Drug screening

1- Results of drug screening and IC_{50} calculations.
-Problems with screening on promastigotes
-What next is needed?
-Other approaches to screening drugs e.g., using genomics without cells
-Two approaches: cells/Omics

Leishmania detection and species identification:

2- Comparison between ITS-1 and cytochrome b PCRs in terms of:
- Sensitivity and specificity. What factors can affect PCR sensitivity?
- Possibility of post amplification analysis that could lead for species identification.

3- qPCR/KDNA:
- Advantages of kDNA PCR (classical PCR) over ITS-1 and cytochrome b PCR systems.
- Importance and need of qPCR-kDNA system.
- Disadvantages of kDNA PCR (classical PCR) over ITS-1 and cytochrome b PCR systems
- Sensitivity and specificity. What factors can affect PCR sensitivity?

4- qPCR-kDNA system followed by HRM:
- Sensitivity compared to other systems.
- If other used PCR systems are suitable for HRM analysis (what type of experiments are needed).

5- ITS1 and Leishmania cytochrome b PCR sequence analysis their suitability for creating phylogenetic tree.
- We have ITS1 and cytochrome b DNA sequence for the Old World *Leishmania* species, can these genes be used for New world *Leishmania* species? What do we need to check?

6- Comparison between different *Leishmania* species identification methods (RFLP, Sequence, HRM)
-What other techniques can be used to identify and type Leishmania?
Advantages/disadvantages

7- Blood meal analysis by RLB.
- Advantages and disadvantages.
- Discuss the reasons for a positive amplification of the mammalian cytochrome b gene and why it could give negative results by RLB.

8- Blood meal analysis by RLB:
- Multiple blood feed behavior in sand fly and its contribution to *Leishmania* transmission.

Challenging question:
- Problem solving of primer cross-reactivity.

- The possibility to develop a quantitative blood meal analysis that can differentiate between old and fresh blood meals.
- Drug testing – how can you adapt for your parasite