CLINICAL METAGENOMICS OF HOSPITAL-ACQUIRED PNEUMONIA

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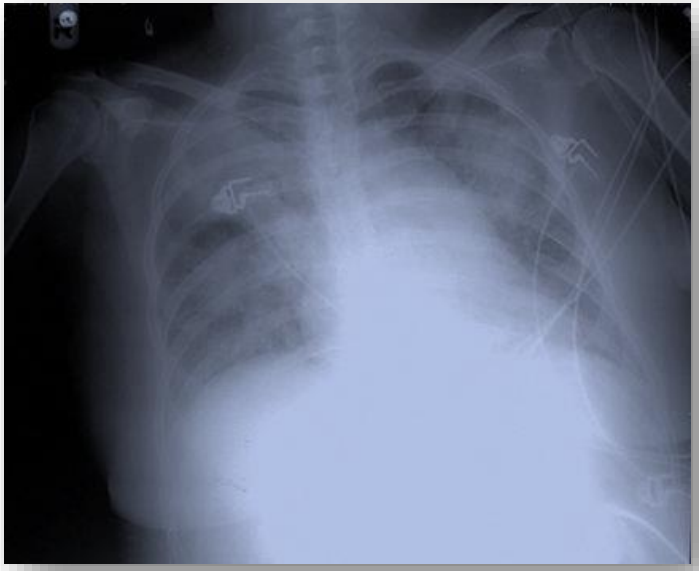
PIONEERING DIAGNOSTICS

Hôpitaux Universitaires Genève

BIOMÉRIEUX

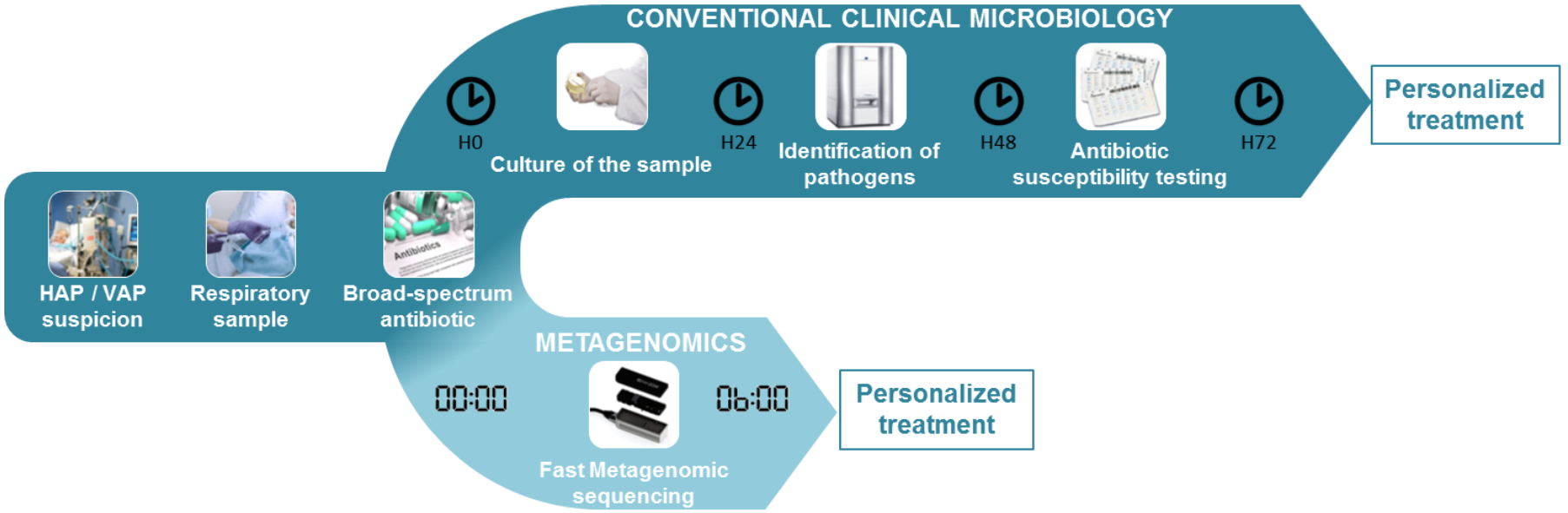
Introduction



- **Hospital-Acquired Pneumonia (HAP) and Ventilator-Associated Pneumonia (VAP)**
  - Common nosocomial infections in intensive care units
  - Highest morbidity and mortality rate of all nosocomial infections

- **Medical need to adjust antibiotic treatment**
  - Rapid identification of pathogens detected above clinical threshold
  - Rapid detection of drug resistances

Project Objectives



- **Bypass the time-consuming culture-dependent methods for identification of pathogens and resistance profiles in 6 hours**
- **Whole Genome Sequencing for exhaustive detection of bacteria :**
  - Conversion of metagenomics data into clinically actionable information
  - Quantification of pathogens to differentiate bacterial colonization from infection
  - Detection of Antibiotic Resistance Determinants and association to detected pathogens

Clinical Metagenomics Workflow for BronchoAlveolar Lavage (BAL) and mini-BAL

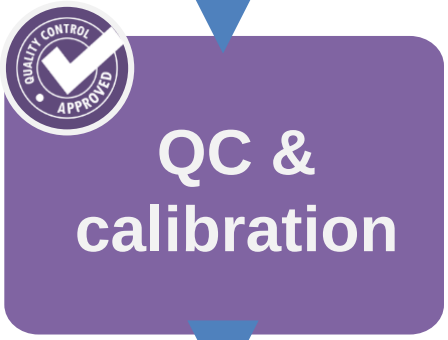
Selective extraction of bacterial DNA

Whole Genome Sequencing

Detection of pathogens and **A**ntibiotic **R**esistance **D**eterminants

Quality **C**ontrol ➔ Confidence for clinical decision

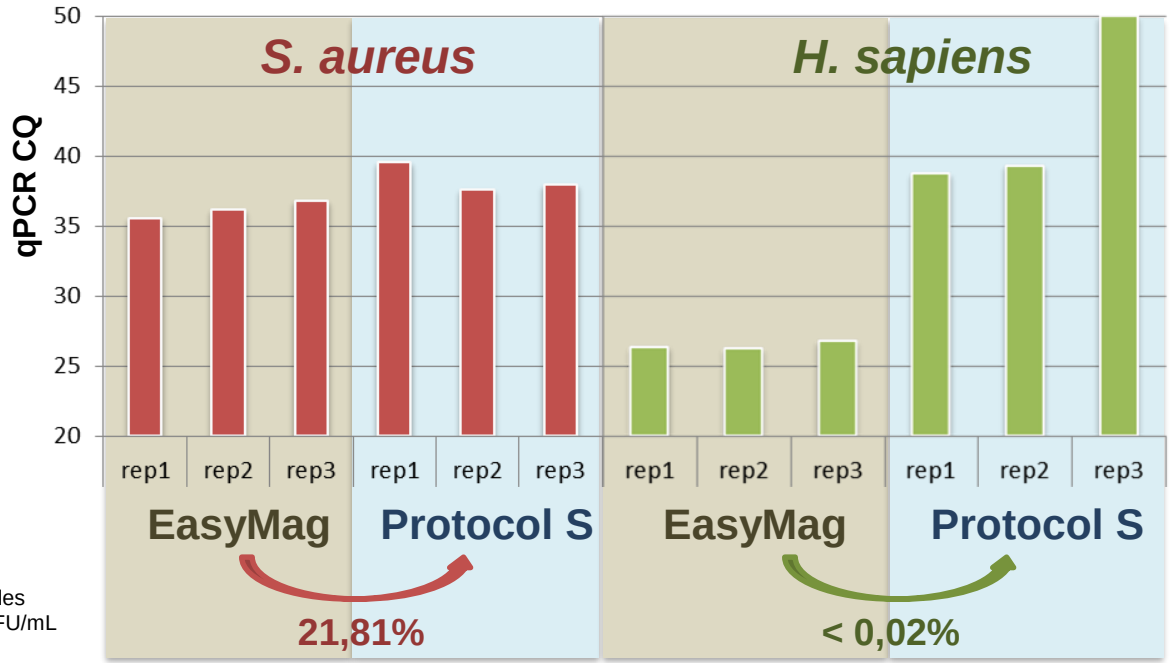
Calibration ➔ Clinical threshold



Clinically relevant information and metagenomic data

Improved sensitivity by specific sample preparation

- **Clinical samples are BAL or mini-BAL**
  - Contain huge quantity of DNA from patient ➔ Impact detection sensitivity and costs
  - Bacterial concentrations ranging from <1^{E}2 CFU/mL to >1^{E}6 CFU/mL
- **Selective sample preparation**
  - Protocol S eliminates 99,98 % of human DNA in BAL samples

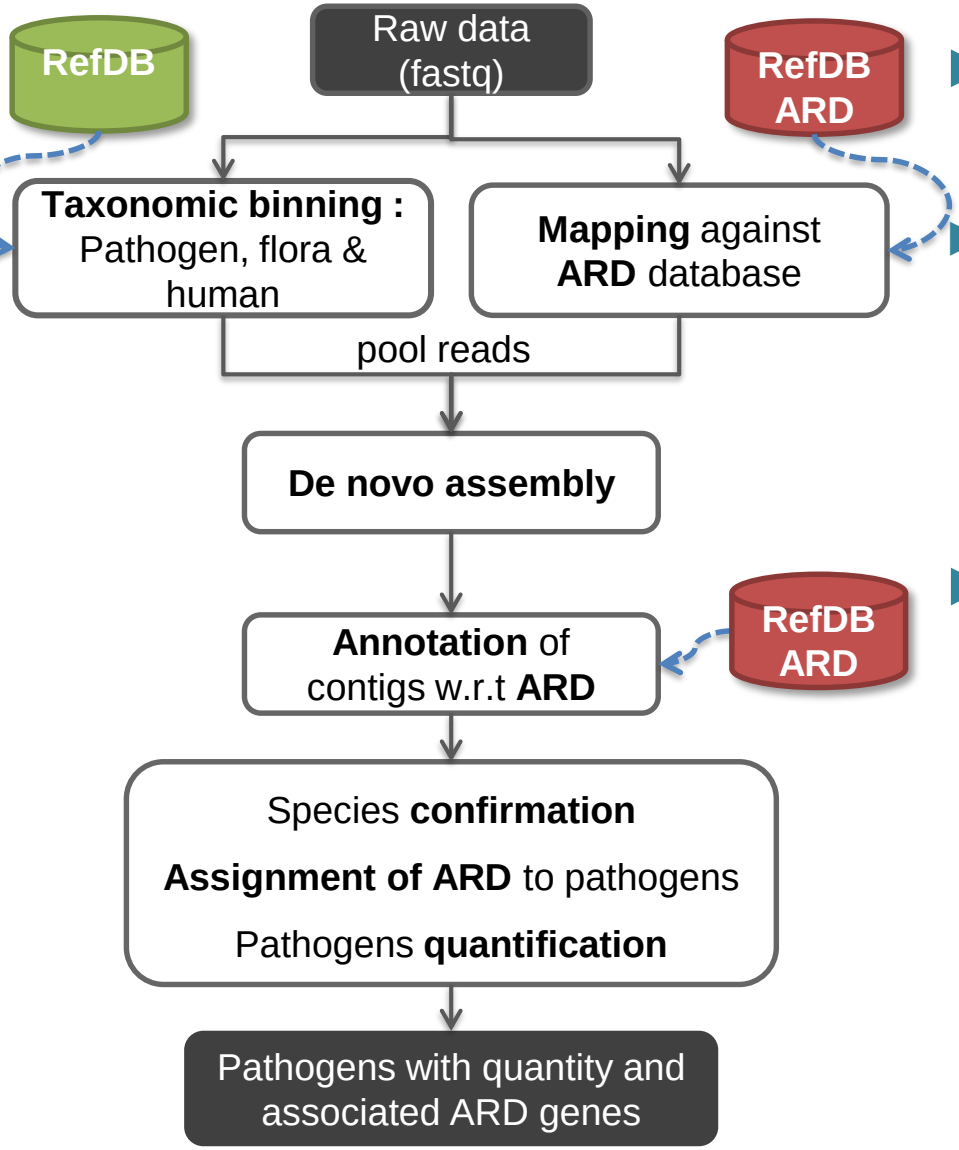


Pool of BAL samples spiked with 1^{E}3 CFU/mL of *S. aureus*

Increase in bacterial/Human DNA ratio > 1 000 X

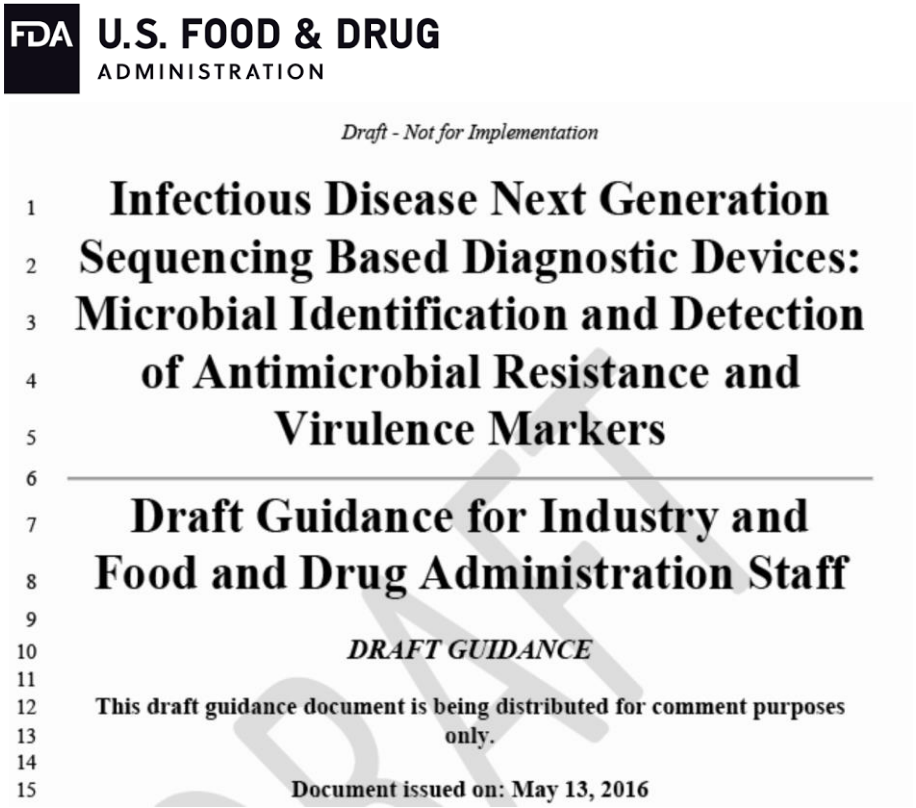
➔ Improvement of pathogen detections

Dedicated bioinformatics pipeline to extract clinical information



- **Dedicated DataBase construction**
- **Separation of taxonomic binning and Antibiotic Resistance Determinants mapping**
- **Extraction of clinical information:**
  - Pathogens identification with specificity checking (16S, MetaPhlAn, BLAST)
  - Quantification of pathogens
  - Association of ARD to pathogens
    - Possible ➔ Resistant pathogens
    - Not possible ➔ Resistant flora ?
    - ARD not present ➔ Sensitive pathogens

Quality control to facilitate clinical decision with confidence



FDA U.S. FOOD & DRUG ADMINISTRATION

Draft - Not for Implementation

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

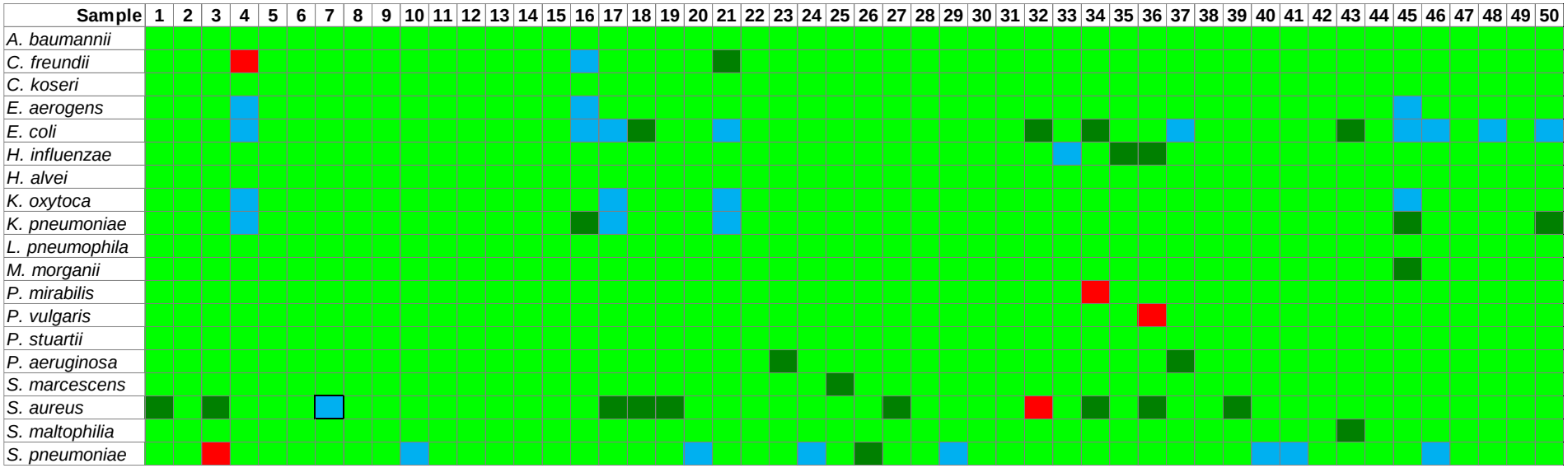
This draft guidance document is being distributed for comment purposes only.

Document issued on: May 13, 2016

- **Sample Processing Control (SPC) designed according to FDA guidance**
  - Spiked at sufficient concentrations into each clinical sample
  - Analyzed simultaneously with the clinical targets
- **FastQC**
  - Sequence reads quality control
- **Specificity checking**
  - Control correct sequence binning of pathogenic bacteria

Correlation of pathogen detection between culture and metagenomics

► Culture and sequencing detections match at 96,4%



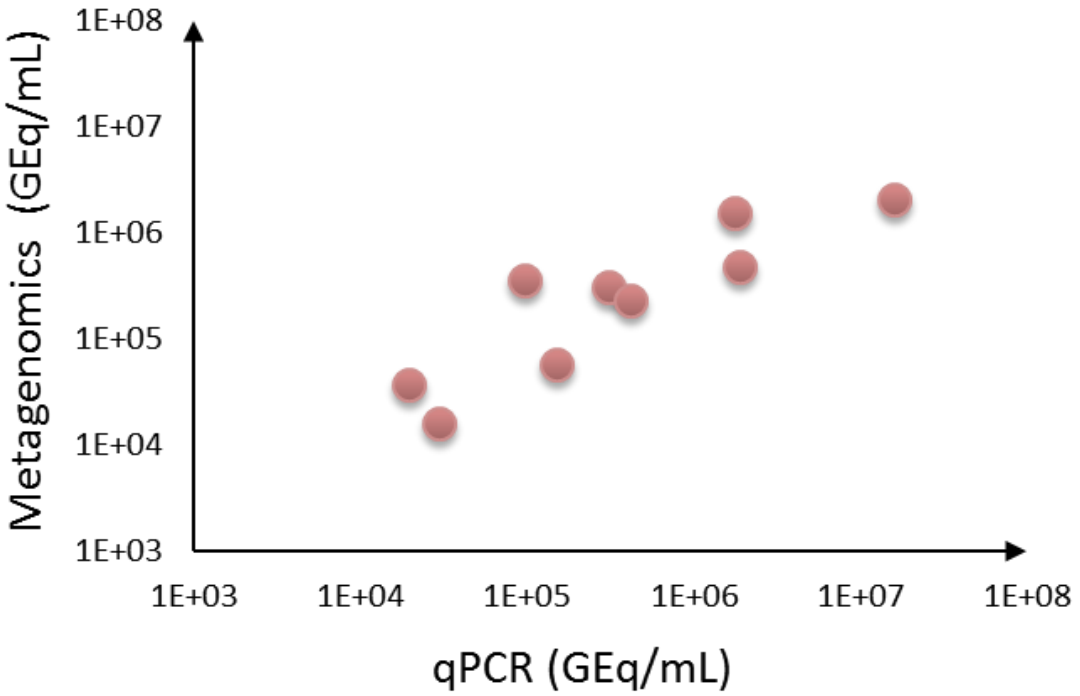
- **Less than 0,5% of "false negative"**
  - Observed in multi-infected patient ➔ competition effect
  - No sample would be declared as negative !
- **3,1 % of "false positive"**
  - Better sensitivity of NGS
  - In sample 7, presence of *S. aureus* DNA was confirmed by qPCR

Culture \ Metagenomics	+	-
+	25	5
-	29	891

Calibration for quantification of bacteria around clinical threshold

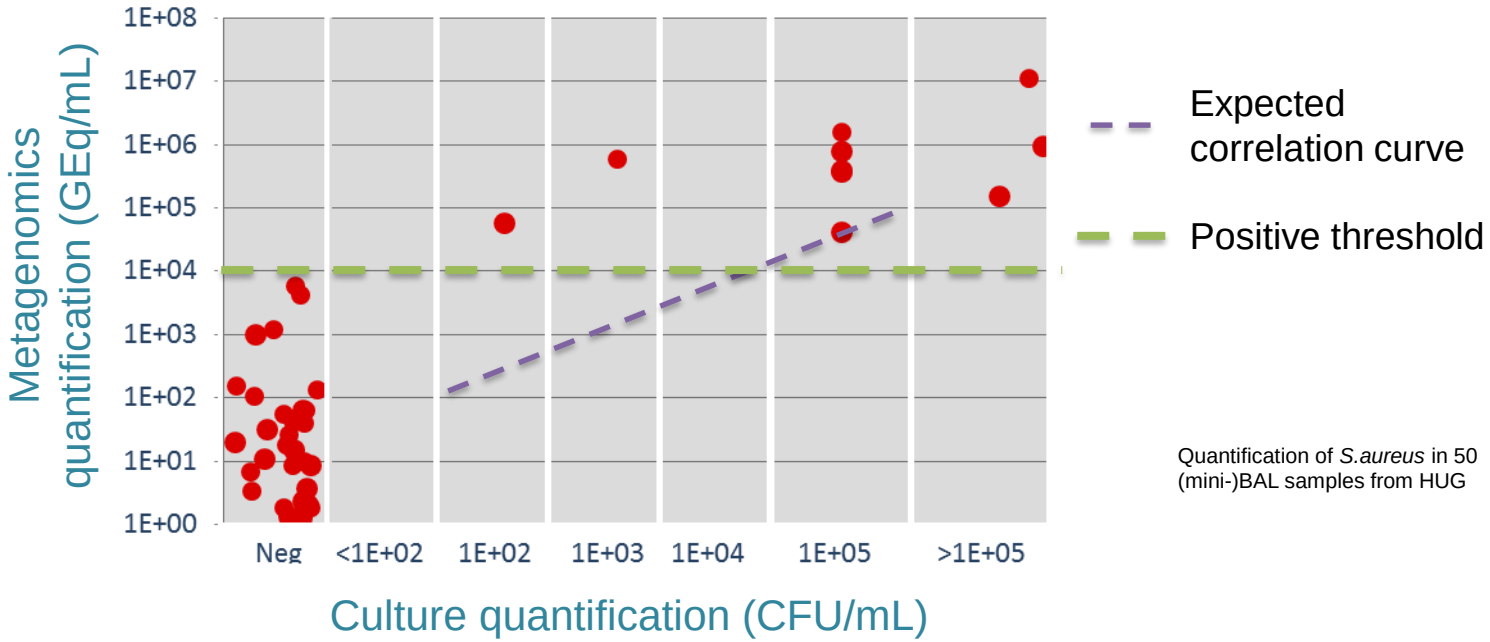
- **Quantitative detection**
  - Internal calibration and software allow for quantifying bacterial genomes in **G**enome **Eq**uivalents (GEq) unit.
  - Metagenomics quantification correlates with real-time PCR

Quantification of *S. aureus* in BAL samples by shotgun sequencing and real-time PCR (qPCR)



Calibration for quantification of bacteria around clinical threshold

- **Bacterial colonization or infection ?**
  - Clinical threshold at 1^E4 CFU/mL for BAL samples and 1^E3 CFU/mL for mini-BAL
  - No direct correlation between CFU and quantified genomes in clinical samples
  - However, culture positive and negative samples can be distinguished by genome quantification



➔ **Needs to adjust clinical threshold when expressed in GEq/mL**

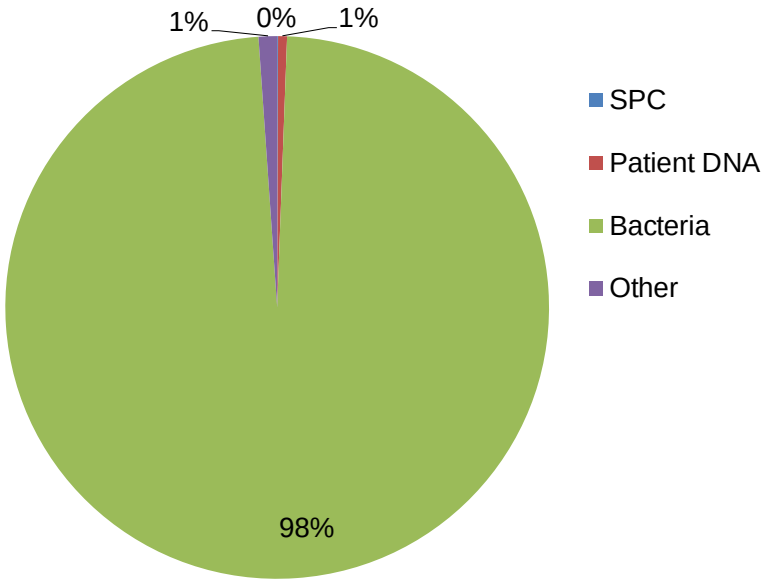
Clinical reporting of metagenomics data

BAL_107

Sample Processing Control

SPC not detected, however *C. freundii* and *K. pneumoniae* are detected above the clinical threshold

DNA content



Species Of Interest

Species	% Reads tot.	Concentration	Specificity control
Acinetobacter baumannii	0,01%		
Citrobacter freundii	**64,15%**	**7,1E+06 GEq/mL**	**YES**
Escherichia coli	1,17%		NO
Haemophilus influenzae	0,00%		
Klebsiella oxytoca	0,37%		NO
Klebsiella pneumoniae	**14,63%**	**1,6E+06 GEq/mL**	**YES**
Pseudomonas aeruginosa	0,00%		
Serratia marcescens	0,01%		
Staphylococcus aureus	0,00%		
Stenotrophomonas maltophi	0,00%		
Streptococcus pneumoniae	0,00%		

Antibiotic Resistance Determinants

	CMY_116	LEN-9
Citrobacter freundii	+	
Klebsiella pneumoniae		+

GTH-589 – *C. freundii* 1E+02 CFU/mL

Positive detection

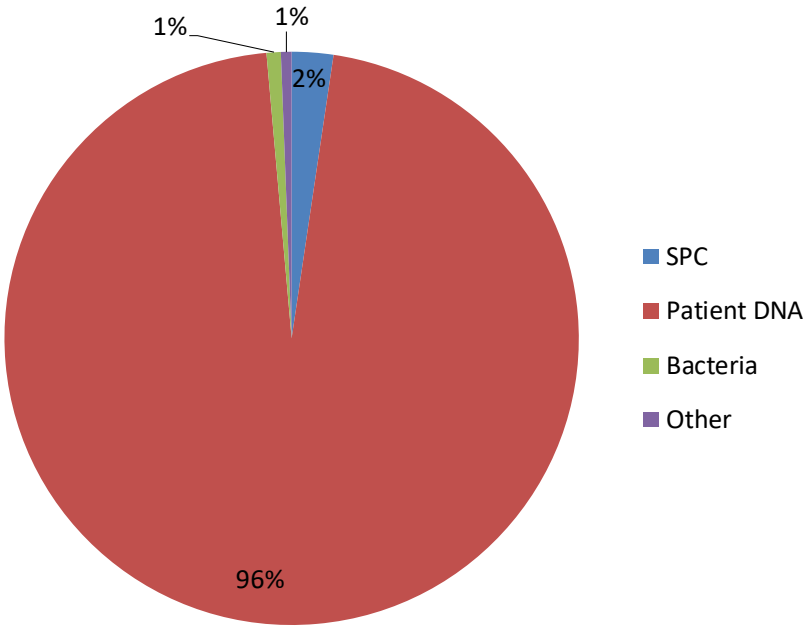
Clinical reporting of metagenomics data

mini-BAL_6

Sample Processing Control

SPC is detected, the limit of detection (7,1E+02 GEq/mL) is below the clinical threshold.

DNA content



Species Of Interest

Species	% Reads tot.	Concentration	Specificity control
Acinetobacter baumannii	0,00%	NEG	
Citrobacter freundii	0,00%	NEG	
Escherichia coli	0,00%	NEG	
Haemophilus influenzae	0,00%	NEG	
Klebsiella oxytoca	0,00%	NEG	
Klebsiella pneumoniae	0,00%	NEG	
Pseudomonas aeruginosa	0,00%	NEG	
Serratia marcescens	0,00%	NEG	
Staphylococcus aureus	0,00%	NEG	
Stenotrophomonas maltophi	0,00%	NEG	
Streptococcus pneumoniae	0,00%	NEG	

Antibiotic Resistance Determinants

No detection

Negative detection

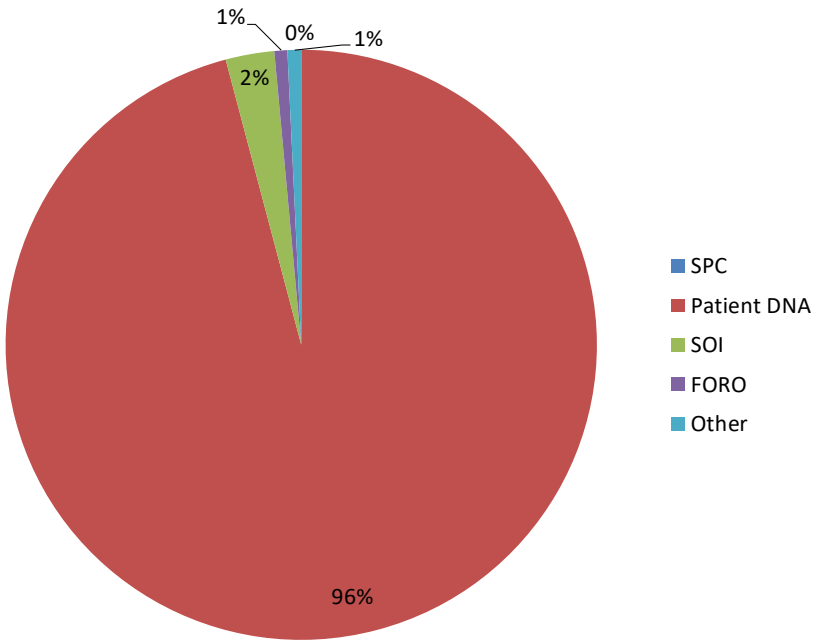
Clinical reporting of metagenomics data

mini-BAL_253

Sample Processing Control

SPC was not detected, the process did not meet the expected efficiency for safe interpretation of results

Microbial Documentation



Species of interest

Species	% Reads tot.	Concentration	Confirmation
Acinetobacter baumannii	0,00%	?	
Citrobacter freundii	0,00%	?	
Escherichia coli	0,02%	?	
Haemophilus influenzae	0,00%	?	
Klebsiella oxytoca	0,00%	?	
Klebsiella pneumoniae	0,02%	?	
Pseudomonas aeruginosa	0,00%	?	
Serratia marcescens	0,00%	?	
Staphylococcus aureus	0,00%	?	
Stenotrophomonas maltophi	0,00%	?	
Streptococcus pneumoniae	0,00%	?	

Antibiotic Resistance Determinants

?

Invalid detection

Conclusion and perspectives

Pathogens detection ✓

Pathogens quantification ✓

Sample Processing Control ✓

ARD detection ✓

ARD association to pathogens ✓

- Validation of the metagenomics workflow
- Inclusion of new centers

Time To Result ✗

- Automation of sample preparation
- Continuous evolution of NGS platform :
  - Gain in speed
  - Sequencing depth
  - Cost reduction

BIOMÉRIEUX

Thank you

HUG Hôpitaux Universitaires Genève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