16S rRNA PCR, DNA microarray, and high-throughput pyrosequencing. We determined the infectious agent for 31 (24%) of 121 previously negative samples. Candidate pathogens were identified for 25 (27%) of 94 unexplained meningitis cases and 6 (16%) of 37 unexplained encephalitis cases. Epstein-Barr virus (15 infections) accounted for most of the identified agents in unexplained meningitis cases, followed by *Escherichia coli* (3), enterovirus (2), human herpesvirus 2 (1), and *Mycobacterium tuberculosis*. Herpesviruses were identified in samples from patients with unexplained encephalitis cases, including varicella-zoster virus (3 infections), human herpesvirus 1 (2), and cytomegalovirus (1). Our study confirms the power of multiplex MassTag PCR as a rapid diagnostic tool for identifying pathogens causing unexplained CNS infections.

Central nervous system (CNS) infections pose a diagnostic challenge because clinical manifestations are not typically pathognomonic for specific pathogens, and a wide range of agents can be causative. An infectious cause of encephalitis is determined for <40%–70% of cases worldwide (1–3). Culture is of limited use, particularly for viral infections. In recent studies, only 1.9% of cerebrospinal fluid

(CSF) viral cultures were positive (3), and <0.1% of CSF cultures recovered viruses other than enteroviruses or herpesviruses (3).

PCR enables sensitive detection of microbial nucleic acids in clinical samples, which may be useful for identifying pathogens that are nonviable, uncultivable, or fastidious. MassTag PCR is a multiplex platform that enables inexpensive, sensitive, and simultaneous detection of multiple pathogens (7–10). Originally implemented for differential diagnosis of respiratory tract infections (7), MassTag PCR has been expanded to several syndrome-based panels for differential diagnosis of hemorrhagic fever and for detection of tick-borne pathogens (9,11).

Amplification and sequencing of the 16S ribosomal RNA (rRNA) gene is a well-established technique for identifying bacterial pathogens (12). Broad-range 16S rRNA PCR with subsequent sequencing is superior to bacterial culture for diagnosing bacterial meningitis, with a sensitivity of 86% and a specificity of 97% (13). It is particularly useful for slow-growing bacteria (e.g., *Mycobacterium tuberculosis*) and for diagnosis of cases that remain culture-negative as a result of antimicrobial drug treatment before lumbar puncture.

Microarray technology also has been applied to the detection and identification of infectious diseases (10,14) and has the potential to test for virtually all known viruses, bacteria, fungi, and parasites (15). The advent of high-throughput sequencing affords unique opportunities for pathogen surveillance and discovery with regard to CNS infections. We have successfully used high-throughput sequencing to identify causative agents of disease for patients with transplant-associated encephalopathy (16) and encephalitis associated with agammaglobulinemia (17).

Our staged molecular approach used complementary tools for pathogen detection and discovery that include syndrome-based multiplex PCRs, DNA microarray, and

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Pathogen	No. of cases (%)	Meningitis cases (%)	Encephalitis cases (%)	No. of HIV (+)	Molecular methods
Virus					
Enterovirus	2 (6.5)	2 (8)	0 (0)	0	MassTag PCR
Herpes simplex-1	2 (6.5)	0 (0)	2 (33)	0	MassTag PCR/Microarray
Herpes simplex-2	1 (3.2)	1 (4)	0 (0)	0	MassTag PCR
Varicella zoster virus	3 (9.7)	0 (0)	3 (50)	0	MassTag PCR
Epstein-Barr virus	16 (51.6)	16 (64)	0 (0)	9	MassTag PCR
Cytomegalovirus	1 (3.1)	0 (0)	1 (17)	0	MassTag PCR
Bacteria					
<i>Mycobacterium tuberculosis</i>	1 (3.2)	1 (4)	0 (0)	0	MassTag PCR
<i>Escherichia coli</i>	5 (16.1)	5 (20)	0 (0)	1	16S rRNA PCR
Total	31 (100)	25 (100)	6 (100)	10	

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Syndromic Surveillance : a Staged Molecular Approach

- Syndromic panel by Mass Tag PCR
 - Respiratory panel
 - CNS infections panel
 - Diarrheal panel
 - Hemorrhagic fever panel





Respiratory Panel

- ***RNA agents***

- FluAM
- FluBM
- FluBHA
- Respiratory syncytial virus group A
- Respiratory syncytial virus group B
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4
- Metapneumoniavirus
- Enterovirus
- Coronavirus-OC43
- Coronavirus-229E
- Coronavirus-SARS

- ***DNA agents***

- Adenovirus
- *Chlamydophila pneumoniae*
- *Legionella pneumophila*
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*



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CNS Infections Panel

- ***RNA agents 14 plex***

- Eastern Equine Encephalitis virus
- Nippah/Hendra virus
- Japanese Encephalitis virus
- Parecho virus
- Powassan virus
- Lacrosse virus
- Lymphocytic Choriomeningitis virus
- St. Louis Encephalitis virus
- Enterovirus
- West Nile virus
- Western Equine Encephalitis virus
- Venezuelan Equine Encephalitis virus
- Rabies virus
- Influenza A virus

- ***DNA agents- 14 plex***

- Adenovirus
- Cytomegalo virus
- Epstein Barr virus
- Varicella Zoster virus
- Herpes Simplex virus 1
- Herpes Simplex virus 2
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Cryptococcus neoformans*
- *Leptospira interrogans*
- *Mycobacterium tuberculosis*
- *Candida albicans*
- *Toxoplasma gondii*



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Hemorrhagic Fever Panel

- Ebola Zaire virus
- Ebola Sudan virus
- Marburg virus
- Lassa virus
- Rift Valley fever virus
- Crimean-Congo hemorrhagic fever
- Hantaan virus
- Seoul virus
- Yellow fever virus
- Kyasanur Forest virus



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Diarrheal Disease Panel

Virus

- Astrovirus
- Rotavirus-A
- Rotavirus-B
- Rotavirus-C
- Norovirus-GI
- Norovirus-GII
- Saporovirus-GI/GII
- Saporovirus-GIV/GV
- Adenovirus-F

Protozoa

- Giardia Lamblia
- Cryptosporidium
- Entamoeba histolytica

Bacteria

- Listeria Monocytogenes
- Clostridium perfringens
- Clostridium difficile
- Campylobacter jejuni
- Campylobacter coli
- Samonella (genus)
- Samonella typhi/paratyphi
- Vibrio (genus)
- Vibrio vulnificus
- Yersinia enterocolitica
- Yersinia pseudotuberculosis
- E. coli- O157:H7
- E. coli-EPEC/EHEC
- E .coli-EPEC/EHEC (O26:H11)
- E. coli-EPEC (typical)
- E. coli-EHEC
- E. coli-EIEC/Shigella
- E. coli-EAEC
- E. coli-stx (shigatoxin)



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Thank you



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