Polyoma Plaque Assay 11/9/67.

8 plates mouse embryo II° cultures.

Sucked off medium and washed 2x c ~5 ml Tris.

Virus stocks.

LP 148 A 1.4 x 10" pfu/ml rec'd from MV.
Diluted as follows 10 ml → 5.0 ml Tris-5% HS = 2.8 x 10^8 pfu/ml. (SAVED)

Made additional dilutions as follows. 10 ml → 5.0 (1:500) ; 10 ml → 4.0 (1:400) → 0.5 → 2.5 (280 pfu/ml)
→ 0.1 → 2.0 (70 pfu/ml)

① 0.2 ml of d.ln ctg ~ 70 pfu/ml. (~14)
② " " " " "
③ 0.2 ml of d.ln " ~280 pfu/ml. (~64)
④ " " " " "

P16/S4K ~ 5 x 10^10 pfu/ml.

Diluted as follows 10 ml → 5.0 ml Tris-5% HS = 10^8 pfu/ml (SAVED)
then
10 ml → 4.0 (1:400) (2.5 x 10^5/ml) ; 10 ml → 4.0 (1:400) 600 pfu → 1:2 (300 pfu/ml)
→ 0.5 → 2.5 (120 pfu/ml) → 1:2 (60 pfu/ml)

⑤ 0.2 ml of d.ln ctg 60 pfu/ml. (~12)
⑥ " " " " "
⑦ 0.2 ml of d.ln ctg 300 pfu/ml (~60)
⑧ " " " " "

Adsorbed for 30' at 37° then overlaid with agar-Eagles - 3.5% HS.
Incubated at 37° for 2 days then shifted to 33°

Polyoma Plaque Assay.

On 11/20 (the 12th day) overlaid with neutral-red agar and put at 33°

On 11/22 (14th day) could see clear plaques on the LP 148 A infected plates but none the P16 plates.

On 11/24 (16th day). nice clear plaques on LP but no easily visible plaques ∴ P16.

On 11/27 (19th day). counted plaques

LP 148 A

		Av	Predicted from titre of stock
①	9	12	~14
②	16		
③	36	33	~64
④	30		

Only few plaques seen with P16 ∴ left at 33°

On 11/30/67

		Expected on basis of titre
⑤	4	~12
⑥	lost	
⑦	6	60
⑧	4	

Either down by factor of 10 (stock is at 3 yrs old) or plating efficiency at 33 is lower

Attempt to make plaques on PY-6 12/2/67

8 ~~plates~~ trays of 6 plates each. Plated at ca 2×10^5 cells/plate on 11/30

Virus stock dilutions

LP14EA 2.8×10^9 pfu/ml

10 ml → 5.0 ml (1:50) ⟶ 0.1 ml → 5.0 ml (1:50) ⟶ 0.6 ml → 6.0 (1:10) 10^3 pfu/ml

Used 0.2 ml aliquots to infect ↓ 0.6 ml → 6.0 (1:10) 10^2 pfu/ml

Plates washed 1x ~ 25.0 ml Tris. Infected for 30' at 37° then overlaid with agar and different media then incubated at 35° or 37°

(see next page)

The first two of each set infected with 200 pfu and the second set infected with 20 pfu

38°

(1) (2)* (3)* (4) — 3.5% HTS + Hurtle — most cells dead - little stain - cells detached and floaty

(9) (10)* (11)* (12) — 0.5% HTS

(17) (18)* (19)* (20) — 0% HTS

[redacted]

(25) (26) (27) (28) — 0% HTS 1:25 Trypsin — Well stained layer although much clumping: easily visible plaques: but only few/plate by 11 days

(33) (34) (35) (36) — 0% HTS 1:100 Trypsin — Less well stained but clearly live cells in layer. Lots of clear areas but not certain these are plaques

(41) (42) (43) (44) — 0% HTS 1:250 Trypsin — Still less well stained but perhaps some plaques

33°

(5) (6)* (7)* (8) — 3.5% HTS + Hurtle — Same as 38°

(13) (14)* (15)* (16) — 0.5% HTS

(21) (22)* (23)* (24) — 0% HTS

(29) (30) (31) (32) — 0% HTS 1:25 Trypsin — Same as 38°

(37) (38) (39) (40) — 0% HTS 1:100 Trypsin — Same as 38

(45) (46) (47) (48) — 0% HTS 1:250 Trypsin — Same as 38°

* stained on 12/7
all others stained a 12/9