Medical and Pharmaceutical Applications of Microbial Biodiversity (MPAMB)

Teaching Unit, 2020-2021

Master 2, Fundamental and Applied Microbiology (MGB)

Session 1-Examination, Feb. 16th, 2021

1. Please describe the evolution of bacterial and viral *in Vitro* diagnostics and genotyping methods since their origin both technically and concerning fields of applications. **3 POINTS. 5 Keywords or alias**

From Phenotypic (growth, identification, culture, microscopy, direct examination) to Immunologic (monoclonal antibody, ELISA) and molecular diagnostics (hybridization principle). WGS and Mass-Spectrometry as the ultimate tool.

2. What are the 11 mandatory vaccines in France ? Against which diseases are they used ? **2,5 POINTS**

Diphterie : Corynebacterium diphteriae
Tetanos
Coqueluche : Bordetella pertussis
Polyomyelite
Rougeole
Oreillons
Rubéole
Hemophilus influenzae B : meningite
Meningocoque : méningite. Neisseira meningitidis
Pneumocoque : pneumonie et méningite. Streptococcus pneumoniae
Hepatite B

3. What are the factors that are contribute to the bacteriophages-bacteria co-evolution process ? **(3 POINTS)**

Both bacterial and Phage factors (genomic escape)

Rceptor Accessibility
Cellular mimicry. (extra-cellular modifications)
Quorum sensing
RM
Abortive infection
Superinfection exclusion (intra-cellular modifications)
CRISPR-cas / acr proteins

4. What is Clinical Research, how is it organized ? Why and What for is it important ? **(2,5 POINTS)**

Definition : the study of drug actions in human beings, split into different phases (I,II,III,IV) required for market approval

1. Why is it important do distinguish Salmonella at a fine taxonomical level? How is it done and give some practical applications. **(3. POINTS)**
2.

To distinguish SEROTYPES, i.e. antigenic formula (O,H,Vi)
It can be done using serotyping (with antibodies) or by many genomic techniques
To distinguish the origin of Food-born infections, to track outbreaks.

3. What are the major requirements for efficient foreign DNA recognition and cleavage during CRISPR interference (example of type I and type II systems) ? **(3. POINTS)**

- **Effective expression of crRNA and *cas* operon and crRNA processing**
- **Complementarity of spacer in crRNA to a protospacer in foreign DNA** (no mismatches in seed region, limited number of mismatches in remaining part)
- **Presence of PAM (protospacer adjacent motif) upstream or downstream** (depending on the type of CRISPR system) **from protospacer in foreign DNA sequence**
- **Presence of active CRISPR effector complex and Cas 3 nuclease** (for type I) **or Cas9 effector protein** (for type II)

4. Briefly describe the main steps of an experimental procedure that will follow the CRISPR adaptation process ? **(3. POINTS)**

- **Set up the plasmid transformation/conjugation or phage infection experiment**
- **Perform PCR analysis of new spacer acquisition in CRISPR arrays with primers to amplify the region between CRISPR leader and first spacer** (specific primer pair for each CRISPR array if several are present in the genome)
- **Analyze the size of PCR fragments (acquisition of new spacers will result in an increase in amplicon size)**
- **Sequence the amplicon to analyze the nature of acquired new spacers in CRISPR arrays**