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MS2 Plaque Assay V.1

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Water TEAM

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DISCLAIMER

ABSTRACT

MS2 plaque assay based on EPA Method 1601 and modified as a spot plating assay (Beck et al., 2009).

PROTOCOL CITATION

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MATERIALS TEXT

- Sterile Tryptic Soy Broth (3%, 30 g/L)
- Bacto Agar
- Tryptic Soy Broth powder
- P10, P100, P1000 pipets and tips, sterile
- 125 mL and 250 mL flasks
- 1L bottles
- Auto-pipette and serological pipet tips
- 100 mm Petri dish
- Water bath
- Autoclave

DISCLAIMER:

MS2 Plaque Assay 1d 7h 15m

1 **Sterilize**: Autoclave pipet tips and growth media at least two days before the plaque assay.

2 **E. coli Famp Overnight Culture:** Add **10 µL** E. coli Famp (ATCC 700891) cyrostock to **10 mL** sterile Tryptic Soy Broth (30 g/L). Incubate **180 rpm, 37°C, 16:00:00** to obtain stationary phase.

3 **E. coli Famp Morning Culture**: Add **0.75 mL** E. coli Overnight Culture to fresh **100 mL** sterile Tryptic Soy Broth (3%) in a 250 or 500 mL flask. Incubate **02:30:00** **180 rpm, 37°C** 2h 30m

to exponential phase.

Remove Morning Culture after the incubation period elapses and leave flask at room temperature. Use host cells within 4-6 hours.

4 **Prepare Soft Tryptic Soy Agar**: Prepare fresh agar on the morning of the plaque assay. Add 30 g/L Tryptic Soy Broth and 7.5 g/L Bacto Agar to distilled water. Mix vigorously. Sterilize in autoclave at **121 °C** **00:45:00** . Remove molten agar from autoclave and place in **48.5 °C** water bath. 45m

Agar must be cooled before adding host cells to prevent thermal inactivation when adding cells to the molten agar.

5 **Sample Preparation**: Prepare MS2 samples by ten-fold serial dilution in micro-centrifuge tubes with **900 µL** 1X PBS. Vortex samples between dilutions.

6 **Spot Plating Assay:** After MS2 samples are diluted, prepare agar plates (10-15 mL) per plate to be used for spot plating. 1d 4h

1. First, prepare negative control plates containing only agar (no host cells).
2. Add **1 mL** of host cells per **50 mL** agar. Mix gently.
3. Add 10-15 mL of molten agar to bottom of Petri dish. Swirl to spread agar evenly across the bottom of the dish. Dry for 5-10 minutes or until the agar is solidified.
4. Label bottom of Petri dishes with sample information and draw grids for applying spots.
5. Spot MS2 samples onto the surface of the agar without touching the agar with the pipet tip. Apply the spots in labeled areas to keep track of sample information. Spot volumes can be anywhere from **1 µL** to **50 µL** . Incubate plates for **12:00:00** to **16:00:00** at **37 °C** .
6. Remove plates from incubator and count plaque forming units (PFU) per spot for each sample and dilution. Record plaque forming units, dilution, sample identification, spot replicates, and spot volume.
7. Calculate PFU/mL for each sample by aggregating total PFU across dilutions and accounting for the total undiluted volume:

PFU/mL = (Total PFU across dilutions) / (Total Undiluted Volume of Sample across dilutions).

Important: Provide enough spacing between spots to avoid spots running. Avoid moving plates before spots dry. Smaller volume spots dry faster than larger volume spots.

WaterTEAM: Spot 10 technical replicates of **10 µL** spots per dilution.

Pour Plating: As an alternative plating method, aliquot agar with host (e.g. in sterile culture tubes or centrifuge tubes) and add MS2 sample (record volume), swirl the tube gently in-between palms, and pour into the bottom of a 100 mm Petri dish. Swirl gently to evenly cover the bottom of the dish and dry completely.

6.1 This spreadsheet set up can be used for recording data and calculating concentrations.

A	B	C	D	E											F		
Sample	Dilution Factor, $1/10^n$	Method	Volume	Counts										Σ Counts	Undiluted Volume	Conc., C	Log10 C
Name	*n*	*(1) Spot plate or (2) Pour plate*	*(1) µL/spot (2) total*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*PFU*	*mL*	*PFU/mL*	
0	3	1	10	6	4	4	5	7	2	6	5	6	2	47	1.00E-04	4.70E+05	5.7
10	2	1	10	7	7	6	6	6	7	8	5	6	6	64	1.00E-03	6.40E+04	4.8
20	2	1	10	3	1	1	1	1	1	1	2	1	0	12	1.00E-03	1.20E+04	4.1
30	2	1	10	2	1	1	0	0	0	0	0	0	0	4	1.00E-03	4.00E+03	3.6
40	2	1	10	1	1	1	0	0	0	0	0	0	0	3	1.00E-03	3.00E+03	3.5
50	1	1	10	1	1	3	1	1	1	0	0	0	0	8	1.00E-02	8.00E+02	2.9
60	0	1	10	3	1	5	1	5	2	7	3	3	1	31	1.00E-01	3.10E+02	2.5

Screenshot of Spreadsheet for Plaque Assay Calculation. Yellow = Data Entry, Blue = Calculation or Formula.

Plaque Assay Calculation.xlsx