**Plaque assay**

**Introduction**

This assay will reveal if the parasites are able or not to be propagated in culture. It will give you a general idea of the fitness but you will not know which step of the lytic cycle is affected. Here only the size of the plaques is informative, not their number.

Materials

- 12 or 24 well plate with HFF

- Crystal violet solution 1x

12.5 g crystal violet in 125 ml ethanol mixed with 500 ml 1% ammonium oxalate

- 4% Paraformaldehyde (PFA)

!! Be careful, crystal violet and PFA are toxic !!

Procedure

- Prepare the HFF plate to receive the parasites: add to each well fresh medium and ± drug (if necessary).

- Dilute 10 μl of parasites from a totally lysed dish in 1ml of medium and then inoculate 20-30 μl into the first well of a 24 or 12w plate and pass 50 μl to the neighboring wells by serial dilution.

- Incubate for 7-8 days without disturbing the wells (the best is to store them in the bottom of the incubator shelf).

- After incubation for 7-8 days, aspirate the medium, gently rinse the infected monolayer with PBS, fix for 10 minute with 4% paraformaldehyde, wash with PBS and stain with crystal violet for 15-30 min (1/5 dilution of crystal violet in water, filter the solution with 0.22 μm filters).

- Remove the crystal violet solution, rinse twice with water and let it dry.

!! Be careful, crystal violet is toxic, provide container to collect the used crystal violet and tips, do not trash into the normal trash can !!