

Assay of Hemoglobin Transforming Factor.

8/30/52

10 ml Eugen-Leventhal Broth inoculated with hypodilized cultures of R.D. strain of H. I. (R.D. 6-27, log culture 270, 2-21-52) obtained from S. Goodgal. Allowed to grow overnight at 37° with very gentle shaking. The culture was quite turbid and contained a great deal of flocculent polysaccharide-like material. This was diluted 1/10 in sidearm tube and shaken gently.

Transforming principle (T.P.) obtained from S. Goodgal (350^{SWA}/ml) diluted in citrate-saline as follows.

Tube #	no. T.P.	(citrate-saline)
1	10 ²	1/10
2	10 ³	1/10, 1/10
3	10 ⁴	1/10, 1/10, 1/10
4	10 ⁵	1/10, 1/10, 1/10
5	10 ⁶	1/10, 1/10, 1/10

Seven tubes prepared containing 1.7 ml Eugen-Leventhal broth. 0.1 ml of above dilutions of T.P. added to tubes 1-6. To tube seven was added 0.1 ml 10² dil. of T.P. and 0.1 ml DNA-X solution 20%/ml. f.c. = 12/ml. ~~1-3-52 gentle shaking~~ Then 0.2 ml of H.I. culture (C.P. = .30 ~ 6x10⁹/ml) added to each tube 30' 37°, gentle shaking. Tubes plated according to following dilutions with 10 ml Eugen-Leventhal agar.

Tube #	dil.	add
1	10 ⁰	add 1.0 ml undil
2	2x10 ²	1/10, 1/10 add 1 " "
3	2x10 ³	1/10, 1/10 " " "
4	2x10 ⁴	1/10 " " "
5	2x10 ⁵	add 0.5 ml undil
6	10 ⁰	add 1.0 ml undil
7	10 ⁰	" " "

agar allowed to harden then incubated 120' 37°

then 10 ml agar containing 500 μ streptomycin/ml added to each.
 agar again allowed to harden and plates incubated 24 hr at 37°.

To .85 ml of cells used in transformation was added 0.15 ml sterile glycerol.
 frozen up at (-70°)

Results

<u>Tube#</u>	<u>dil of T.P.</u>	<u>count</u>	<u>Total count</u>	<u>cells/ml transformed</u>	<u>μ DNA</u> <u>$\times 10^1$</u>	<u>cells transformed</u> <u>$\times 10^6$</u>
1	not-p.	0	0	0	0	0
2	10 ²	424 x 4	1690	3.4 x 10 ⁶	.35	1 x 10 ⁶
3	10 ³	265 } x 4 240 }	1010	2.2 x 10 ⁶	.35	6.3 x 10 ⁶
4	10 ⁴	350 } x 4 264 }	1225	2.5 x 10 ⁵	.035	7.2 x 10 ⁶
5	10 ⁵	224 } x 4 219 }	888	1.8 x 10 ⁴	.0035	5.1 x 10 ⁶
6	10 ⁶	174	174	1.7 x 10 ³	.00035	4.9 x 10 ⁶
7	10 ⁷ + DNAase (1/2)	70	70	7 x 10 ²		

mean # cells transformed per μ DNA
 under these conditions: $6 \times 10^7 \pm 0.9 \times 10^7$