Paediatric Viral Infections: Enteroviruses and CMV

Maria Craig

Viruses in July 2004

St George Hospital
Children’s Hospital Westmead
School of Women’s & Children’s Health, University of NSW
Outline

- Enteroviruses
  - Molecular virology
  - Congenital Infections
  - Paediatric Infections
  - Diagnosis

- HCMV
  - Molecular virology
  - Congenital Infections
  - Paediatric Infections
  - Diagnosis
Background

- Enteroviruses are common agents in paediatric infections
- Ubiquitous
  - ~ 50 billion per year
- Transmitted by faecal oral route (infants are the “most efficient” transmitters)
  - coxsackie A21 spread by respiratory secretions
  - enterovirus 70 shed in tears, spread via fingers & fomites
- Shed in the upper respiratory tract for 1-3 weeks & in faeces for up to 8 weeks after primary infection
- Cause a wide spectrum of common and uncommon illnesses
  - Often asymptomatic or mild illness
  - Severe infection & death
Picornaviruses

- Diverse family > 200 serotypes
- ‘Oldest' known viruses
  - records from Egypt ~ 1400 BC
- FMDV was one of the first viruses to be recognised - Loeffler and Frosch 1898
- Polio was first recognised as a viral disease by Landsteiner & Popper in 1909
Picornavirus phylogeny
Enterovirus structure

- Small icosahedral, non-enveloped viruses
- 27 –30 nm, 7500 - 8500 nucleotides long
- Simple viral capsid & single positive strand RNA
  - capsid is composed of 60 densely packed copies of capsid proteins VP1, VP2, VP3 & VP4
- Antigenic diversity is due to capsid protein variation (VP1 – VP3)
- VP4 functions as an anchor to the viral capsid
  - destabilisation of VP4 results in viral uncoating
- Replication cycle is rapid, usually 8 hours
  - Occurs in cytoplasm
Enterovirus Genome Structure

Capsid

5’ UTR

Structural proteins

Non-structural proteins

3’ UTR

750 bp

50-100 bp

Translation, virulence, IRES

Mediates attachment & entry into target cell

Important for (-) strand synthesis

AAA
Genetic Subtyping of EVs

“Classical” subtyping was based on:
- disease caused in suckling mice
  - CAVs vs CBVs
- particle density & pH sensitivity
- Enteric Cytopathic Human Orphan viruses

After 1969, new EVs were given numbers
- EV 68: pneumonia
- EV 70: acute haemorrhagic conjunctivits
- EV 71: meningitis & rhombencephalitis
Current Enterovirus nomenclature

- Human enterovirus A (HEV A):
  - CV -A2, -A3, -A4, -A5, -A6, -A7, -A8, -A10, -A12, -A14, -A16, EV-71

- Human enterovirus B (HEV B):
  - CV -B1 to -B6, CV-A9, Echovirus (E) -1 to -9, E-11 to -21, E-24 to -27, E-29 to -33, EV-69

- Human enterovirus C (HEV C):
  - CV -A1, -A11, -A13, -A15, -A17 to -A22, -A24

- Human enterovirus D (HEV D):
  - Enterovirus (EV) -68, -70
Genus Enterovirus cont.

- Poliovirus
  - although close to HEV C, separate species due to unique clinical features and receptor usage
- Bovine enterovirus
- Porcine enterovirus A
- Porcine enterovirus B
- Unassigned enteroviruses
  - mostly Simian EVs
- New classification (A-D) based on 3’ & 5’ UTR
Phylogeny of Enteroviruses

- VP1 CAPSID: 4 main groups
  - Cluster A: CAVs, EV 71
  - Cluster B: CAV 6, CBV 1, E26, EV 69
  - Cluster C: CAV 19, CAV 24, PV 1
  - Cluster D: EV 68, EV 70

- Virus evolution - EVs probably all derived from a single virus
  - capsid proteins are targets of host immune surveillance, so allow EVs to broaden their “niche”
  - EV diversity is reflected in the variety of cell surface molecules they recognise as they enter host cells (at least 6 membrane proteins interact with EVs)
Phylogenetic analysis of the VP1 gene
Enterovirus Infections

- Exanthema – Hand foot & mouth disease
- Non-focal acute febrile illness
  - ~ 50 – 60% of infants < 3 mths
- Respiratory illness
- Gastroenteritis
- Encephalitis / Meningitis
- Myocarditis
- SIDS
- Acute haemorrhagic conjunctivitis (EV 70/CAV 24)
- Pancreatitits / Type 1 diabetes
Hand, foot and mouth disease
Congenital EV infections

- Case reports
- Clinical features
  - Cerebral palsy
  - Diabetes
  - Hepatitis
  - Jaundice
  - Thrombocytopenia
  - Generalised infection
Neonatal Enterovirus infection

- Represents a significant proportion of “PUO”
- Presenting features include
  - asymptomatic/mild infection
  - poor feeding, lethargy, convulsions, tremor, hypotonia, diarrhoea
- Clinical manifestations include
  - hepatic necrosis, meningoencephalitis, myocarditis, fever, rash, sepsis, respiratory illness/pneumonia
- The absence of maternal symptoms does not preclude infection in the neonate
- Early onset < 6 days
  - usually due to maternal transmission
- Late onset ≥ 7 days
  - postnatal maternal/ family member /nosocomial transmission
Neonatal Enterovirus infection

- Investigations
  - Infant (and maternal) samples
  - Culture, PCR, serology

- Treatment
  - IVIG
  - Polio vaccine
  - Pleconaril – limited experience
Respiratory illness

- Jartti et al, Emerg Infect Dis June 2004
- 2-year prospective study in Finland
  - 293 hospitalized children
  - NPA: enteroviruses (25%), rhinovirus (24%), non-typable rhino/enterovirus (16%) were found most frequently; RSV (27%)
  - In older children, respiratory picornaviruses dominated (65% of children ages 1-2 years and 82% of children ages ≥3 years)
Myopericarditis

- CVBs are cardiomyotrophic
- ~33 – 50% of sporadic cases
- Most cases in epidemics
- ~5% fatality rate
- Viral replication in the myocardium peaks within 3-7 days & persists for 7-10 days in immunocompetent hosts,
  - longer in the immunocompromised
- Adolescents / young adults at highest risk;
  - Males twice the risk of females
EV 71 epidemics

- South East Asia & Australia
- 1997 - 1999
- Hand, foot, and mouth disease (common)
- Severe disease, including pulmonary oedema & invasive CNS disease
  - aseptic meningitis, Guillain-Barre´ syndrome, acute transverse myelitis, acute cerebellar ataxia, opso-myoclonus syndrome, BIH
    - McMinn et al, Clin Inf Dis 2000
  - Survival related to Rx with ?pleconaril, steroids, IVIG, vigorous resuscitation, afterload reduction
    - Nolan et al J Neurology 2002

Survival related to Rx with pleconaril, steroids, IVIG, vigorous resuscitation, afterload reduction
## Clinical features – EV 71 cases

<table>
<thead>
<tr>
<th>Patient</th>
<th>Greatest deficit</th>
<th>Acute MRI lesions (day)</th>
<th>Late MRI lesions</th>
<th>Long term deficit (17-86 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, no resp effort, AFP all 4 limbs</td>
<td>whole brainstem cervical*</td>
<td>whole brainstem whole spine</td>
<td>Died 9 weeks into illness, only grimace, weak movement of eyes &amp; R hand</td>
</tr>
<tr>
<td>2</td>
<td>Facial weakness, bulbar dysfunction, poor resp effort, AFP all 4 limbs, UL L&gt;R</td>
<td>medulla cervical</td>
<td>L cervical (C4 only)*</td>
<td>Normal other than weak L shoulder &amp; elbow</td>
</tr>
<tr>
<td>3</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, poor resp effort, AFP UL R&gt;L, LLs strong</td>
<td>pons medulla medulla</td>
<td>ND</td>
<td>Normal other than weak R shoulder &amp; R elbow flexion</td>
</tr>
<tr>
<td>4</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, no resp effort, myoclonus, urinary retention, AFP all 4 limbs</td>
<td>pons medulla medulla</td>
<td>medulla whole spine</td>
<td>Weak gag, some resp effort but ventilator dependent, L UL weakness, other limbs normal &amp; walking independently</td>
</tr>
<tr>
<td>5</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, no resp effort, myoclonus, urinary retention, AFP all 4 limbs</td>
<td>pons medulla whole spine</td>
<td>whole brainstem whole spine</td>
<td>No gag, some independent resp effort but ventilator dependent, functional ULs (L weaker than R), severe weakness LLs</td>
</tr>
<tr>
<td>6</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, no resp effort, urinary retention, AFP all 4 limbs</td>
<td>pons medulla whole spine</td>
<td>medulla whole spine</td>
<td>Fully ventilator dependent, only movement is head nod, facial expression and very limited R hand function</td>
</tr>
<tr>
<td>7</td>
<td>Ophthalmoplegia, facial weakness, bulbar dysfunction, no resp effort, AFP UL, some LL movement</td>
<td>whole brainstem cerebellum cervical*</td>
<td>medulla whole spine</td>
<td>Normal strength, diaphragm pacing allows independent daytime ventilation, nocturnal ventilation still required</td>
</tr>
</tbody>
</table>
Figure 1. MRI of a 9-month-old female infant (patient 2) with enterovirus 71–associated neurological disease. A, axial gradient-echo T1-weighted MRI done 3 days after the onset of acute transverse myelitis, showing a high signal lesion centered in the dorsal column white matter of the cervical cord. B, midsagittal turbo spin-echo T2-weighted MRI scan done during the same examination as in A, showing a lesion from C2 to T2 (arrows) with mild cord expansion.
Type 1 diabetes and EVs

- Enteroviral association with type 1 diabetes is well known, but not well understood
- Mechanism of involvement in diabetes pathogenesis is unclear
  - molecular mimicry
  - innocent bystander
  - direct infection
- Early studies suggested predominance of Coxsackieviruses (B4)
Amino acid sequence homology between GAD65 and Enterovirus 2C protein
The evidence

- **When:**
  - Enteroviruses at diagnosis
  - Prospective studies of children at risk
  - In utero infection
  - Temporal association with Ab conversion

- **How:**
  - Serologic studies
  - Studies of pancreata and cultured islets
  - Animal studies
  - Detection of RNA in serum, buffy coat, stool
EV and Diabetes Study

- Case-control study
- 206 children from Western Sydney diagnosed between April 1997 - Sept 1999
- 160 age matched healthy controls
- Plasma & stools samples collected for RT-PCR from diabetic & control subjects
- Serum and DNA in diabetic children
  - HLA typing & diabetes –associated autoantibody analysis
  - ELISA for heterotypical IgM, IgA and IgG
## Results

<table>
<thead>
<tr>
<th></th>
<th>Diabetes</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>62/206</td>
<td>6/160</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(30%)</td>
<td>(4%)</td>
<td></td>
</tr>
<tr>
<td>Stool</td>
<td>26/110</td>
<td>4/25</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(24%)</td>
<td>(16%)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>58/206</td>
<td>3/160</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(28%)</td>
<td>(2%)</td>
<td></td>
</tr>
</tbody>
</table>

Craig et al, J Inf Dis 2003
Age distribution

![Age distribution chart](chart.png)
Seasonal pattern of infection

Number of cases

month & year of diagnosis

rna +

no

yes
Multiple enterovirus subtypes

- Enterovirus 71 17 (28%)
- Coxsackie B1 14 (23%)
- Coxsackie B3 7 (11%)
- ECHO 30 4 (7%)
- CAVs 3 (5%)
- Other 11 (18%)
- Not typable 5 (8%)
- Polio (Sabin) - excluded 2
Further analysis

- Negative association with genetic predisposition (DR3 or DQB1*02) implies a “viral subgroup” of type 1 diabetes
- Children with high C-peptide at diagnosis (>90th percentile) were less likely to be enterovirus RNA positive
- Severe DKA at diagnosis (pH <7.1) was significantly associated with enterovirus RNA positivity
- No association with autoimmune markers
- No association with gender, BMI, history of infection
Polio

- In 1988, the World Health Assembly resolved to eradicate poliomyelitis globally.
- Countries with endemic polio decreased from 125 in 1988 to six in 2003.
- But in 2003, 10 countries reported poliovirus importations,
  - West and Central Africa (8), Southern Africa (Botswana), and Middle East (Lebanon).
FIGURE. Number* and location of virus-confirmed poliomyelitis cases, January–April 2003 and January–April 2004.  

† As of May 18, 2004.
# Acute flaccid paralysis - worldwide

## TABLE. Acute flaccid paralysis (AFP) and poliomyelitis cases, by World Health Organization region and country, 2003 and 2004*

<table>
<thead>
<tr>
<th>Region/Country</th>
<th>No. reported AFP cases 2003</th>
<th>No. reported AFP cases 2004</th>
<th>Nonpolio AFP rate 2003</th>
<th>Nonpolio AFP rate 2004</th>
<th>% persons with AFP with adequate specimens 2003</th>
<th>% persons with AFP with adequate specimens 2004</th>
<th>Virus-confirmed cases total 2003</th>
<th>Virus-confirmed cases total 2004</th>
<th>Virus-confirmed cases January–April 2003</th>
<th>Virus-confirmed cases January–April 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td>8,184</td>
<td>2,745</td>
<td>2.6</td>
<td>2.7</td>
<td>88</td>
<td>91</td>
<td>446</td>
<td>162</td>
<td>34</td>
<td>162</td>
</tr>
<tr>
<td>Nigeria</td>
<td>3,318</td>
<td>1,425</td>
<td>6.0</td>
<td>7.9</td>
<td>91</td>
<td>91</td>
<td>355</td>
<td>133</td>
<td>32</td>
<td>133</td>
</tr>
<tr>
<td>Niger</td>
<td>175</td>
<td>80</td>
<td>2.4</td>
<td>3.6</td>
<td>79</td>
<td>88</td>
<td>40</td>
<td>12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>5,294</td>
<td>1,798</td>
<td>2.4</td>
<td>2.3</td>
<td>90</td>
<td>90</td>
<td>113</td>
<td>15</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Pakistan</td>
<td>2,270</td>
<td>742</td>
<td>3.0</td>
<td>2.8</td>
<td>90</td>
<td>90</td>
<td>103</td>
<td>12</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>599</td>
<td>212</td>
<td>3.9</td>
<td>3.9</td>
<td>88</td>
<td>91</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Egypt</td>
<td>608</td>
<td>268</td>
<td>2.5</td>
<td>2.7</td>
<td>93</td>
<td>94</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>South-East Asian</td>
<td>11,305</td>
<td>3,360</td>
<td>1.9</td>
<td>1.1</td>
<td>83</td>
<td>85</td>
<td>225</td>
<td>8</td>
<td>77</td>
<td>8</td>
</tr>
<tr>
<td>India</td>
<td>8,524</td>
<td>2,543</td>
<td>2.0</td>
<td>1.1</td>
<td>81</td>
<td>84</td>
<td>225</td>
<td>8</td>
<td>77</td>
<td>8</td>
</tr>
<tr>
<td>American</td>
<td>2,229</td>
<td>488</td>
<td>1.3</td>
<td>0.8</td>
<td>80</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>European</td>
<td>1,639</td>
<td>491</td>
<td>1.2</td>
<td>1.0</td>
<td>82</td>
<td>81</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>6,397</td>
<td>1,313</td>
<td>1.4</td>
<td>0.9</td>
<td>88</td>
<td>85</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Worldwide</td>
<td>35,048</td>
<td>10,195</td>
<td>1.9</td>
<td>1.5</td>
<td>86</td>
<td>87</td>
<td>784</td>
<td>185</td>
<td>135</td>
<td>185</td>
</tr>
</tbody>
</table>

*2004 data are cases reported during January–April, as of May 18, 2004.
† Data presented only from countries with indigenous polio during 2003. Values do not add to regional and global totals.
§ Per 100,000 children aged <15 years; annualized for 2004.
‖ Two stool specimens collected at an interval of at least 24 hours within 14 days of paralysis onset and adequately shipped to the laboratory.
Diagnosis of Enterovirus Infection

- Viral culture
- Serology
  - Complement Fixation
  - Neutralisation
  - ELISA
- PCR
Viral culture

- Traditional, “gold standard”
- Relatively sensitive, and yields an isolate that can be further serotyped for clinical or epidemiologic purposes

BUT
- takes 3 - 7 days
- expensive
- requires cell lines
- some types eg CAVs difficult to culture
PCR

- Rapid result ~ hours
- Increased sensitivity compared with culture (some studies > 90%)
- Detects multiple subtypes in one assay
- Use of specific primers (eg VP1) or sequencing allows genotyping of isolates
- Can improve patient management
  - decreased hospital length of stay for children with enteroviral meningitis
ELISA

- Low sensitivity
- High specificity for IgM
- Useful for retrospective diagnosis
- Cheap, large number of specimens can be processed
- Depends on background immunity of population
## PCR vs ELISA for EVs

<table>
<thead>
<tr>
<th>ELISA</th>
<th>No. ELISA positive/ No. PCR-Positive</th>
<th>Sensitivity (%)</th>
<th>No. ELISA positive/ No. PCR negative</th>
<th>%</th>
<th>P-value</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>20/68</td>
<td>29</td>
<td>70/297</td>
<td>24</td>
<td>0.3</td>
<td>76</td>
</tr>
<tr>
<td>IgM</td>
<td>19/68</td>
<td>28</td>
<td>11/297</td>
<td>4</td>
<td>&lt;0.001</td>
<td><strong>96</strong></td>
</tr>
<tr>
<td>IgG</td>
<td>37/68</td>
<td><strong>54</strong></td>
<td>102/297</td>
<td>34</td>
<td>0.002</td>
<td>66</td>
</tr>
<tr>
<td>IgM or IgG</td>
<td>48/68</td>
<td>71</td>
<td>107/297</td>
<td>36</td>
<td>&lt;0.001</td>
<td><strong>64</strong></td>
</tr>
<tr>
<td>IgA or IgG</td>
<td>42/68</td>
<td>62</td>
<td>137/297</td>
<td>46</td>
<td>0.02</td>
<td><strong>54</strong></td>
</tr>
<tr>
<td>IgA or IgM</td>
<td>32/68</td>
<td>47</td>
<td>78/297</td>
<td>26</td>
<td><strong>0.001</strong></td>
<td>74</td>
</tr>
<tr>
<td>IgA, IgM or IgG</td>
<td>50/68</td>
<td><strong>74</strong></td>
<td>141/297</td>
<td>47</td>
<td>&lt;0.001</td>
<td><strong>53</strong></td>
</tr>
</tbody>
</table>

Craig et al, J Clin Micro 2003
"You're fired, Jack. The lab results just came back, and you tested positive for Coke."
Human Cytomegalovirus (HCMV)

- DNA virus – *Herpesviridae*
  - Large, enveloped viruses
  - Properties of latency and reactivation
- Genome consists of DS DNA ~ 200 kilobase pairs
  - codes for more than 200 ORFs
- Subtyping based on variation in glycoprotein B (gB)
  - Correlates with viral tropism *in vivo*
  - Variation in gB may influence CMV virulence
- Ubiquitous
  - adult seropositivity rate ~ 60 - 100%
  - Aust Red Cross seropositivity rates
    - ~ 40% at 20 yrs to 70% at 50 yrs
- Transmission
  - breast feeding, sexual contact, vertical transmission, spread from children, transplanted organs
Human CMV infection

- Mainly asymptomatic in healthy individuals
  - 10% have mononucleosis-like illness
    - malaise, persistent fever, myalgia, cervical lymphadenopathy
    - less common pneumonia, hepatitis
    - laboratory findings include atypical lymphocytes, mild thrombocytopenia and elevated liver enzymes
  - infection is self-limited
    - viral excretion may be prolonged
    - CMV persists throughout life
- Severe infection in immunocompromised host, fetuses and neonates
Reactivation vs reinfection

- Recurrent infection (intermittent excretion of virus from single or multiple sites)
  - Reactivation of an endogenous virus (more common)
  - Exposure to a new virus strain from an endogenous source (less common)
  - Mixed infection may also occur

- Reasons for recurrence
  - Low grade chronic infection following primary infection, with intermittent detection of virus due to low copy numbers
  - Reactivation of latent virus in response to stimuli eg pregnancy
CMV diagnosis

- Viral culture – MRC5
  - > 2 weeks
- Serology
  - IgM, IgG
  - IgM Avidity
- PCR
  - Qualitative & quantitative
  - *In situ*
  - Multiplex
<table>
<thead>
<tr>
<th>Test</th>
<th>Principal uses</th>
<th>Potential problems</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen detection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus culture</td>
<td>Virus detection</td>
<td>Long time to result (3–4 weeks)</td>
<td>Urine, blood, tissue</td>
</tr>
<tr>
<td></td>
<td>Virus for further study using PCR,</td>
<td>Expensive set-up costs for virus culture laboratory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>genotyping, antiviral susceptibility</td>
<td>Confusion of cytopathic effect (CPE) with adenovirus CPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>testing</td>
<td>Specimen contamination</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Culture positivity with reactivation ± disease</td>
<td></td>
</tr>
<tr>
<td>Direct immunofluorescence</td>
<td>Rapid detection of virus</td>
<td>Culture positivity with reactivation ± disease</td>
<td>Blood, urine</td>
</tr>
<tr>
<td></td>
<td>– results available in 48 h</td>
<td>Limited culture is still necessary</td>
<td></td>
</tr>
<tr>
<td>Nucleic acid testing</td>
<td>Rapid detection of virus</td>
<td>Cost of individual test high</td>
<td>Urine, blood, CSF, Tissue</td>
</tr>
<tr>
<td></td>
<td>Quantification of viral load</td>
<td>Contamination resulting in false positives</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus strain typing</td>
<td>Various techniques (PCR, bDNA, NASBA, TMA)</td>
<td>Tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acceptable for diagnosis if correlated with active CMV infection</td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td>Definitive demonstration of tissue</td>
<td>Need for a clinical procedure</td>
<td>Tissue</td>
</tr>
<tr>
<td></td>
<td>damage</td>
<td>False negative rate high</td>
<td></td>
</tr>
<tr>
<td>In situ hybridization</td>
<td>Definitive demonstration of CMV</td>
<td></td>
<td>Tissue</td>
</tr>
<tr>
<td>Antibody detection:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG-EIA</td>
<td>Show previous infection</td>
<td>Seroconversion takes 2–3 weeks, needs two samples</td>
<td>Serum</td>
</tr>
<tr>
<td></td>
<td>Show recent infection with seroconversion</td>
<td>False seroconversion with administration of blood products or immunoglobulin (Ig)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Avidity shows acute infection</td>
<td>Detect recent infection (avidity &lt; 60%)</td>
<td></td>
</tr>
<tr>
<td>IgM-EIA</td>
<td></td>
<td>Seropositivity for 2 years post acute infection in 5%</td>
<td>Serum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cross-reactivity with EBV (rare)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensitivity of single cord blood IgM 70–80%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antigenic heterogeneity in clinical isolates</td>
<td></td>
</tr>
<tr>
<td>Complement fixation</td>
<td>Demonstration of rising titre</td>
<td>False negative in 2–5%</td>
<td>Serum</td>
</tr>
</tbody>
</table>

Trincado & Rawlinson, JPCH 2001
Congenital CMV

- Most common cause of congenital infection
  - 0.3 - 2.4% of neonates are infected with CMV
  - Increased rate if premature (4.8% < 34 weeks)
    - Panhani et al, Scand J Infect Dis 1994
  - Higher in populations of lower SES
  - ~10% symptomatic
  - 10 - 30% mortality
- Primary infection or reactivation
Epidemiology

- 1 - 3% of pregnant women develop primary CMV infection
  - 30 – 40% of infants are congenitally infected
  - Of these
    - 10 – 15% symptomatic
    - 20 – 30% mortality (DIC, hepatic dysfunction, bacterial superinfection)
    - 70 – 80% of symptomatic infants will develop complications in first few years of life
  - 5% -10% infected but asymptomatic infants at birth will develop later sequelae

- In women who have CMV infection at least 6 months prior to conception
  - ~1% infants are congenitally infected
  - Most asymptomatic
Clinical features - congenital CMV

- Asymptomatic
  - reactivation >>> primary infection *
- Microcephaly*
- Thrombocytopenia*, petechiae
- IUGR*, prematurity
- Hepatosplenomegaly* / jaundice
- Sensorineural hearing impairment
  - 40% severe – impaired communication/learning
  - 80% detected > 1 year old
- Cerebral Palsy / Mental retardation
- Chorioretinitis

* Most common findings: Boppana et al, Ped Inf Dis J 1992
Symptomatic vs asymptomatic

- More severe or atypical manifestations & higher mortality in preterm infants (NB small nos)
  - Yamamoto et al, Paed Inf Dis J 2001
  - Perlman et al, Ann Neurol 1992

- Earlier studies suggested symptomatic congenital CMV infection usually associated with primary maternal infection

- Recent studies show symptomatic congenital infection in highly seropositive populations
  - Ahlfors et al, Scand J Infect Dis 1999
  - Boppana et al, Pediatrics 1999
  - Yamamoto et al, Paed Inf Dis J 2001
Diagnosis of congenital infection

- Amniotic fluid testing
  - Multiplex PCR
- Cordocentesis
  - Fetal blood
- Urine culture
- Serology
# AF testing for CMV

<table>
<thead>
<tr>
<th>Author</th>
<th>Time of collection</th>
<th>Total cases</th>
<th>PCR +ve Results</th>
<th>Post natal isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CMV</td>
<td>VZV</td>
</tr>
<tr>
<td>McLean et al. 1995</td>
<td>1994</td>
<td>277</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Mouly et al. 1997</td>
<td>1989-94</td>
<td>107</td>
<td>ND</td>
<td>8.4%</td>
</tr>
<tr>
<td>Liesnard et al. 2000</td>
<td>1985-98</td>
<td>237</td>
<td>29%</td>
<td>ND</td>
</tr>
<tr>
<td>Lipitz et al. 1997</td>
<td>1992-95</td>
<td>66</td>
<td>35%</td>
<td>ND</td>
</tr>
</tbody>
</table>

R = Random trial, T = Targeted trial
Multiplex PCR
Acknowledgements

- Bill Rawlinson, Gillian Scott, Sian Munro
- Leighton Clancy, David Liuwantara, Sharon Chow
- Peter Robertson, Ross Whybin
- Margaret Lloyd, Neville Howard, Martin Silink (CHW)
- Caroline Ford