New proposals for WHO International Standards for Human Herpesvirus 6 and Adenovirus

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Proposed 1st WHO International for Human Herpesvirus 6 (HHV-6) for NAT-based assays
Background

- High worldwide seroprevalence (>95%).
- Variants A & B, only variant B causally linked with disease.
- HHV-6B infection associated with febrile illness in children <2 yrs.
- Following infection HHV-6 establishes life-long latency with periodic reactivations.
- Chromosomal integration of HHV6 genome found in ~1-2% of population worldwide. These individuals have persistently high levels of HHV-6 DNA in whole blood (>6 log₁₀ copies/mL).
- HHV-6B frequently reactivates in immunocompromised transplant recipients and occasionally leads to severe disease, particularly encephalitis, and increased mortality (HHV-6B encephalitis treated with anti-herpetic drugs).
- Critical to distinguish active infection from CI HHV-6.
Rationale

- Quantitative PCR is widely used in management of HHV-6 infection and disease

- Published European recommendations for management of HHV-6 infections in HSCT patients (Ljungman P., et al, European Conference on Infections in Leukemia. Bone Marrow Transplant. 2008;42:227-40), recommend use of quantitative PCR for both diagnosis of HHV-6 disease and the exclusion of CI HHV-6

- Range of different NAT methods in use, both in-house and commercial

- Results from QCMD EQA programme ‘demonstrated large variation in quantification of HHV-6 … highlighted the need for standardisation of HHV-6 viral load determination’
Proposal and source materials

• Proposal to develop 1st WHO International Standard for HHV-6 for NAT-based assays presented to and endorsed by the WHO Expert Committee for Biological Standardisation (ECBS) October 2010

• Candidate standard
  – Prototype HHV-6B laboratory strain Z29
  – Whole virus prepared in cell culture (to standardise entire assay)
  – Formulate in universal buffer (10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose) for dilution in matrix appropriate to individual assay system
  – Concentration ~ 1x10^7 genomes/mL (IU when established)
Collaborative study and intended use

- Collaborative study
  - Evaluate candidate standard against other HHV-6 samples (e.g. HHV-6 A, different strains, clinical samples)
  - Participants to represent clinical/reference laboratories, IVD manufacturers

- To ECBS earliest 2012

- Intended use by clinical and reference laboratories, IVD manufacturers, and EQA providers, to calibrate secondary references used in HHV-6 NAT-based assays.
Proposed 1st WHO International for Adenovirus for NAT-based assays
Background

- Common worldwide infection
- 52 serotypes, 7 subgroups
- Cause wide spectrum of diseases in immunocompetent, particularly children, manifesting as respiratory and gastrointestinal diseases, cystitis and conjunctivitis.
- Persists following 1 infection
- Significant cause of morbidity and mortality in immunocompromised individuals, particularly, in paediatric haemotopoietic stem cell transplantation (HSCT).
- Treatment with cidofovir (nephrotoxic).
Rationale

• Quantitative PCR is the recommended method for identifying HSCT patients at risk of potentially fatal disseminated adenovirus infection, and for the pre-emptive initiation and monitoring of antiviral therapy (van Tol et al. Bone Marrow Transplant. 2005;35 Suppl 1:S73-6).

• Early and accurate diagnosis by NAT important to identify risk of adenovirus-associated disease and reduce incidence of illness and death.

• Range of different NAT methods in use, both in-house and commercial.

• Variability in results from different methods makes it difficult to interpret and compare results, and develop uniform therapeutic strategies.
Proposal and source materials

- Proposal to develop 1st WHO International Standard for Adenovirus for NAT-based assays presented to and endorsed by the WHO ECBS October 2010

- Candidate standard
  - Subgroup C - associated with persistent infection, common in young children, causes disseminated disease; subtype 2 - prevalent worldwide
  - Whole virus, grown in cell culture (to standardise entire assay)
  - Formulate in universal buffer (10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose) for further dilution in matrix appropriate to assay
  - Concentration ~1x10^7 – 1x10^8 genomes/mL (IU when established)
Collaborative study and intended use

- Collaborative study
  - Evaluate candidate standard against other AdV samples (e.g. different subgroups/subtypes, clinical samples)
  - Participants to represent clinical/reference laboratories, IVD manufacturers

- To ECBS earliest 2012

- Intended use by clinical and reference laboratories, IVD manufacturers, EQA providers to calibrate secondary references used in adenovirus NAT-based assays.