**Title:** Impact of deep-sequencing for the identification of viruses in pediatric lung transplant recipients

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**Background:** Respiratory viruses have been linked epidemiologically to bronchiolitis obliterans syndrome in lung transplant recipients. While molecular diagnostics have improved the recovery of respiratory viruses, deep sequencing (DS) provides an opportunity to explore beyond the routine capacity of available platforms.

**Methods:** As part of an ongoing study in the Clinical Trials in Organ Transplantation in Children, prospective serial nasopharyngeal (NP) and bronchoalveolar lavage (BAL) specimens were collected. A subset was interrogated by 1) respiratory multiplex PCR (Luminex xTAG) that identifies 17 viral species but does not differentiate enterovirus from rhinovirus and 2) next generation sequencing using the Roche/454 Life Sciences platform (DS) to an average depth of 25,000 reads per sample that were then analyzed using a customized bioinformatic pipeline to define viruses present in each sample. Concordance between samples was evaluated.

**Results:** Eighty samples (44 NP, 36 BAL with 31 paired NP/BAL sets) from 11 subjects were explored. Concordance between samples occurred in 72/80 samples; PCR and DS were both positive in five samples (1 each with adenovirus and RSV; 3 with enterovirus/rhinovirus). PCR and DS were negative in 67 samples. Only one of the methods detected a virus in 10% of the samples (8/80), all from NP specimens. In three PCR negative specimens, DS was positive (influenza C, human papillomavirus 14 and rhinovirus). DS was negative in five samples positive by PCR (one with human metapneumovirus and four with enterovirus/rhinovirus). DS had the added capability to differentiate enterovirus from rhinovirus.

**Conclusions:** As diagnostic techniques emerge, identification of potential viral pathogens is expanding. PCR may be more sensitive for specific viral targets. Deep sequencing can provide identification of new and unsuspected viruses not available by PCR.