Standards for microbial community molecular profiling

Jim F Huggett[1], Denise O'Sullivan[1], Thomas Laver[2], Sasithon Temisak[1], Ramnath Elaswarapu[1], Gavin Nixon[1], David Studholme[2] & Carole A Foy[1]

[1]LGC, Queens Road, Teddington, TW11 0LY. [2]Biosciences, University of Exeter, EX4 4QD. jim.huggett@lgcgroup.com

Introduction

- Advanced molecular methods, like next generation sequencing, offer the possibility to measure whole microbial populations (microbiome)
- These 'metagenomic' approaches offer the potential to understand industrial, clinical and environmental microbiology
- The complexity offered by metagenomic analysis is a considerable challenge for standardisation
- There is a lack of well defined materials to demonstrate data/platform comparability and knowledge on how best to produce and certify such materials

Aim

To identify standardisation needs and develop material to aid data comparability and permit evaluation of metagenomics platforms

Methods

- **Panel comprising 10 bacterial (table 1) selected to reflect a range of gram +ve and gram –ve human pathogens**
- **Bacterial gDNA sourced from ATCC**
- **gDNA quantified using absorbance at A260 (Nanodrop)**
- **Genome copy number used to define relative bacterial abundance**
- **Panel prepared to reflect a microbiome comprising dominant organisms and those present at a minority**
- **Difference between most and least abundant bacterial genome covers three orders of magnitude**
- **Initial experiments examined panel using the following massively parallel/next generation sequencing (NGS) approaches (figure 1):**
  - Illumina Hi seq whole metagenome (shotgun) sequencing
  - Roche 454 FLX shotgun sequencing
  - Roche 454 Junior amplicon (bacterial 16S ribosomal gene) sequencing

Conclusion

- **We have developed a QC material consisting of a panel of 10 bacteria**
- **Initial experiments highlight the potential applicability of the microbial material to metagenomic studies and further work is required to:**
  - investigate value assignment of the QC material
  - further define and identify sources of bias using NGS
  - analyse material using other non-NGS based methods
- **We are now producing a large batch of material for further analysis**
- **Panel will allow us to identify challenges associated with ensuring comparability when performing microbial profiling**

Results

Gram +ves	Gram -ves
Staphylococcus aureus *	*Pseudomonas aeruginosa*
Streptococcus pneumoniae	*Klebsiella pneumoniae*
Streptococcus pyogenes	*Acinetobacter baumannii*
Streptococcus agalactiae	*Escherichia coli*
Enterococcus faecalis	*Neisseria meningitides*

Table 1 components of the bacterial panel (*both methicillin resistant and susceptible strains included)

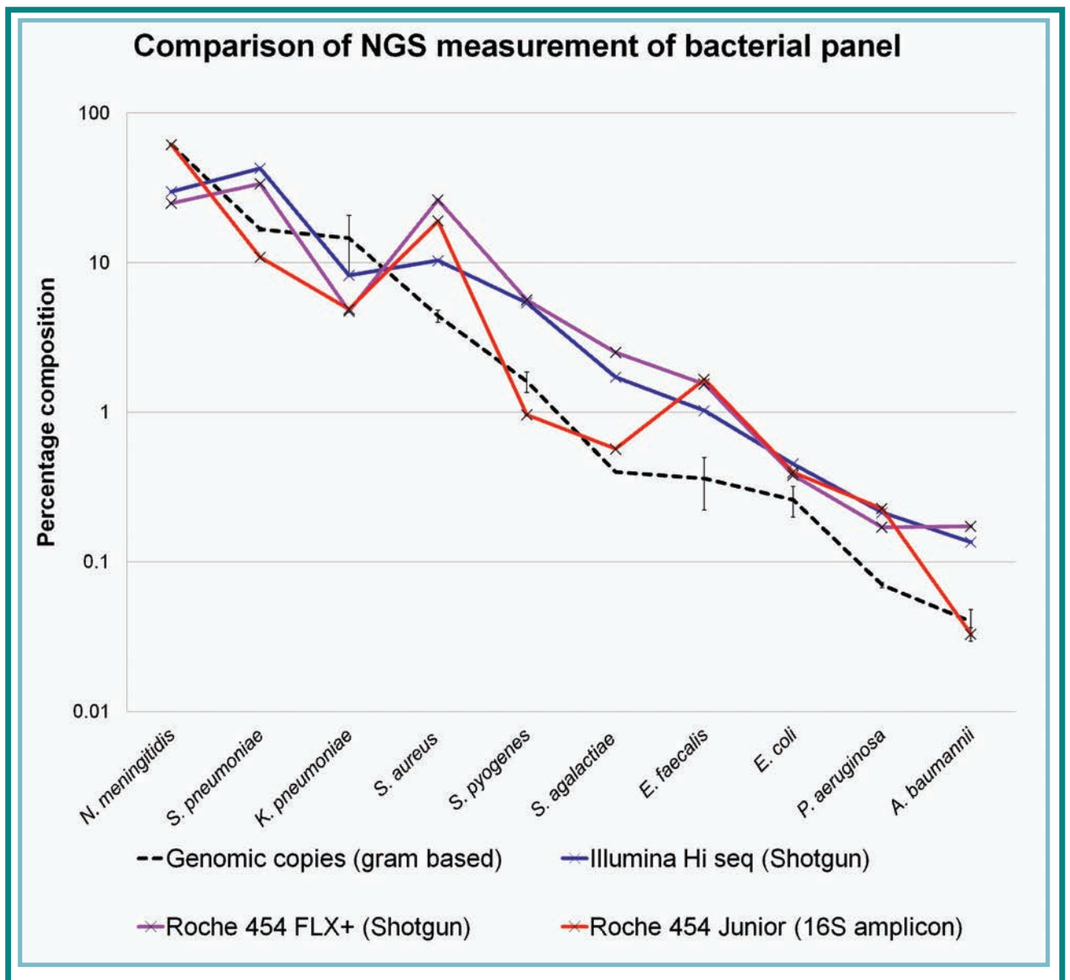

Figure 1. Comparison of NGS instrument analysis of the proportions of the mixed bacterial panel.
Error bars = 95% CI. NB single NGS run for each instrument

Preliminary analysis of material by Next Generation Sequencing suggests differences can occur between:

1. The NGS analysis results and weight based assigned value
2. The different NGS techniques used

These findings highlight the need for further work into A) assigning values to these types of materials and B) defining the biases that can occur between different technologies.

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Setting standards in analytical science