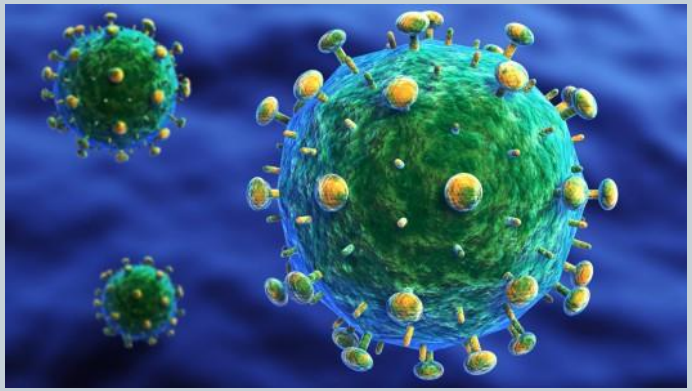
RNA PCR, Proviral DNA and Emerging Trends in Infant HIV Diagnosis



BRENDAN MCMULLAN
INFECTIOUS DISEASES, SYDNEY CHILDREN'S HOSPITAL
SCHOOL OF WOMEN'S AND CHILDREN'S HEALTH, UNSW

SYDNEY CHILDREN'S HOSPITAL RANDWICK

OUTLINE

- Charlie (1)
- Background: Infants vs adults
- Proviral DNA PCR
- RNA PCR
- Other methods
- Charlie (2)

Charlie

- Charlie is 4 days old
- His mother, Sue, has been on ARVs throughout pregnancy and viral load is undetectable
- *How likely is Charlie to be infected?*
- *How can infection be excluded?*

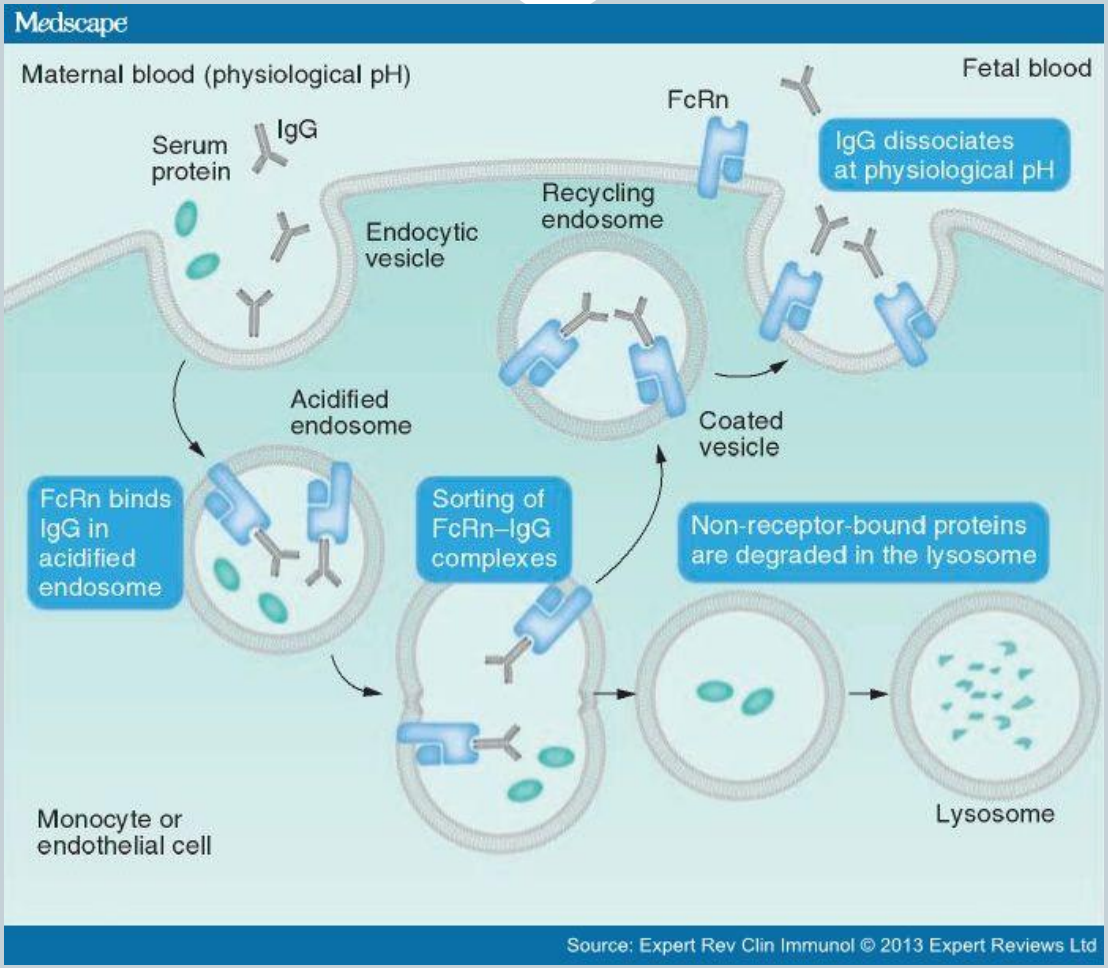
Mother-to-child Transmission (MTCT) of HIV

- The mechanism of infection for the vast majority of children with HIV
- Without intervention MTCT is between 25-40%
- With appropriate interventions, risk of transmission is <<1%

Standard adult HIV Testing

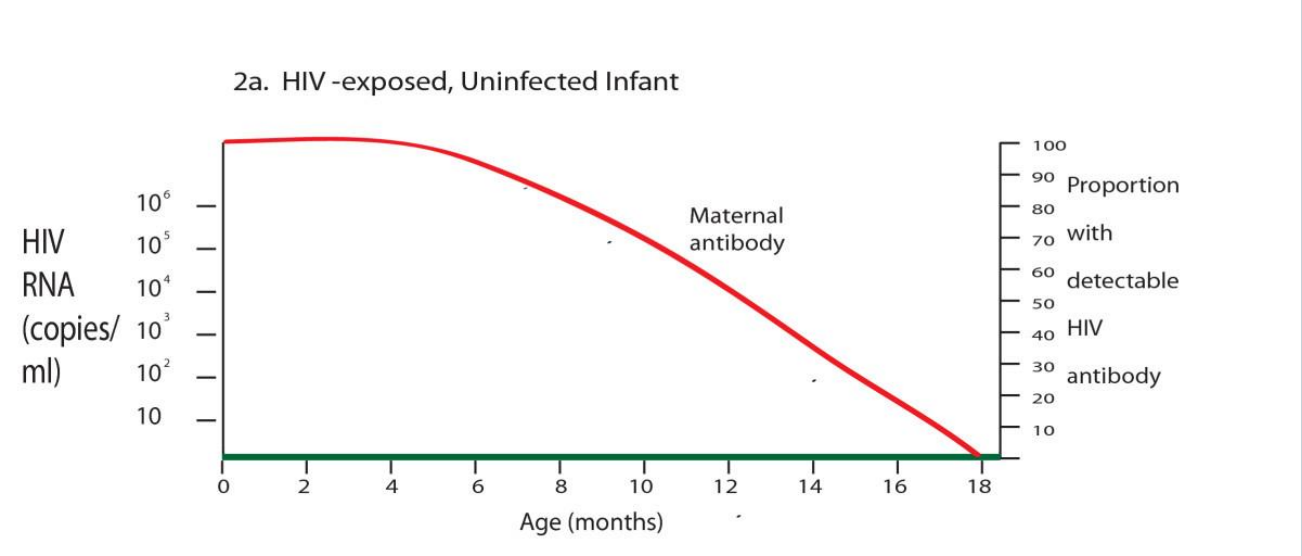
- Screening with 4th generation ELISA
- Confirmation with supplementary tests and Western Blot
- Subsequent testing for HIV RNA viral load and resistance genotyping
- Other options include rapid tests
  - (screening)

Diagnostic issues in infants



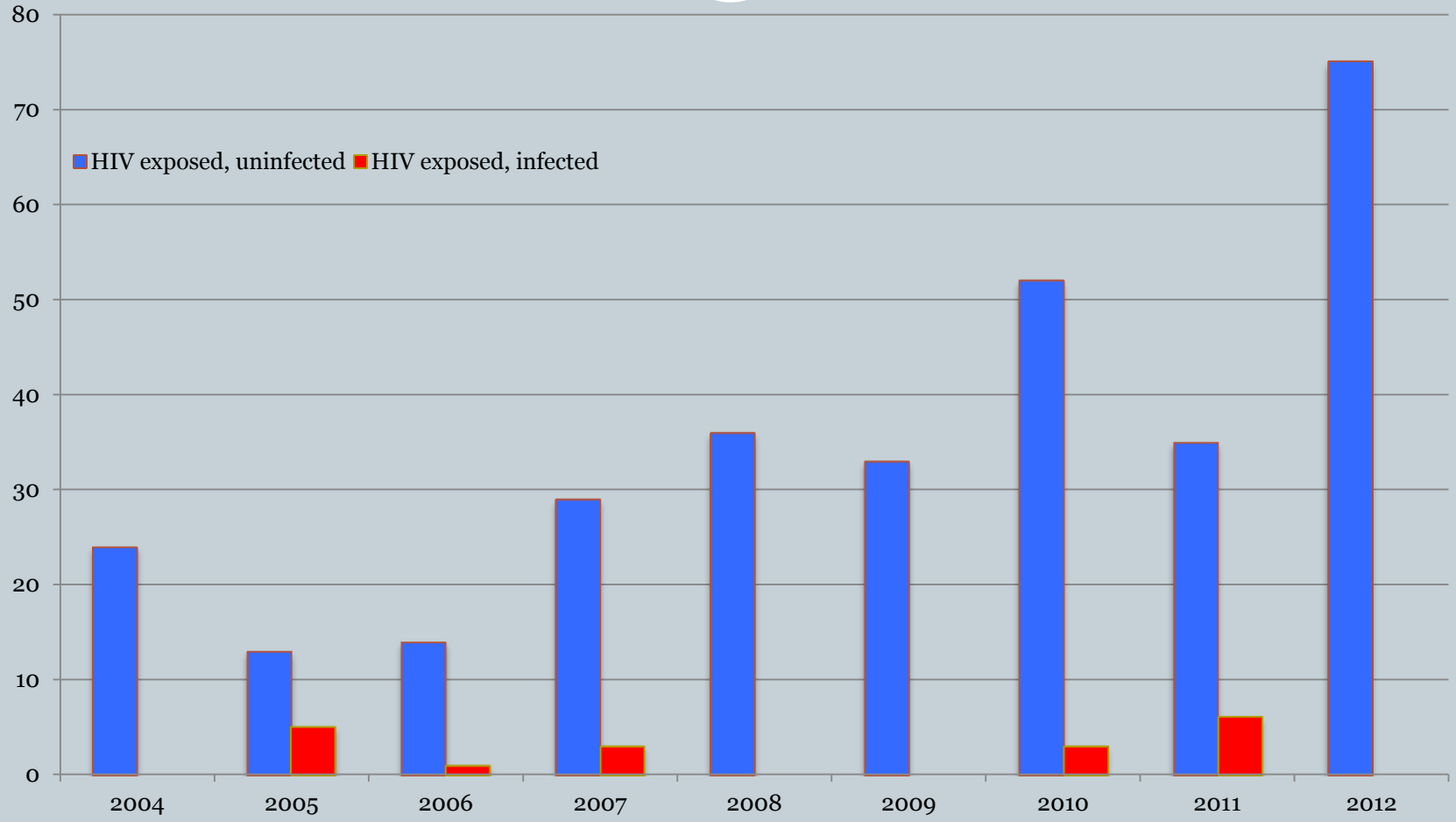
Infant HIV testing

- Virologic only (prior to 18 months)
- Serologic testing (after ~18 months)

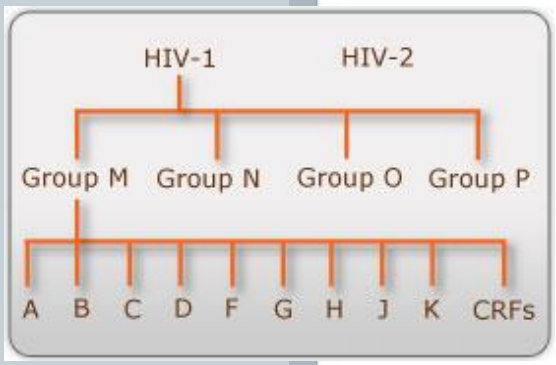


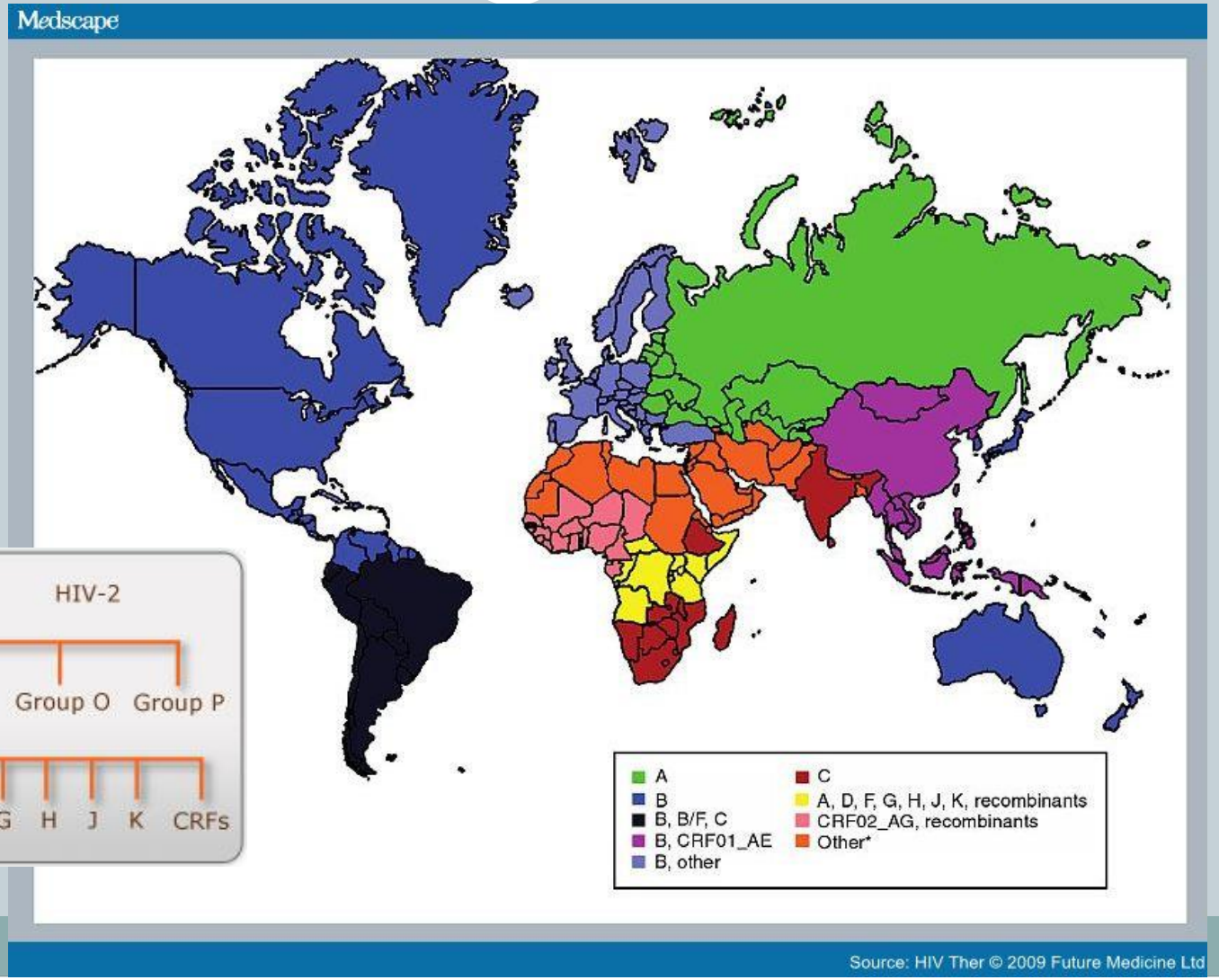
Ciaranello et al. BMC Medicine 2011, 9:59

HIV-exposed uninfected children (APSU)



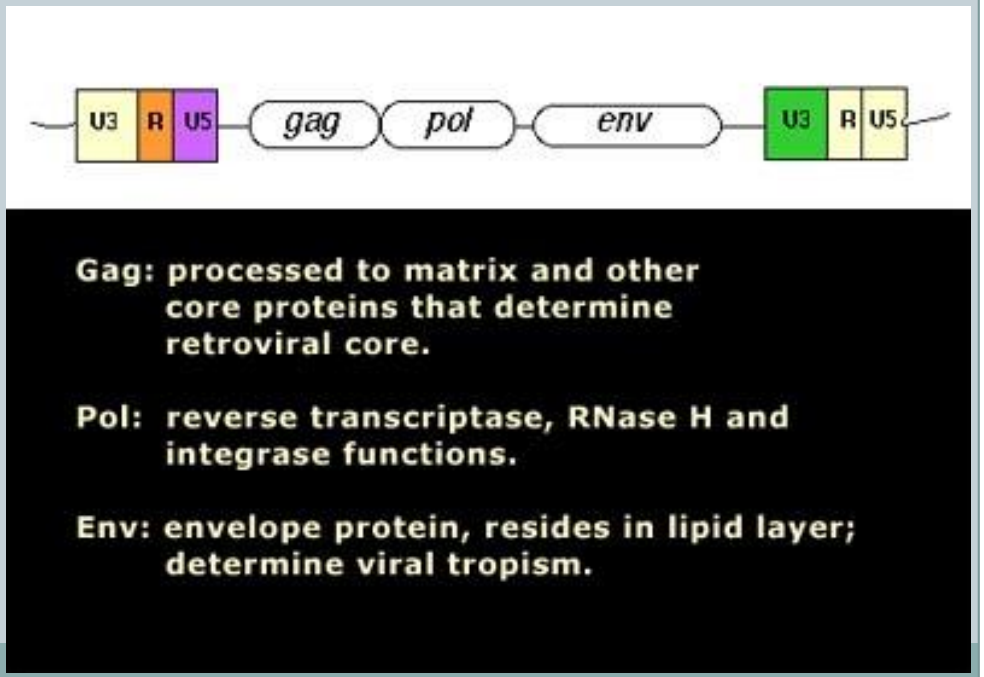
HIV Groups and Subtypes





HIV Proviral DNA PCR

- Qualitative PCR
- Target is HIV-1 *gag* gene
- Limit of detection can be 10 copies/mL (assay and sample-dependent)
- Limitations
  - Transport
  - Access to testing
  - Non-subtype B virus



HIV RNA PCR

- Designed as viral load PCR
- Levels relatively low at birth but high by 1-2 months of age
- Appears as sensitive and specific as DNA PCRs
- More widely available than DNA PCRs

- Limitations
  - Potential interference from antiretroviral therapy
  - Possible false positives with low copy numbers
  - Blood volume requirements

BHIVA 2014 diagnosis of HIV infection in infants

- Gold standard test in infancy was HIV DNA PCR on peripheral blood lymphocytes
  - Equal or increased early sensitivity RNA PCR

- Infants infected intrapartum may have low peripheral blood HIV levels, so HIV DNA/RNA may not be amplified from all infected infants at birth.
  - Positive HIV DNA/RNA <72 hours = presumptive intrauterine transmission
  - Within first weeks sensitivity of the viral diagnostic tests increase dramatically - by 3 months 100% of non-breastfed HIV-positive infants are likely to be detected

BHIVA 2014 diagnosis of HIV infection in infants

- *Situations when infant should be tested using DNA PCR include*
  - Low volume sample: RNA assays commonly require greater volume (at least 1mL plasma).
  - MTCT may have occurred in utero, and subsequent maternal antiretroviral therapy could lead to a false-negative RNA result in an infected infant. (eg high risk such as late-presenting mother)

- Maternal sample should always be obtained for HIV DNA or RNA amplification with the first infant sample to confirm primers used detect the maternal virus

US NIH guidelines – August 2015

- HIV infection in infants should be diagnosed using HIV nucleic acid amplification virologic assays, include DNA and RNA PCR.
- Virologic diagnostic testing at birth should be considered for infants at high risk
- Virologic diagnostic testing should be considered 2-4 weeks after cessation of antiretroviral (ARV)
- Non-subtype B HIV may not be detected by commercial NAT, particularly HIV DNA PCR
  - Newer HIV RNA assays have improved detection of non-subtype B HIV, but there are still variants that are poorly detected - use newer HIV RNA assays should be used in this case

US NIH guidelines – August 2015

HIV proviral DNA PCR

- HIV DNA PCR detects specific HIV viral DNA in peripheral blood mononuclear cells.
- Specificity of the HIV DNA PCR is 99.8% at birth and 100% at ages 1, 3, and 6 months.
- Sensitivity of test at birth is 55% but increases to >90% by 2-4wks and 100% at 3mths

HIV RNA PCR

- HIV quantitative RNA assays detect extracellular viral RNA in plasma.
- Specificity (for results ≥5,000 copies/mL)* is 100% at birth and 1, 3, 6mths and comparable to HIV DNA PCR.
- Sensitivity is 25% to 58% during the first weeks of life, 89% at age 1 month, and 90% to 100% by age 2 to 3 months.

*HIV RNA levels <5,000 copies/mL may not be reproducible and should be repeated.

US NIH guidelines – August 2015

- HIV RNA can also:
  - be used as supplemental test for infants who with initial positive HIV DNA
  - provide virologic confirmation of infection and are less expensive and assesses baseline viral load
  - potentially be more sensitive than HIV DNA PCR for detecting HIV non-subtype B
- HIV DNA:
  - remains positive in most on ART
  - Whereas HIV RNA could potentially be affected by maternal ART or infant cART

ASID Perinatal algorithms (2014)

HIV PCR DNA or RNA can be used – highly specific and equivalent sensitivity

Table 2: Suggested Testing Regimen

TIME OF TESTING	TEST	
	PCR – Proviral DNA or HIV RNA[c]	HIV Antibody
Week 1	+	No
Week 6	+	No
3 months	+	No
6 months	No	No
12 months	No *(clinical visit only)*	No
18 months		Yes *(to document clearance of maternal HIV antibodies and confirm infant's HIV-ve status)*

- Testing should occur at least 2 weeks and 2 months after antiretroviral prophylaxis is ceased, hence testing at 6 weeks and 3 months.
- Whilst testing at 6 and 12 months is no longer recommended, clinical visits here provide the opportunities for clinical assessment, routine childhood immunisations and maintenance of contact with family

DNA or RNA?

- Sensitivity/Specificity
  - Both effective strategies in standard-risk settings
  - Additional issues if high-risk, infant cART, unusual genotype
- Practicalities
  - Sample volume
  - Local or send-away test
  - Turn-around time

What about serology?

- Case report:
  - Infant born to HIV-positive mother
  - Maternal VL negative at delivery
  - Infant completed 6 weeks zidovudine
  - Negative DNA PCRs at birth, 6 weeks, 4 months
  - Reportedly no breastfeeding/premastication
  - Declared uninfected

  - 20 months – diarrhoea, failure to thrive, oral thrush, lymphadenopathy – diagnosed with HIV1 with reactive serology and HIV1 RNA VL of 1.3 million/mL
  - ?Need for routine recommendation about confirmatory 18-month serology

Puertolas, Clin Pediatrics 2015 ePub ahead of print

International comparison

Time of testing	Australia	UK	US
1 week	DNA/RNA	DNA/RNA	(High risk)
2-3 weeks	No	(High risk)	DNA/RNA
6 weeks	DNA/RNA	DNA/RNA	DNA/RNA (1-2m)
3 months	DNA/RNA	DNA/RNA	No
6 months	No	No	DNA/RNA
18 months	Ag/Ab	Ag/Ab (Breastfed)	Not routine

Recommendations with limited evidence:
*Use RNA if non-B subtype HIV
*Use DNA if mother on cART
*Additional testing if high-risk

DBS and Diagnosis

- Use of dried blood spots for testing
  - Infant diagnosis
  - Remote settings
  - Home collection
- Total Nucleic Acid PCR (Xpert®)
- Qualitative RNA PCR (Aptima™)

Total Nucleic Acid Testing

- Cepheid Xpert® HIV-1 Qual
  - "Total" Nucleic Acid PCR = RNA and proviral DNA
  - 100µl of whole blood or 1 dried blood spot
  - 90 minute run-time

Charlie

- Charlie is 18 months old now
- His HIV RNA tests were negative at 5 days, 6 weeks and 3 months and Ag/Ab test was non-reactive at 18 months
- He is uninfected and discharged from HIV follow-up

Acknowledgements

- Dr Emma Best, Starship Hospital, Auckland
- SEALS Virology
- Dr Alex Carrera, Sydpath HIV Laboratory

References

- Burgard M, Blanche S, Jasseron C, et al. Performance of HIV-1 DNA or HIV-1 RNA tests for early diagnosis of perinatal HIV-1 infection during anti-retroviral prophylaxis. J Pediatr 2012;160:60-6.
- British HIV Association guidelines for the management of HIV infection in pregnant women 2012 (2014 interim review). HIV Medicine (2014), 15 (Suppl. 5), 1–77.
- Catlett, B. Performance evaluation of the Aptima HIV-1 Quant Dx assay for detection of HIV-1 in plasma and dried blood spots (DBS). Presentation from nrl.gov.au, accessed 5 May 2016.
- Lambert JS, Harris DR, Stiehm ER et al. Performance characteristics of HIV-1 culture and HIV-1 DNA and RNA amplification assays for early diagnosis of perinatal HIV-1 infection. J Acquir Immune Defic Sydnr 2003; 34: 512–519.
- Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf. Accessed (18 March 2016)
- Puertolas, Late ELISA Testing in Infants Born to HIV-Positive Mothers. Clin Pediatrics 2015 ePub ahead of print

Further information and useful resources

- http://www.asid.net.au/resources/clinical-guidelines
- http://aidsinfo.nih.gov/guidelines
- www.bhiva.org.uk