9/22/83

Infection of CB

306
314
317
312 } LAV; 18 hrs later α IF (from Montagnier

9/23/83

LW47 . . . co-cult.

LW47 x CB 317♂

991[cc] / 0906 } x 314 CB ♂

F1624 JM♂ 1p. culture - lamp O.K.

11/9/8?

Cloning of HUT 78
Capillary technique; without feeder
Total clones 21; med. RPMI 1640+20% FCS
Cl 19. - Nicole O.K.

11/10/8

Contr single-cell cloning.
15 out of 21 - One cell
HOS - does not grow - discarded.
Ti 7.4 - O.K viability 70%
HUT 78 - O.K - " - 65%
change cult. media; Moore & Esu

11/11/?

Cl 19 - chem. med. 10% TCGF
HUT 78 / V cell number. 6x10^5/ml
Ti 7.4 / V " " 4.3x10^5/ml.

11/15/83

Isolation of HTLV/AIDS

Cells: HUT 78 (perfore'n) . 2×10⁶ cells.
infected with sup. from W 6233, W 6542, F 6367 } RT±

Cultured in flasks 25 cm². RPMI 1640-20%

Culture tho Sarsey per RT ~~HUT 78/in/ ###~~ ~~Ti 7.4/in/ ##~~
Moaeni: BMq +
Spence Ollie, Hemophiliacs LAS, B Hagas; 3 flask; 2 amp. Kimiko Frazier. DNA-Veffi; 2 amp. from ATL - Japan Broder. c. carrier

11/16/83

Cloning of HUT 78 cells

Using PB feeder and capillary technique

10^5 cells PB and 1 cell/well

Total isolated cells . 35

Cultured in RPMI 1640+20% FCS

BM♂ AIDS ZAIRE - 3 amp.
Yan 11/16/83
BMq -"-"- Moaeni 2 amp.
PB ♀ Moaeni 2 amp.
PB ♂ Yan Moaeni 2 amp.
} C-carrier 5/3

11/21/83

Clones - HUT 78 outgrowth
5; plated in costar wells / 1.5 ml RPMI 1640 20%.

Clones PB feeder - contamination; discarded.
Cl 19 (TCGF 10%) Spl. subcultured.
doubling time 24 hrs.
HUT 78/V; Ti 7.4/V - O.K.

11/22/84

HUT 78/V; Ti 7.4/V = MOV.
expanded.
Cl. 19 subcultured. Cells to Nicole

HUT 78 infected again. ? Few cells multinucl. } F 6367 } W 6233 } W 6592 } sup.

Infection of cocultivation. [11/24/8]

Cl 19 }
Cl 22 } Recipient FT4/TK donor. / Mif-C
Cl 207 }

ratio 2:1 cultured RPMI 1640+20% + 15% TCGF.

HUT 78 clones
1. good expand. conc. 9.4×10^5 cells/ml
2. ? don't grow
3. very good. exp. conc. 11.1×10^5 cells/ml
4. excellent exp. (fast growing) " $1,5 \times 10^6$ cells/ml
5. ?

Infection sup HUT 78/MOV ↓ HT4
usual procedure.

N 200461

12/5/8

HT/Mov for concentration

Harvested sup from 12 flask à 1,5 l.

15 min 8000 gr.

20 " 6 000 g.

60 " ∂ 50 000 g. rot 35 ; 3 x the same tubes.

Resusp in Dulbecco PBS : (∩ 3 ml)

Cells - for antigen (Schmpbach)

Conc. Mov - Serum -

HT/III pooled. for conc. cells.

Clones - Changed media.

12/28/84

Conc. of culture fluids
from HT/HUT

Collected approx. ~1.2 l.

15' 800g. Sorvall. Cells frozen for
20' 7000g. Sorvall. Ag-detection or nucleic acid.
60' 50 000g. Spinco
Spray. RF.

N 200456

Cone. virus HT/Mev. 11 2 84
for Szrang. RT.
cells for Schupbach.

Second cone. sup. from AIDS LAS

W 7644 NEG P-19
W 7645 NEG RT
W 7647 NEG Cone. for not done
W 7675 NEG not done
W 7650 NEG P19 HT infection RT P-24 NEG
W 7780 NEG P19
W 7777 NEG P-19 RT NEG, P-24 NEG

HT/V. pool + cone. 7 wk after passage treatment.

Frequent contamination in bl
Szrang. serolog. O.K!!! 11 3 84