Disclosures - Non

Molecular Diagnosis of CNS Infectious Diseases

World Congress of Neurology
Santiago de Chile
CNS Infections Teaching Course

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Objectives

- Learn about the use and abuse of molecular diagnostic testing for CNS infections
- See some specific examples
- Examine the latest developments in this area which may become routine in future

- Illustrative Interactive Cases
- Recent Research
  - Our group and others

Key Messages

- Put effort into getting the right samples
- Only asks for tests where the diagnosis is possible
- PCR is appropriate for many CNS infections
  - Viruses, bacteria, TBM
  - HIV disease
  - But not all infections
- Think about which other tests may be helpful
- Future
  - Role of dual infections
  - Deep sequencing approaches may help identify new pathogens
  - Host response approaches will also prove useful

Learning Points from this case

- Encephalitis Presentation can be subtle, fluctuant
- Clues
  - Lethargy Drowsiness, Sleeping 15hrs/day, Severe Headache, Hiccoughs, SIADH
- Beware “atypical pneumonia”
- Molecular diagnostic tests don’t work unless you DO the investigation (LP), chase the result and act on it

Why encephalitis is missed

- Wrongly attributing a patient's **fever and confusion**
  - "urinary tract infection"
  - "chest infection"
- Failure to **recognise a febrile illness**,
  - "afebrile on admission"
- **Ignoring a relative** says patient behaviour, "not quite right"
  - "Glasgow coma score =15"
- Wrongly attributing clouding of consciousness
  - **"drugs or alcohol"**
- Failure to properly investigate a patient with a fever and **seizure**

- Failure to do a lumbar puncture, even though there are no contraindications

Diagnosis of HSV in Adults

- 3 of 93 had negative initial PCR
- Approximately 3-5% of adults with HSV encephalitis will have negative initial CSF by PCR

Raschilas F, Wolff M, Delatour F, et al. Outcome of and prognostic factors for herpes simplex encephalitis in adult patients: results of a multicenter study. Clin Infect Dis **2002**; 35:254–60.

If clinical history & imaging/LP consistent with HSV encephalitis, treat even if 1st CSF negative, repeat at CSF 24-48 hours

How good is PCR for diagnosis in Children

- 38 children with HSV encephalitis (CSF PCR +ve)
- In 8 children CSF WCC <10/mm^3, & Protein Normal
  - 4 of 19 samples drawn on day 1 of illness had no cells
- Initial CSF was HSV PCR negative in 8 of 33 children, before day 3 of illness

Limits of Early Diagnosis of Herpes Simplex Encephalitis in Children: A Retrospective Study of 38 Cases

Xavier De Tiège,[1a] Bénédicte Héron,[1] Pierre Lebon,[2] Gérard Ponsot,[1] and Flore Rozenberg[2]

[1]Service de Neuropédiatrie and [2]Laboratoire de Virologie, Hôpital Saint Vincent de Paul, and Faculté de Médecine Cochin, Paris, France

Clinical Infectious Diseases 2003; 36:1335–9

Early CSF may be normal & be PCR Neg
If suspicion high, continue aciclovir, repeat CSF after 24-48 hrs. Early CSF occasionally completely normal (cells protein)

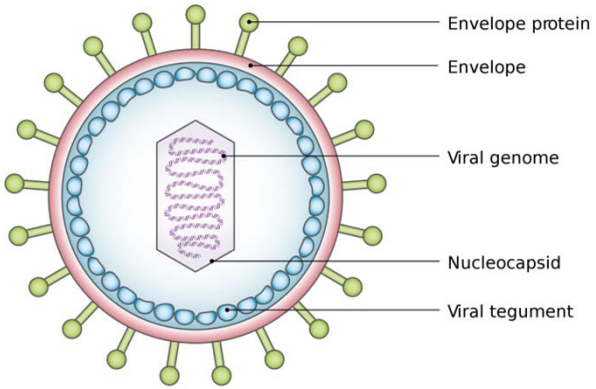
How good is HSV PCR at diagnosing HSV encephalitis?

- 3 pfu or 200gEq/ml

- Sensitivity 96% and the specificity 99% when CSF is studied between 48 h and 10 days from the onset of symptoms
  - (Lakeman and Whitley, 1995; Tebas et al.,1998).

Diagnosis of CNS Infections – the basics

Pathogen



Host

Composition	**Detection through**:
Whole virion	Culture / Electron Microscopy
Nucleic Acid	
- DNA	PCR
- RNA	(Next generation sequencing)
Antigens / proteins	ELISA / Immunofluorescence

Host Response

Antibodies by ELISA
(mRNA expression)

How to ensure you get the right sample (CSF) and the right test requested

- A simple lumbar puncture pack helps
- A CNS infection “set” on the computer helps

Viral causes of encephalitis

Groups	Viruses
Herpes viruses (family Herpesviridae)	
	Herpes simplex virus type 1
	Herpes simplex virus type 2
	Varicella zoster virus type 1
	Epstein-Barr virus
	Cytomegalovirus
	Human herpes virus 6 & 7
Enteroviruses (Family Picornaviridae)	
	Enterovirus 70
	Enterovirus 71
	Poliovirus
	Coxsackieviruses, Echoviruses, Parechovirus
Paramyxoviruses (family Paramyxoviridae)	
	Measles virus
	Mumps virus
Others (rarer causes)	
	Influenza viruses, Adenovirus, Parvovirus B19, Lymphocytic choreomeningitis virus, Rubella virus,
Zoonotic viruses	
	Rabies, other lyssaviruses
	Nipah virus
Arboviruses (most are also zoonotic)	
Flaviviruses (family Flaviviridae)	
	West Nile virus
	Japanese encephalitis virus
	Tick-borne encephalitis
	Dengue
Alphaviruses (Family Togaviridae)	
	Western, Eastern and Venezuelan Equine encephalitis virus
	Chikungunya
Bunyaviruses	
	Lacrosse virus
Coltiviruses	
	Colorado tick fever
Vesiculoviruses	
	Chandipura virus

CNS infections

Bacteria

Small bacteria (mostly intracellular)
- Mycoplasma
- Chlamydia
- Rickettsia (including scrub typhus, rocky mountain spotted fever)
- Erlichiosis
- Coxiella (Q fever)
- Bartonella (Cat Scratch fever)
- *Tropherema whipplei* (Whipple's disease)
- Brucellosis
- Typhoid fever

Spirochetes
- Syphilis
- Lyme Neuroborreliosis
- Leptospirosis
- Borrelia recurrentis (Relapsing fever)

Other bacteria
- Subacute bacterial endocarditis
- Listeria
- Nocardia
- Actinomycosis
- Parameningeal infection
- Abscess

Parasites
- Malaria
- Trypanosomiasis
- Amoebic encephalitis - *Naegleria fowleri*
- Cysticercosis
- Echinococcus
- Trichinosis
- Amoebiasis

Fungi
- Cryptococcus
- Candidiasis
- Coccidiomycosis
- Histoplasmosis
- North American blastomycosis

Para/Post infectious causes

- Acute disseminated encephalomyelitis
- Viral illnesses with febrile convulsions
- Shigella
- Viral infections associated with swollen fontanelle
- Guillain-Barré syndrome

Non infectious diseases

Vascular
- Vasculitis
- Systemic lupus erythematosis
- Behcet's disease
- Sub-arachnoid & sub-dural haemorrhage
- Ischaemic cerebrovascular accidents

Neoplastic
- Primary brain tumour
- Metastases
- Paraneoplastic limbic encephalitis

Metabolic encephalopathy
- Hepatic encephalopathy
- Renal encephalopathy
- Hypoglycaemia
- Reye's syndrome
- Toxic encephalopathy (alcohol, drugs)
- Hashimoto's disease

Other
- Drug reactions
- Epilepsy
- Hysteria
- Voltage gated K channel limbic encephalitis

Non-Viral causes of encephalitis and encephalopathy

Eg Epidemiology of viral CNS infections

- Some pathogens constant across globe,
  - e.g. HSV
- Others varies with geography
  - Esp Arthropod-borne & Zoonotic
- HIV and other immunocompromise has changed epidemiology
  - CMV, EBV, Toxoplasma more important
- Vaccination has changed epidemiology in some places
  - Polio, Measles, Mumps, Japanese encephalitis

Arboviruses – growing in importance

- West Nile virus in USA and Europe
- Tick-borne encephalitis virus in Europe
- Dengue and Chikungunya spreading

- Impact of
  - climate change?
  - Changing agricultural practice
  - People movement
  - etc

Why PCR so useful in CNS Infections?

- Specific
- Very helpful in viral CNS infections (culture hard)
- Unlike blood, CSF doesn't contain PCR inhibitors (heme, endonucleases, and exonucleases), which can lead to false negatives
- CSF is sterile (unlike resp or GI tract), so detection usually means causation, so fewer false positives

PCR of the CSF especially useful for

- Some Viral CNS Infections
  - Herpes viruses (HSV, VZV, CMV, EBV, HHV6 & 7)
  - Some Enteroviruses
- Some Bacteria
  - MTB
- Some spirochetes
  - Borrelia

Limitations

- False negatives can occur
  - Pathogen no longer detectable because cleared
  - PCR inhibitors in bloody tap
  - Poor handling of sample – too many freeze thaws
- False positives can occur
  - Contamination at collection or in the lab
  - Detection of latent, non causal pathogen (e.g. EBV)

False Positives

- Only request PCR if the pre-probability testing is high
- If 5% false positive rate; then 1 in 20 tests will be falsely positive.
- If you don’t think disease X is likely, what will you do if the PCR is positive for disease X!

	Toxoplasm	PCNSL	TB	PML	HAD
Clinical	Acute febrile; focal signs; Sz	Subacute dementia; focal signs (Sz)	Subacute meningitis; focal signs, Sz	Gradual onset, visual, other focal	Chronic cognitive, motor, behaviour
Imaging	Multiple SOLs in basal ganglia and grey/white matter	Single-few SOLs periventricular / anywhere	Single-few small SOLs, or abscesses	Subcortical white matter, esp posterior; T1 hypodense	Diffuse white matter, T1 isodense
Oedema	Marked	Moderate	Marked	None	None
Enhancement	Ring	Homogeneous	Ring, and meningeal	None	None

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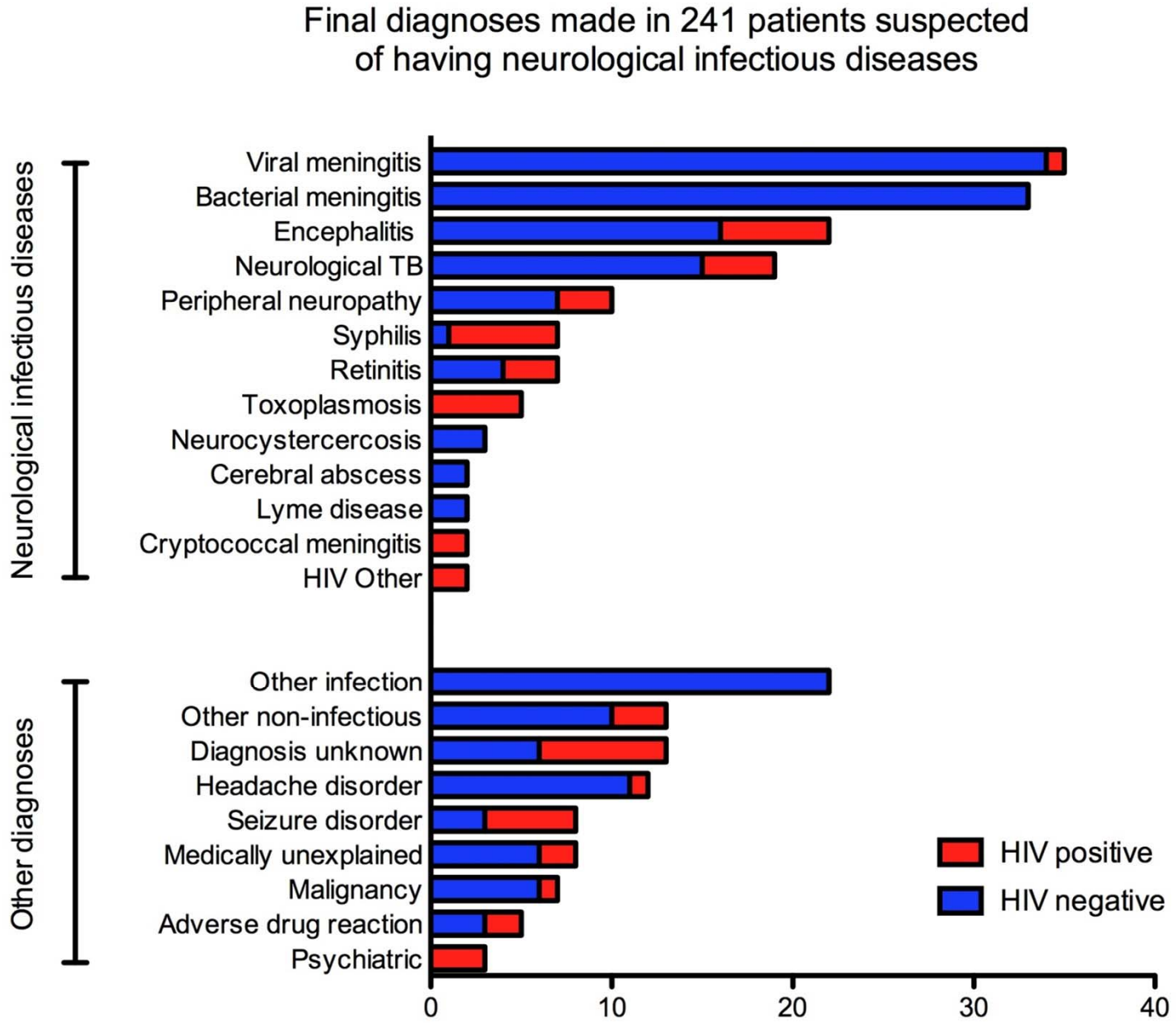
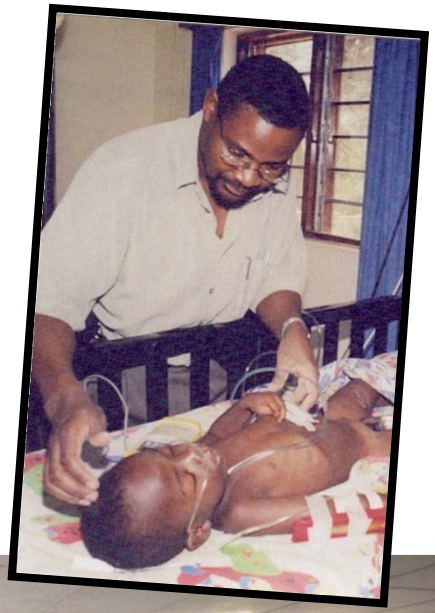


The role of a specialist neurological infectious diseases service at a secondary and tertiary hospital in North West United Kingdom Turtle L, Whiteley J, Jung A, Crocker D, Nicholson A, Beadsworth MB, Miller A, Beeching N, Solomon T , 2015

Next generation sequencing

- Limitation: PCR is specific, will only test for what you ask for
- Next generation (Deep, High throughput) sequencing is non-specific
  - Amplifies everything in the sample
  - Align all sequences
  - Ignore sequences not of interest
  - See what is left

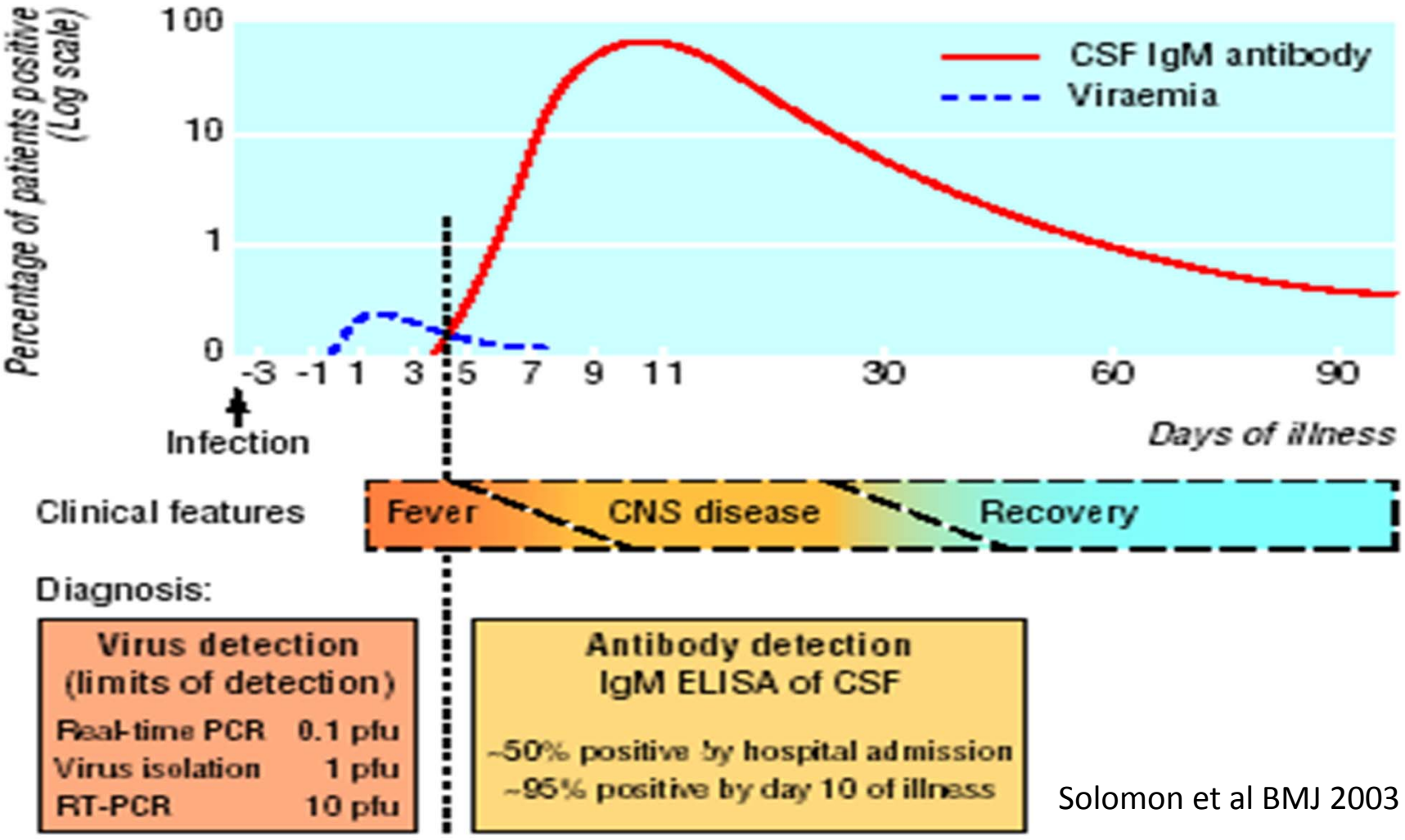
Viral Central Nervous System Infections in Children from a Malaria-Endemic Area of Malawi: a Prospective Cohort Study



- 513 children with suspected CNS infection
  - Excluded bacterial
  - 94 (18%) died.
- 163 (32%) had *P. falciparum* parasitaemia, of whom 34 died;
- 133 (26%) had at least one virus detected in the central nervous system (CNS) by polymerase chain reaction (PCR), with 43 deaths.
- Twelve different viruses were detected,
  - adenovirus most common (42 patients).
- **45 (9%) children had both parasitaemia and viral infection;**
  - 27 (35%) of 78 diagnosed clinically with cerebral malaria.

Mallewa et al. Lancet Global Health 2013

Diagnostic arbovirology
Japanese encephalitis, West Nile virus



Solomon et al BMJ 2003

Detection of NS1 antigen

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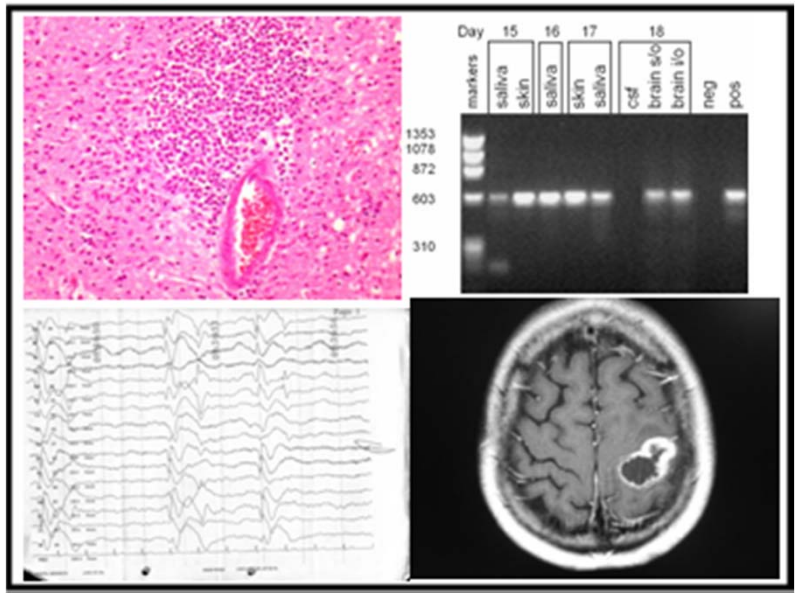
Liverpool Neurological Infectious Diseases Course

Liverpool Medical Institution



Feedback from previous courses:

"Would unreservedly recommend to others"

"An excellent 2 days!! The best course for a long time"

Convenors: Prof Tom Solomon, Enitan Carrol, Rachel Kneen & Dr Nick Beeching

www.liv.ac.uk/neuroidcourse email braininfections@liv.ac.uk

References

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