Accreditation of a clinical metagenomics pipeline

Dr Christophe Rodriguez

Microbiology Dpt, INSERM U955 Team 18, Head of Platform NGS IMRB, NRC hepatitis B, C, delta, Henri Mondor University Hospital, Creteil, France,





Introduction



- COFRAC is the French committee responsible for translating recommendations of quality norms into practice and responsible of French lab accreditation.
- In France, accreditation is mandatory for all medical tests according to standard 15189 (17025 for hygiene).
- However, for innovative analyzes, a 3-year period has been granted for compliance after the start of the activity.
- In this context, a guide for accreditation of NGS analyzes has been produced by experts from oncology, onco-hematology, genetics and microbiology.

Accreditation in Mondor

- Undiagnosed infectious diseases by Shotgun Metagenomic (15189)
- Microbial genomic for Hygiene (17025) ٠





Clinicians

Infectious disease syndrome







NGS platform











Extraction and pre-PCR lib

Post-PCR lib

Sequencing

Analysis

Lab organizations







Analysis Specifications for Lab type 1/2

- List of necessary documents (specific to informatic for medical use of NGS)
 - Description of the informatic organization
 - Bioinformatic Pipeline qualification procedure
 - Method validation file (specific for bioinformatic)

Mondor Informatic organization



Pipeline life cycle



Validation method files



VMF Recommendations



	Vérification (portée A)			Validation (p		rtée B)
CRITERES A EVALUER	Méthode quantitative	Méthode qualitativ		Méthode quantitative		Méthode ualitative
Fidélité (répétabilité et fidélité intermédiaire)	Essai	Essai		Essai		Essai
Justesse/exactitude (approche)	Essai	Essai		Essai	$\langle \langle $	Essai
Incertitudes/facteurs de variabilité et évaluation	Essai	Maîtrise de facteurs de variabilité	6	Essai	> 1 > 1	aîtrise des acteurs de variabilité
Comparaison avec méthode déjà utilisée au laboratoire ou autre méthode du laboratoire (appareil en miroir ⁹ , EBMD) et analyse des discordances ¹⁰	Essai	Essai	NIN.	Essai		Essai
Intervalle de mesure (Limite de quantification et limites de linéarité)	Bibliographie	OPIL		Essai		1
Interférences (lipémie, hémoglobine plasmatique, bilirubine, médicaments,)	Bibliographie	Bibliograph	0	Essai		Essai
Contamination entre échantillons (s'il y a lieu)	Bibliographie	Bibliograph	9	Essai		Essai
Robustesse	Bibliographie	Bibliograph	e	Essai		Essai
Stabilité réactifs (après ouverture, embarqués)	Bibliographie	Bibliograph	e	Essai		Essai
Intervalle de rétérence (valeurs usuelles)	Bibliographie (fournisseur ou autre, s'assurer de la cohérence avec l'état de l'art)	Bibliograph	e	Essai		Essai
Limite de détection	/	Bibliograph	е	/		Essai
Spécificité/sensibilité analytique	/	Bibliograph	e	/		Essai
Le dossier doit conclure sur l'avis d'aptitude ¹¹ de la méthode ou du système analytique.						

Tableau résumé des performances à évaluer lors d'une vérification/validation de méthode quantitative ou qualitative (selon NATA note n°17 – juin 2012) :

Fidelity assay: Repeatability and reproducibility

Trueness: Sen, Spe, NPV, PPV

Uncertainty: (based on trueness and fidelity)

Comparison with other method

Level of measurements and scaling

Interferences

Contamination, Robustness, stability : NA

Positivity cut off for interpretation

From GTA04 COFRAC

Data preparation

- Aims
 - Produce data with characteristics similar to experimental ones
 - Set of data which simulates various experimental conditions
 - Set of data which match conditions for evaluation
- Specifications of data
 - Synthetic -> Guarantee of known results
 - Mixing of different genomes (reference to choose among virus, bacteria fungi) including human genome
 - DNA/RNA
 - Mutations (Sequencing errors, mutations of the micro-organisms)
 - Fragmentation
 - Sequence length
 - Quality of sequencing
 - Different dilutions
 - Control of random

=> Software dedicated to this task: RandomRead

Data preparation



MetaMIC performances

- <u>Fidelity assay</u>: Repeatability and reproducibility
 - No difference between the both (no "operator", no lot...) -> Only one test is possible
 - Objective -> coefficient of variation must be 0 whatever pathogen consider -> Stability of the software is required
 - Subset of pathogens and dilution is enough (no interest to test a broad spectrum)
- Contamination, Robustness, stability
 - Not assessed for Softwares

MetaMIC performances



Analysis comparison

- Pertinence of the method ?
 - All comparison with MetaMIC were always significantly in favour of MetaMIC
 - Are we the best or is there any bias ?
 - Data set test were produced following an "experimental protocol" and the software was designed to fit with these parameters
 => Analysis is biased by the experimental protocol
 - The conclusion of comparison study will be (I hope): your analysis method is the best for your experimental method... Which is a limited, but interesting, result from the quality point of view.

Question: Probability to interpret a positive sample -> positive?

Variable : dilution factor microorganism/human, percentage of informative sequence in your system (#sensitivity of the system)









Conclusion I

Some important Informatic/bioinformatic NGS specific points must be controlled to reach accreditation:

- Define your organization: what is performed by your laboratory ?
- Define the way of your data: are you sure all in under control ? Access must be secure and open only to people which have the knowledge and are accredited.
- Define the life cycle of your Software: How you can secure your update ?

Conclusion II

Validation method must be done specifically for your software:

- To evaluate the stability of your results (reproducibility)
- To evaluate the sensitivity/specificity...
- To evaluate the better experimental condition to maximize the probability to correctly interpret your results.
- The quality of the test data set is critical

Validation method of your software must be completed with a real-life experience (another validation method file) to confirm the results

Finally, 15189 is not so easy to reach but analysis evaluation give us many information to better understand how to improve and understand the mechanism of calculation of the results with very limited experimental test.

Many Thanks

MetaMIC Project

Vanessa Demontant **Guillaume Gricourt** Melissa Ndebi Anais Nguyen Christophe Rodriguez Jean-Michel Pawlotsky **Emilie Sitterle Paul-Louis Woerther**





UPEC