

Current
Cultivation of
Bth. Viruses

MAR 26 1954

Mahoney Virus - Serial Passages with Large Inocula
in Special Tubes from KP 33

Purpose - to determine how many passages can be made in this manner without loss of properties exhibited by KP 33

Mahoney KP 33 of 7/28/53 (C-1-1) - ampule recently retitered and found to have 10^7 TCID₅₀/ml
Inoculum - 0.1 ml of 1:10 dilution per tube with 4 ml of medium.

Special Tubes - many pieces of kidney tissue (2 @ 20) + 5% serum added to medium to provide large outgrowth of epithelial cells; medium changed at 7 days after single washing. New medium without serum added.

Passage 34 MAR 26 1954 - 1:45 PM. - Tube series 41 of 3/15/54 - medium changed 3/22/54; pH to-day @ 6.9
12 areas of epithelial outgrowth, each about 8 mm. in diameter.

3/27/54 1:50 PM. 4 + change

3/28/54 11:45 AM off harvested stored A-4-6

Passage 35 APR 6 1954 Tube Series 52 X of 3/30/54 av 15 areas with tissue implants of ca 4-10 mm in dia. Original medium removed tubes washed with Hank's compl. & refilled with 4 ml. fresh medium.

11:00 AM 0.17 ml of 1:10 dil. of previous passage used as inoculum into each of 2 tubes

APR 7 1954

9:30 AM 4 ml off change in both tubes

APR 3 1954

off at 9:00 AM stored A-4-7

Passage 36 APR 8 1954 10:00 AM Tube Series 52 X of 3/30/54 av 12 areas of ca 10 mm. in dia of epith. cell growth. Original med. removed washed & new med. added like previous pass.

0.17 ml of 1:10 dil. KP-35 used as inoc. into each of 2 tubes

APR 9 1954

4:00 PM 4 ml off

APR 10 1954

off 9:30 AM stored A-4-2

Makoney Virus

- Passage 37 APR 10 1954 Tube Series 52 X of 5/30/54 ca 8-10 areas of epithelial cell growth of ca 10-12mm in dia.
 Medium replaced as in prev. pass. Inoculum into each of 2 tubes consisted of 0.17ml of 1:10 dil. of previous pass.
 off 9:30 AM harvested stored A-1-5
- Passage 38 APR 11 1954 APR 12 1954 Tube Series 53 of 4-3-54 ca 10 areas of growth of from 5-8mm ⁱⁿ dia. & about 4-5 pieces of tissue implant. Since no rabbit serum was contained in the medium of this group of tissue ^{tubes} medium replacement was done. 0.17ml of 1:20 dil of previous passage was used for inoculum since each tube contained only 2 ml. of medium.
 off 11:30 AM
- Passage 39 APR 13 1954 APR 14 1954 APR 14 1954 harvested 10:00 AM stored A-7-7
 Tube Series 54 X of 4/6/54 about 14 areas of growth 2-6mm in dia & about 10 pieces of tissue implant
 medium replaced after washing tubes with Hank's comp
 0.17ml of 1:10 dil of previous passage used for inoculum
- Passage 40 APR 15 1954 APR 16 1954 APR 16 1954 harvested stored A-7-7
 Tube Series 54 X of 4/6/54 about 10 areas of epithelial cell growth 2-6mm in dia. & about 10 pieces of tissue impl.
 medium replaced after washing tubes with Hank's comp. 0.17ml. of 1:10 dil previous passage used as inoculum
 9:00 AM off
- Passage 41 APR 17 1954 APR 18 1954 APR 18 1954 harvested 1:00 PM stored A-5-7
 Tube Series 54 X of 4/6/54 media replaced as usual on 4/17/54 ca. 14 areas of epithelial cell growth ca 10mm in dia
 0.17ml of 1:10 dil previous passage used as inoculum only one tube inoculated
 9:00 AM off
 APR 19 1954 APR 20 1954 harvested stored A-5-7

MAR 26 1954

Y-8k Virus - Serial Passages with large inocula in Special Tubes - from KP 51

Purpose - to determine how many passages can be made in this manner without loss of properties exhibited by KP 51

Virus - Y-8k, KP 51 of 9-23-53 (C-2-1)

Inoculum - 0.1 ml. of 1:10 dilution per tube with 4 ml of medium

Special Tubes - About 20 pieces of kidney - outgrowth aided by 5% serum - medium removed at 7 days, tube washed, and new medium added without serum

Passage 52

MAR 26 1954 1:45 PM - Tube series 41 of 3/15/54 - medium changed 3/21/54 - washed + new 4ml added. pH now @ 6.9 - 16 areas of epithelial outgrowth each @ 8mm. in diameter

3/27/54 1:30 P.M. 4 change

3/28/54 11:45 AM off harvested stored A-4-6

Passage 53

APR 6 1954 Tube Series 52-X of 3/30/54 av. 14-18 areas of tissue mound + plaques of ep. cells of ca. 5 - 10 mm. in dia. Reg. medium removed. tubes washed with Hank's comp + replace with reg. medium (4ml) 0.17ml of 1:10 dil. of previous passage used as inoculum into each of 2 tubes

APR 7 1954 9:30 AM 4 off change in both tubes

APR 8 1954 off at 9:00 AM stored A-4-7

Passage 54

APR 8 1954 10:00 AM Tube Series 52X of 3/30/54 used for transfer av. ca 12 areas of ca 10mm in dia. of epith. cell growth. medium changed as in pre oc. 0.17ml. of 1:10 dil KP 33 used as inoc. into each of 2 tubes

APR 9 1954 4:00 P.M. 4 off

APR 10 1954 9:00 AM off stored A-4-7

Passage 55

APR 10 1954 Tube Series 52X of 3/30/54 ca. 8-10 areas of epith. cell growth of about 10-12 mm in dia. Medium replaced as in previous pass. Inoculum consisted of 0.1 ml of 1:10 dil. of previous passage

APR 11 1954 off 9:30 AM harvested stored A-1-5

Y-5K Virus

Passage 56 APR 12 1954 Tube Series 53 of 4/3/54 5ml reg. series with no added rabbit serum medium was not replaced. Tube has ca. of 8 plaques of growth of from 5-8mm. in dia. with about 6 areas of implants. 0.17ml of 1:20 dil of previous passage material was used as inoculum instead of a 1:10 dil. for only 2ml. of medium was contained in each tube.

APR 13 1954

off 11:30 AM

APR 14 1954

harvested 10:00 AM

Passage 57 APR 14 1954

stored A-7-7

10:00 AM

Tube Series 54X of 4-6-54 used ca. 14 areas of ep. cell gr. of about 3-6 mm in dia. ca 10 pieces of tissue implants medium replaced as usual 0.17ml. of 1:10 dil previous passage used as inoculum

APR 15 1954

11:00 AM off

APR 16 1954

harvested

stored A-7-7

Passage 58

APR 16 1954

9:30 AM

Tube Series of 54X of 4/6/54 ca 8-12 areas of epith cell outgrowth 4-8 mm in dia. medium replaced as usual 0.17ml. of 1:10 dil prev. passage used as inoculum

APR 17 1954

9:00 AM off

harvested 1:00 P.M. stored A-5-7

APR 18 1954

Passage 59

APR 18 1954

Tube Series 54X of 4/6/54 media replaced as usual on 4/11/54 ca. 14 areas of epith cell growth ca. 10 mm in dia. 0.17ml. of 1:10 dil. previous passage used as inoculum only one tube used in passage

APR 19 1954

9:00 AM off

APR 20 1954

harvested stored A-5-7

Leon Virus - Serial Passages with Large Inocula in Special Tubes - from KP 34

Purpose - to determine how many passages can be made in this manner without loss of properties exhibited by KP 51

Virus - Leon, KP 34 of 9-11-53 (C-2-1)

Inoculum - 0.1 ml. of 1:10 dilution per tube with 4 ml of medium

Special Tubes - About 20 pieces of kidney - outgrowth aided by 5% serum - medium removed at 7 days, tube washed, and new medium ^{without serum} added.

Passage 35 - MAR 26 1954 - 1:45 PM - Tube of series 41 of 3/15/54 - medium changed 3/22/54 - washed - + 4 ml of serum-free medium added. pH now @ 6.9
16 areas, @ 8 mm each, of epithelial outgrowth.

3/27/54 + exchange 1:30 P.M.

3/28/54 off

3/29/54 harvested 9:00 AM stored A-4-5

Passage 36 APR 6 1954 Tubes of Series 52-X of 3/30/54 av. 14-18 areas of tissue implant with epithelial cell growth extending from ca. 4-10 mm radia. Big med. removed. tube washed with Hanks compl. & refilled with 4 ml. org medium
11:00 AM. 0.1 ml of 1:10 dil of previous passage used as inoculum into each of 2 tubes

APR 7 1954 9:30 AM 2 x 2 + 2 cytopathogenic change in tube

APR 3 1954 9:00 AM off stored A-4-7

Passage 37 APR 8 1954 10:00 AM Tube Series 52X of 3/30/54 used for transfer ca. ca 12 areas of epith cell growth medium changed as prev. sec.
0.1 ml. of 1:10 dil KP 36 used as inoc. into each of 2 tubes

APR 9 1954 4:00 P.M. off

APR 10 1954 9:00 AM off stored A-4-4

STANDARD B & P

STANDARD B & P "NOTEAR"

Leon Tivine

Passage 38 APR 10 1954 Tube Series 52X of 3/30/54 ca. 8-10 areas of epith. cell growth of about 10-12 mm. in dia. Original medium removed tube washed with Hank's comp. 4ml. of new medium added inoculum into these 2 tubes consisted of 0.17ml.

1:10 dil. of previous passage
APR 11 1954

Passage 39 APR 12 1954 Regular tube series 53 of 4/3/54 ca. 8-10 areas of epithelial growth & with ca. 6 pieces of implants 2ml. of 2 medium cont. in each tube not replaced for 700 rabbit serum in medium inoculum consisted of 0.17ml. of 1:20 dil. of previous passage

APR 13 1954 11:30 AM

APR 14 1954 harvested 10:00 AM stored A-7-7

Passage 40 APR 14 1954 Tube Series 54X of 4-6-54 ca. 14 areas of epith. growth with outgr of 2-6 mm in dia ca 10 tissue implants medium replaced after washing tube 0.17ml. of 1:10 dil. prev. pass. used for inoculum

APR 15 1954 11:00 A.M. 11:00 A.M. APR 16 1954 harvested stored A-7-7

Passage 41 APR 16 1954 Tube Series 54X of 4-6-54 ca 8-12 areas of growth 8-10mm. in dia. 4-5 tissue implants medium replaced as usual 0.17ml of 1:10 dil previous passage used as inoculum

APR 17 1954 9:00 AM

APR 18 1954 harvested 1:00 P.M. stored A-5-7

Passage 42 APR 18 1954 Tube Series 54X of 4/6/54 ca. 8-12 areas of epith. cell growth ca. 10mm. in dia. media replaced as usual on 4/17/54 0.17ml. of 1:10 dil. previous passage used as inoculum. Only one tube used in passage

APR 19 1954 9:00 AM

APR 20 1954 harvested stored A-5-7