Respiratory metagenomics network: validation outline.

Authors: Luke Blagdon Snell, Adela-Alcolea Medina.

Inclusion criteria:

- Samples from patients admitted to ICU
- For adults, lower respiratory tract samples only (sputum, ETT/TA, BAL, NDL, pleural fluid)
- For paediatric sites, other respiratory tract samples can be processed.

Protocols:

- Strictly following the version 1.0 bead-beating protocol provided.
- Aim to run the samples from start to loading the library on the same day.
- Alternatively, samples can be left incubating at 4 degrees after the PCR stage overnight before continuing the library preparation.

Analysis

- Analysis of sequencing data should utilise the NOE Bioinformatics pipeline v3.7, with reference to the Reporting Guidelines v1.0

Sample number and pathogen composition:

- Aim for n = 50-100
- A range of sample types depending on the sample type that each site most frequently processed.
- Aim to test a range of gram negative, gram positive, viral and fungal positives.
- Aim to test ~25% samples negative by culture.
- Any unusual or atypical organisms would be appreciated, for instance *Pneumocystis jirroveci*, *Mycoplasma pneumoniae*, *Nocardia sp.*, *Chlamydia psittaci*.
- Samples can be run up to 72 hours post sampling-date , however this is likely to compromise detection of RNA viruses and exact effects on organisms is unknown.
- Samples should be fresh (not frozen)

Run controls:

- For each run a positive control and negative matrix control should be used
- Positive control: Zeptometrix RP2.1
- Internal control: Tobacco mosaic virus. This should be added to each sample and negative matrix control.

Sample and metadata collection:

- Pre-defined meta-data should be collected for samples.
- This includes data such as samples type and sample date.
- Minimal patient data such as age, gender, ward, admission date, ventilation status.

Confirmatory testing:

- Please save any residual original sample, extract, and dsDNA elute. Residual samples may be frozen at –20°C after processing.
- We aim to confirm any discrepant results using these residual materials (exact details to be follow).

References

- For previous examples of metagenomics workflow validation please refer to:
    1. Charalampous, T., Alcolea-Medina, A., Snell, L.B. et al. Genome Med 13, 182 (2021). https://doi.org/10.1186/s13073-021-00991-y
    2. Adela Alcolea-Medina, Christopher Alder, Luke Snell et al. Research Square https://doi.org/10.21203/rs.3.rs-3148464/v1