Figure 1

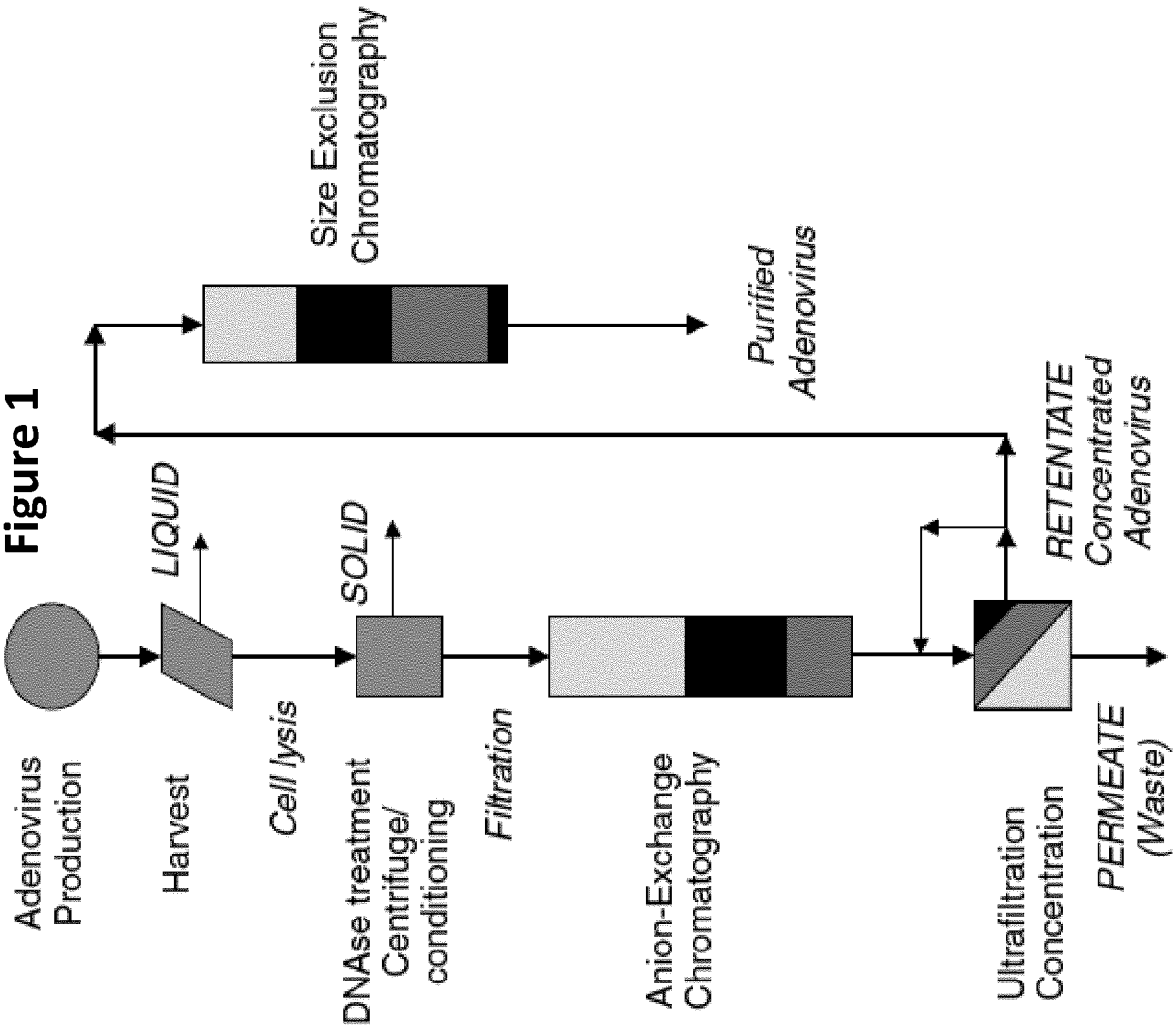

Adenovirus Production
Harvest
LIQUID
Cell lysis
DNAse treatment Centrifuge/ conditioning
SOLID
Filtration
Anion-Exchange Chromatography
Size Exclusion Chromatography
Purified Adenovirus
Ultrafiltration Concentration
RETENTATE Concentrated Adenovirus
PERMEATE (Waste)

Figure 2

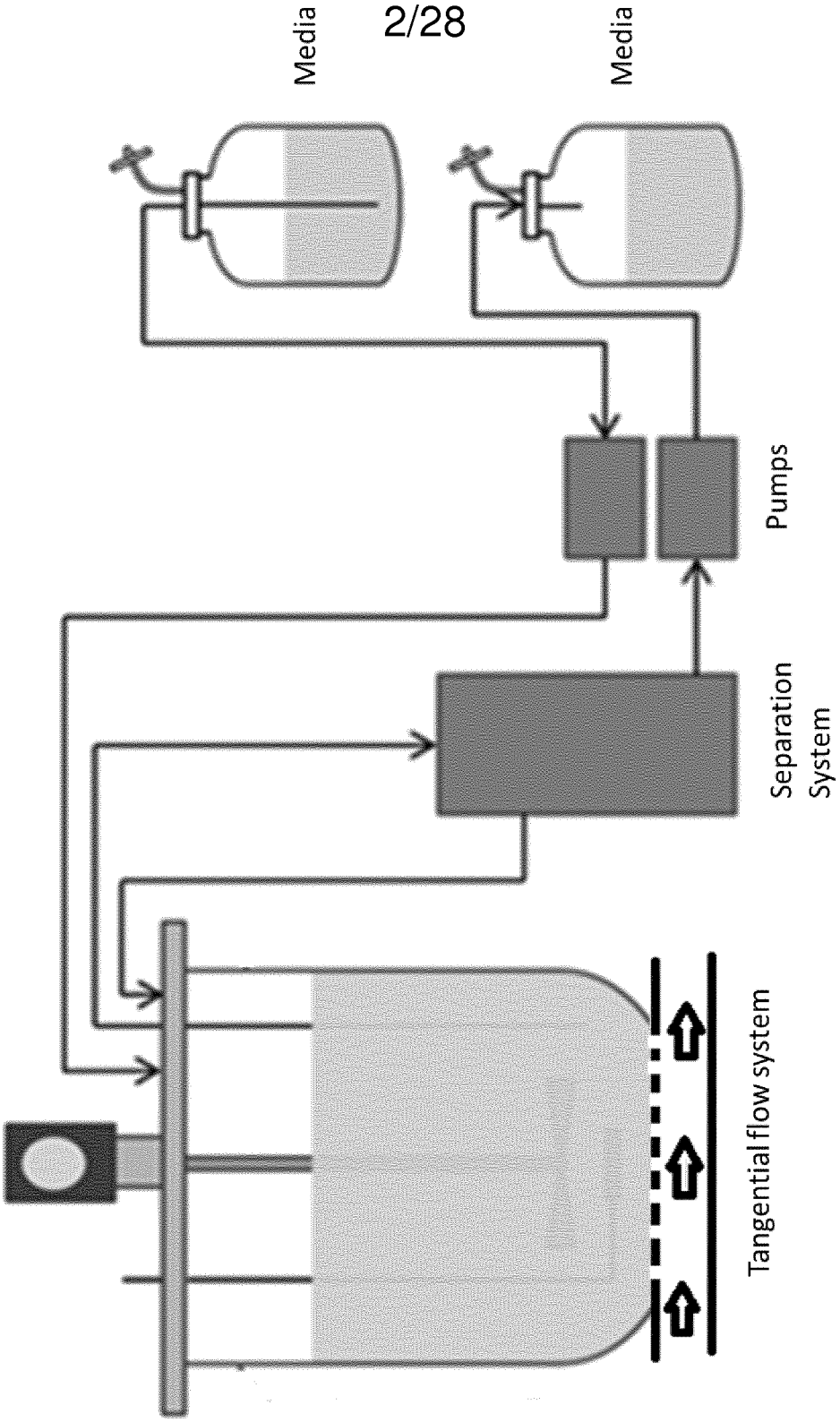

Media
Media
Pumps
Separation System
Tangential flow system

Figure 3

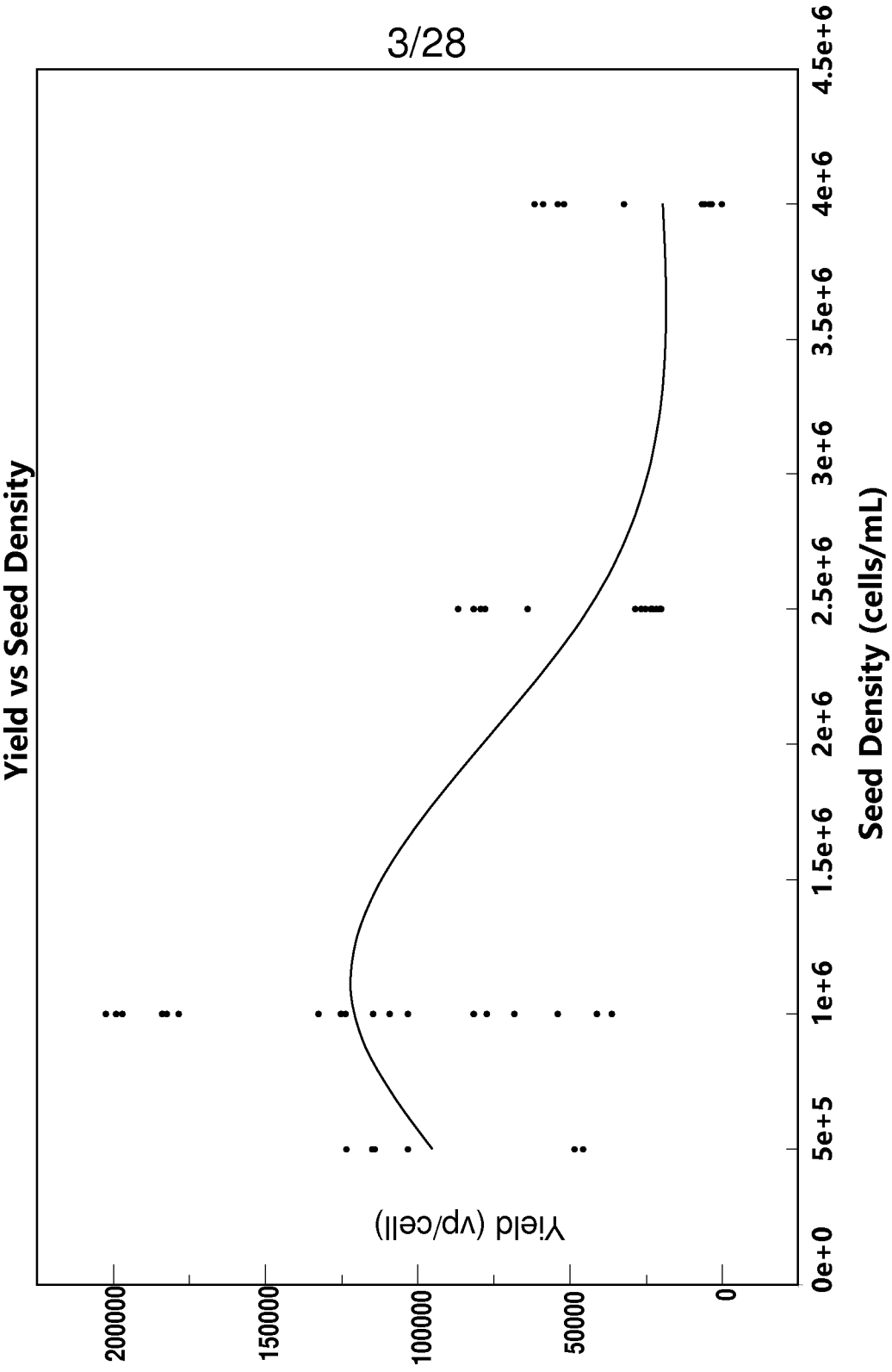

Yield vs Seed Density
Yield (vp/cell)
Seed Density (cells/mL)
0
50000
100000
150000
200000
0e+0
5e+5
1e+6
1.5e+6
2e+6
2.5e+6
3e+6
3.5e+6
4e+6
4.5e+6

Figure 4

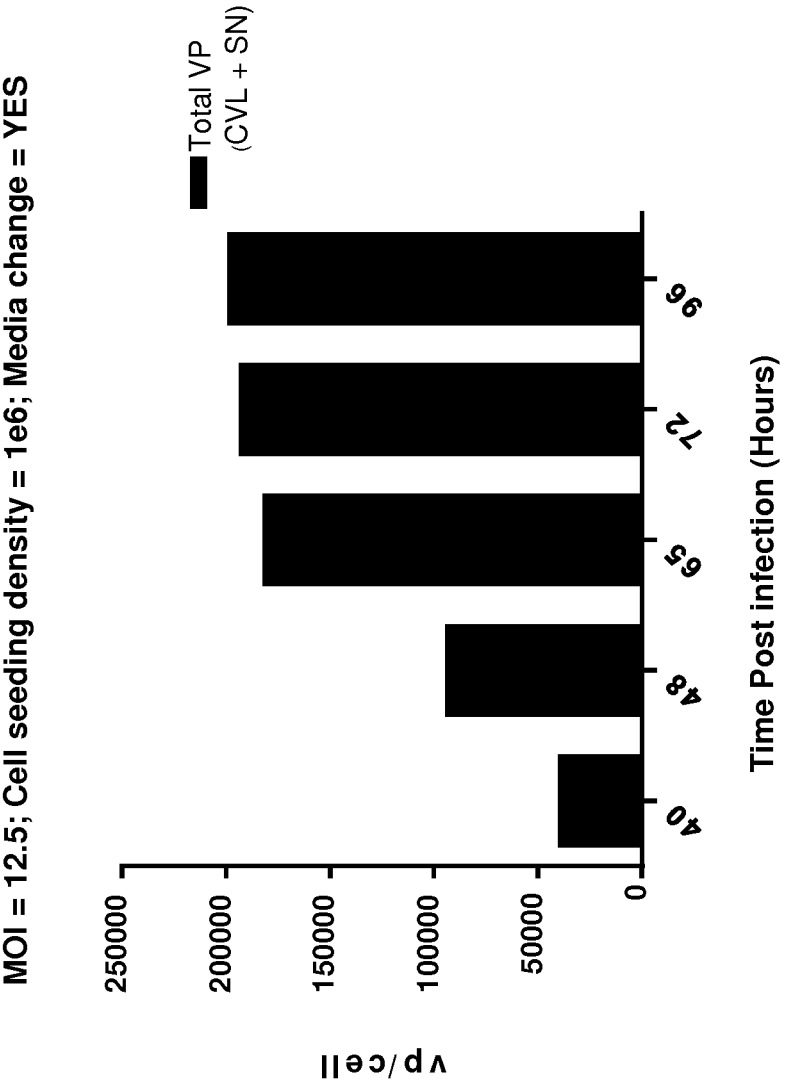

DOE Experiment Flasks A & B
MOI = 12.5; Cell seeding density = 1e6; Media change = YES
Total VP
(CVL + SN)
vp/cell
250000
200000
150000
100000
50000
0
40
48
65
72
96
Time Post infection (Hours)

Figure 5

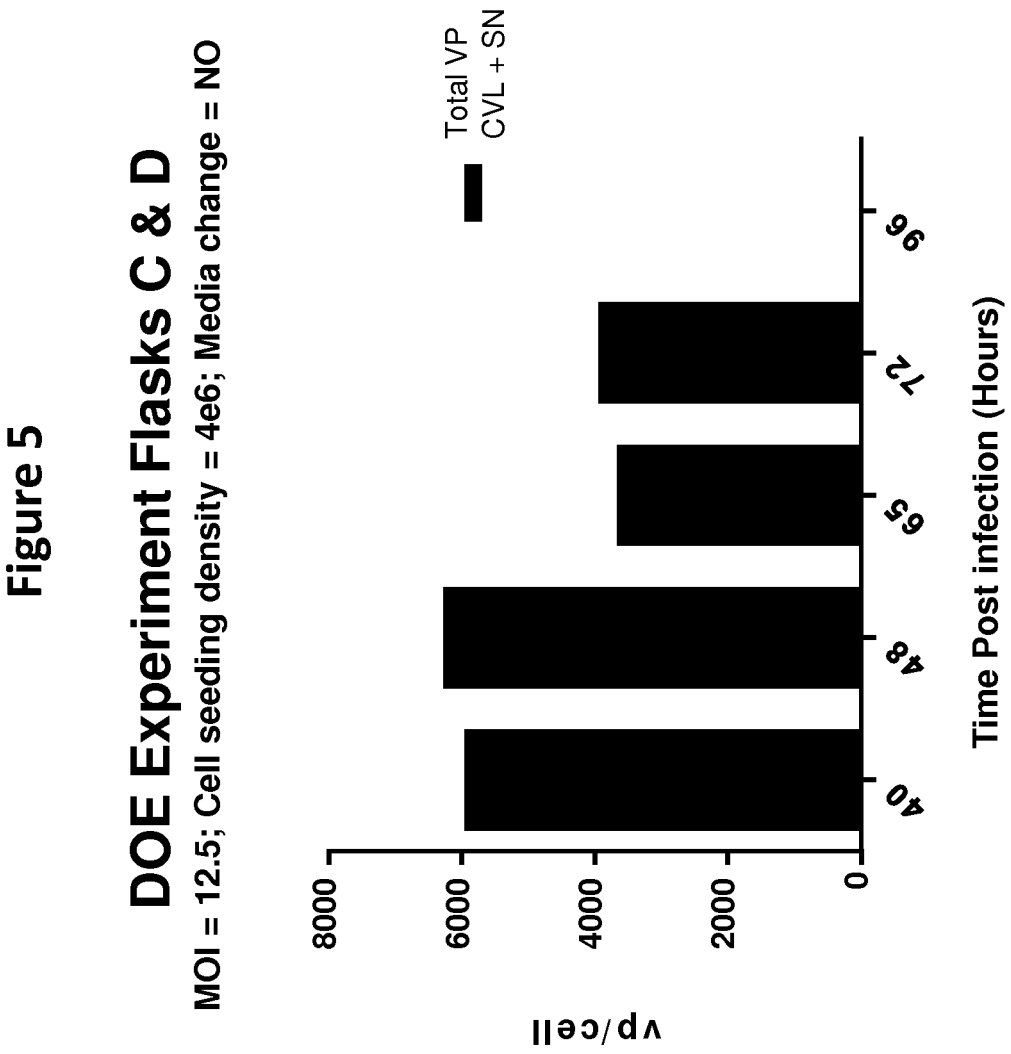

DOE Experiment Flasks C & D
MOI = 12.5; Cell seeding density = 4e6; Media change = NO
Total VP
CVL + SN
vp/cell
8000
6000
4000
2000
0
40
48
65
72
96
Time Post infection (Hours)

Figure 6

Benefit of Media Change on Virus Yield at increasing DOI's and Seeding Densities

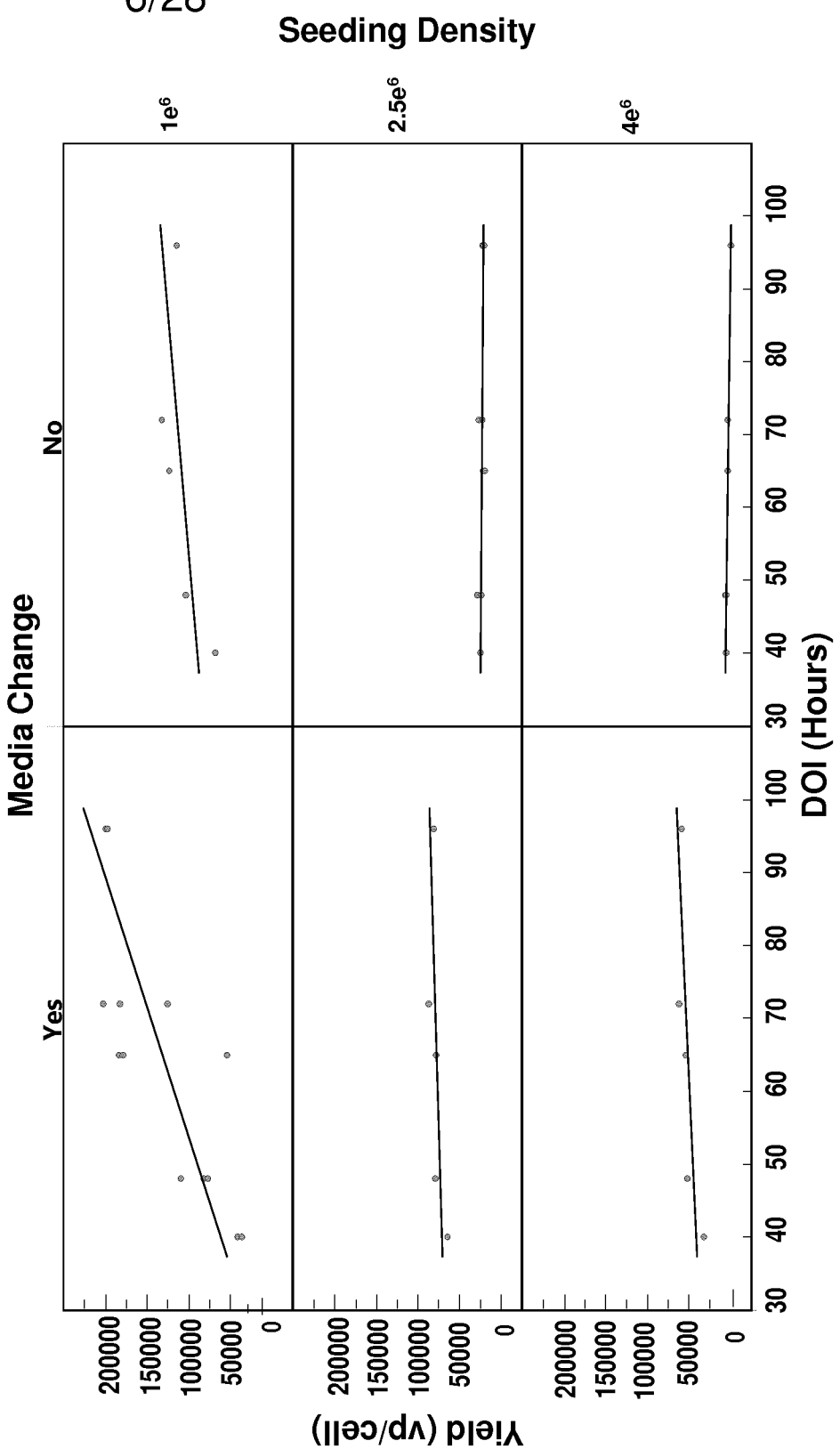


Figure 7

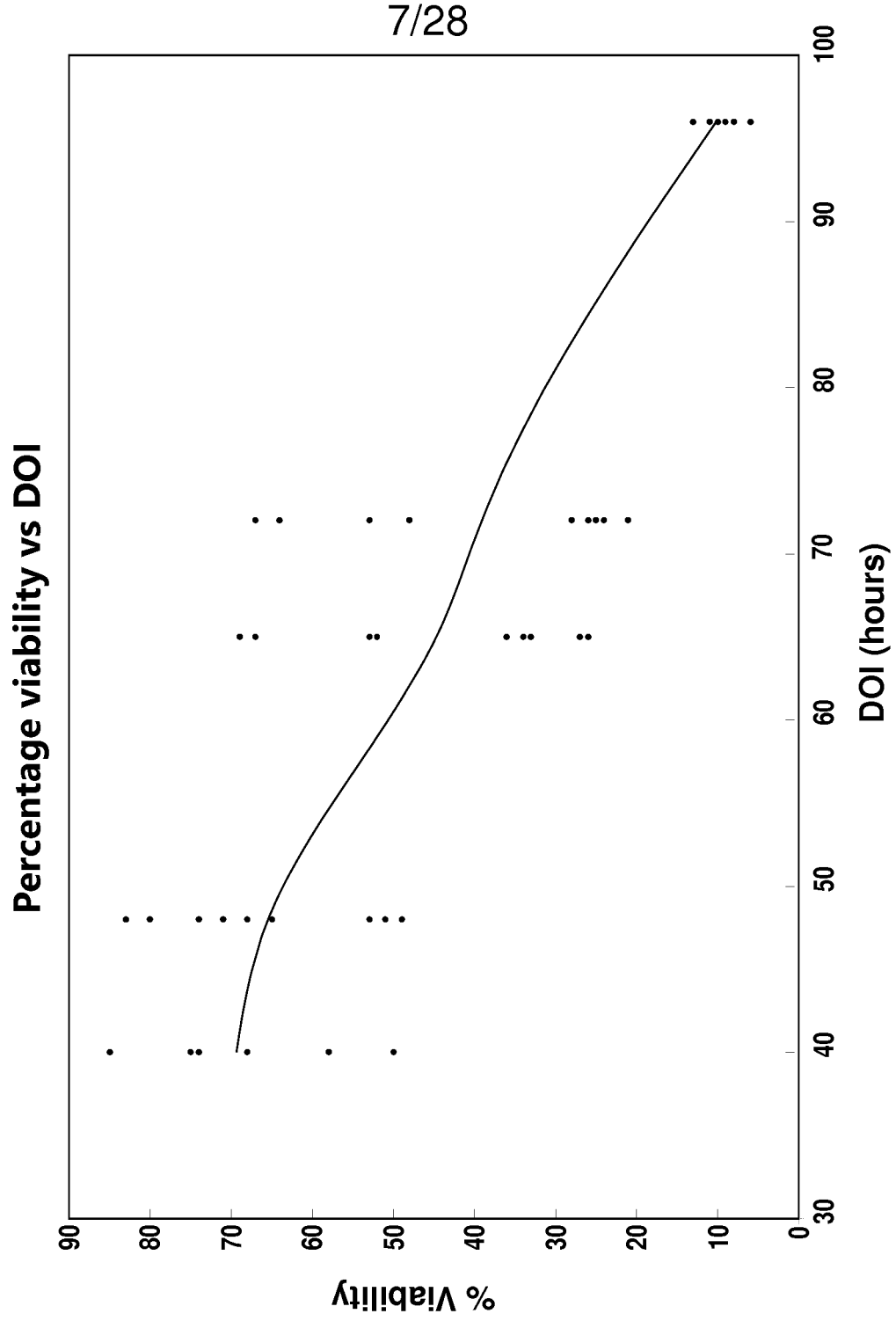

Percentage viability vs DOI
% Viability
DOI (hours)
90
80
70
60
50
40
30
20
10
0
30
40
50
60
70
80
90
100

Figure 8

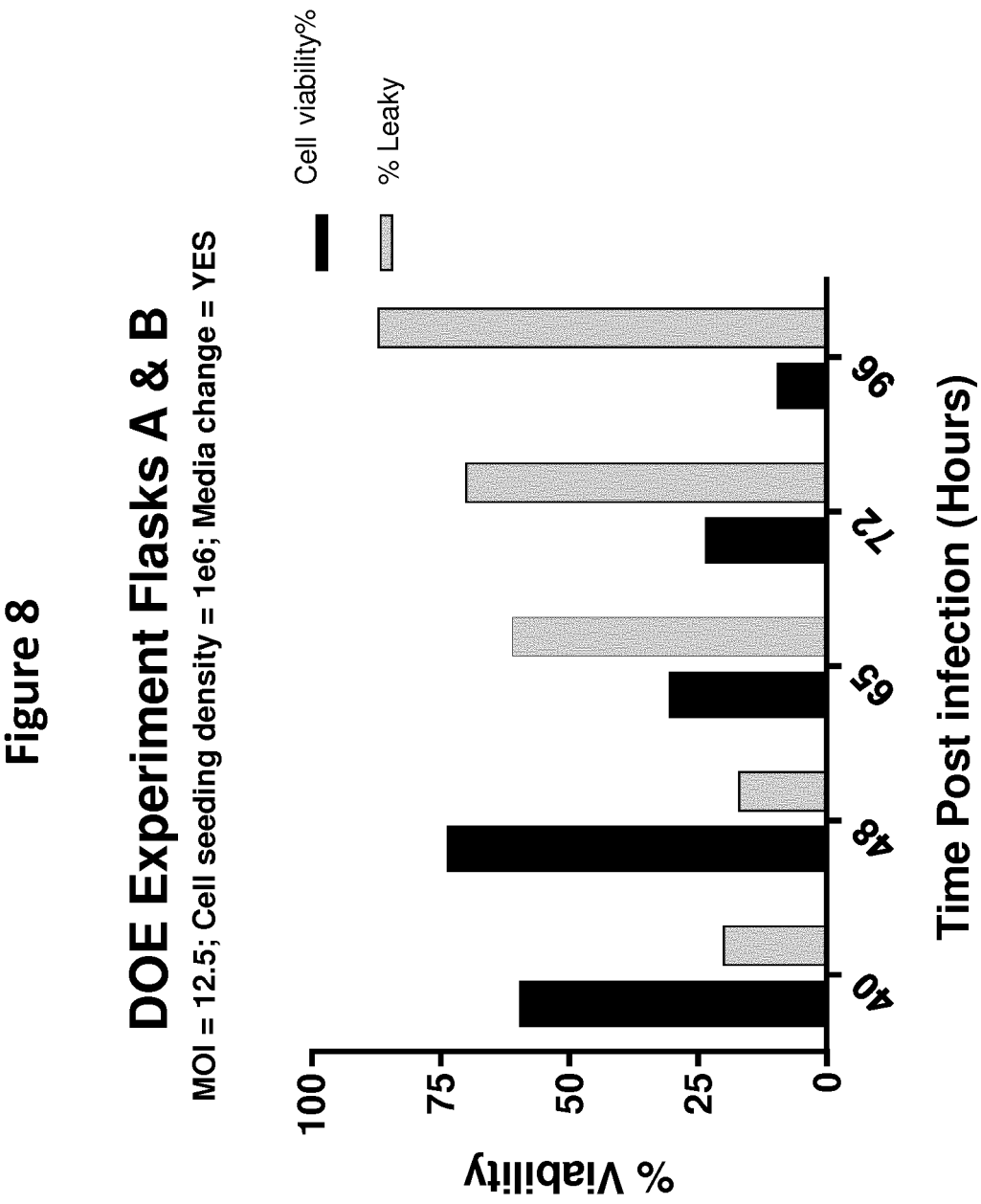

DOE Experiment Flasks A & B
MOI = 12.5; Cell seeding density = 1e6; Media change = YES
Cell viability%
% Leaky
% Viability
100
75
50
25
0
40
48
65
72
96
Time Post infection (Hours)

Figure 9

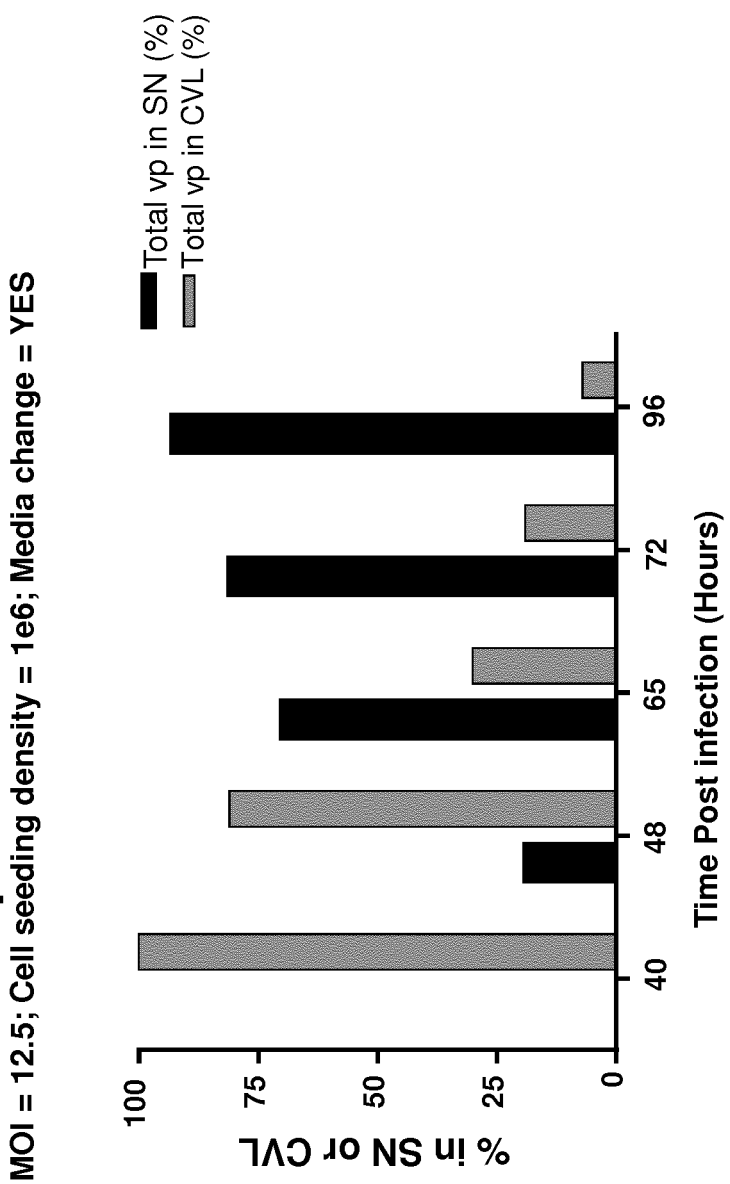

DOE Experiment Flasks A & B
MOI = 12.5; Cell seeding density = 1e6; Media change = YES
Total vp in SN (%)
Total vp in CVL (%)
% in SN or CVL
100
75
50
25
0
40
48
65
72
96
Time Post infection (Hours)

Figure 10

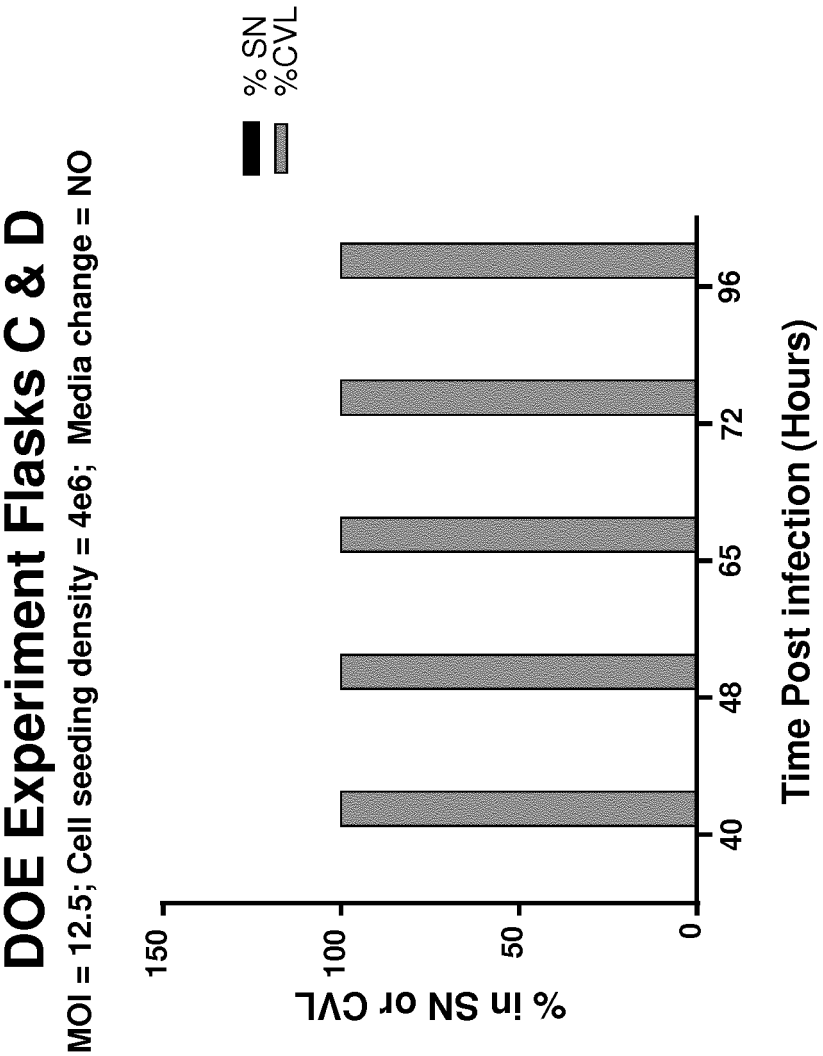

DOE Experiment Flasks C & D
MOI = 12.5; Cell seeding density = 4e6; Media change = NO
% SN
%CVL
% in SN or CVL
150
100
50
0
40
48
65
72
96
Time Post infection (Hours)

Figure 11:
12.5 ppc and a cell density of 1 x10^6 cell/ml.

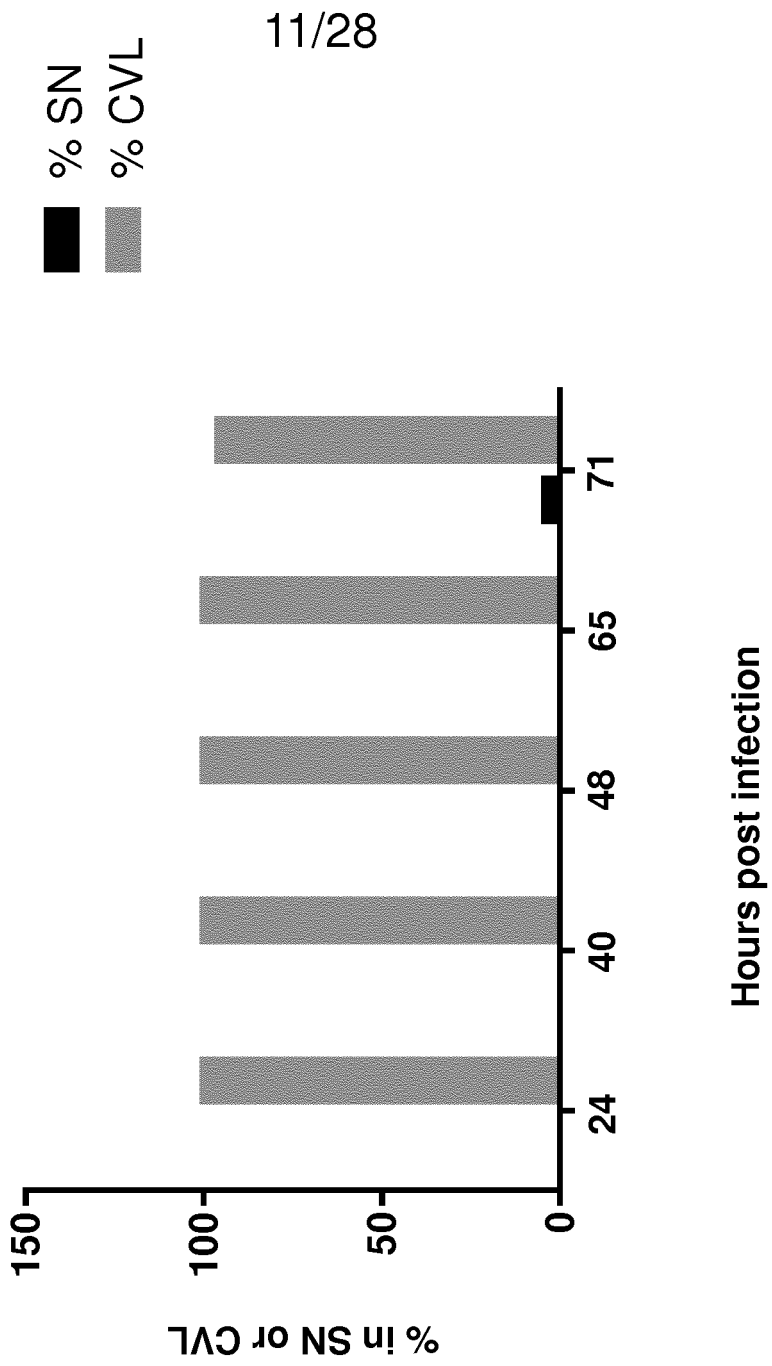


Figure 12:
50 ppc and a cell density of 1.9 x10^6 cell/ml.

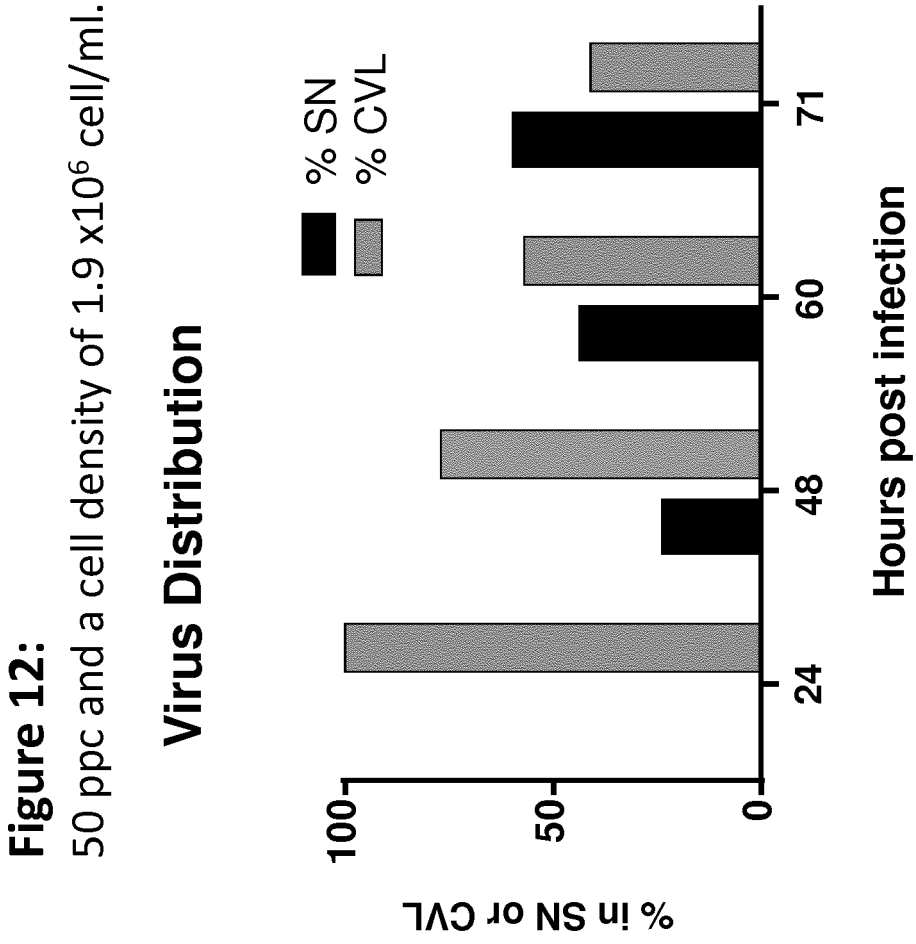


Figure 13:
12.5 ppc and seed density of 1 x 10^6 vp/ml

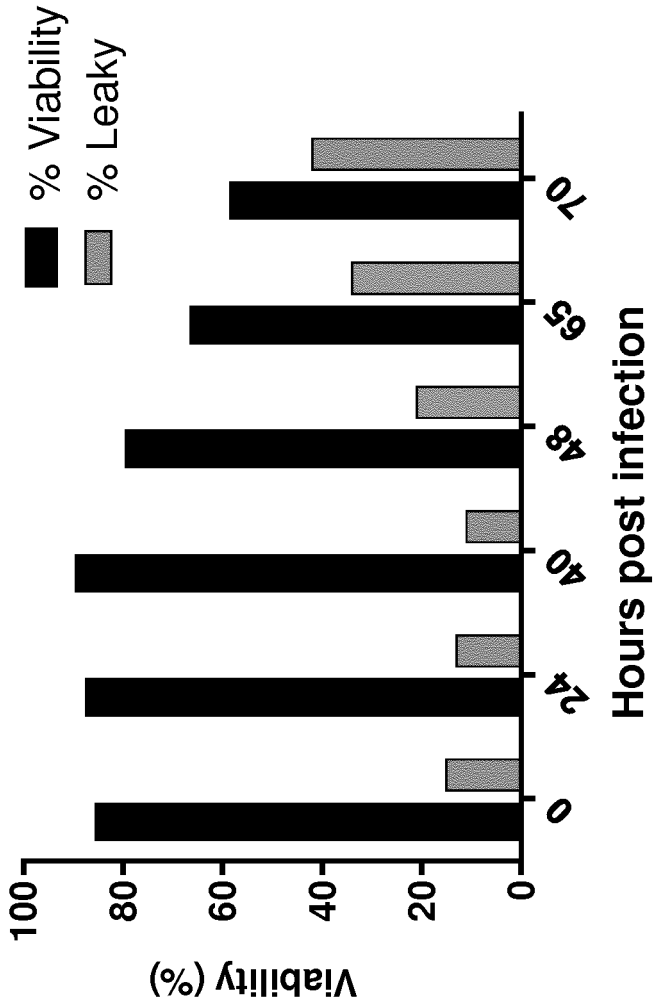


Figure 14:
50 ppc and seed density of 1.9 x 10^6 vp/ml

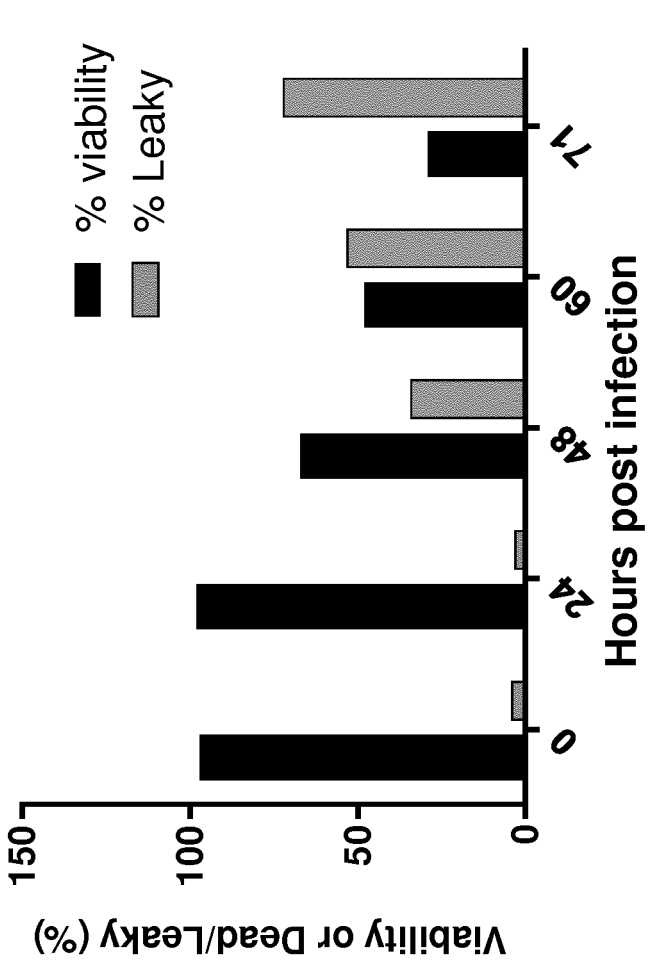


Figure 15

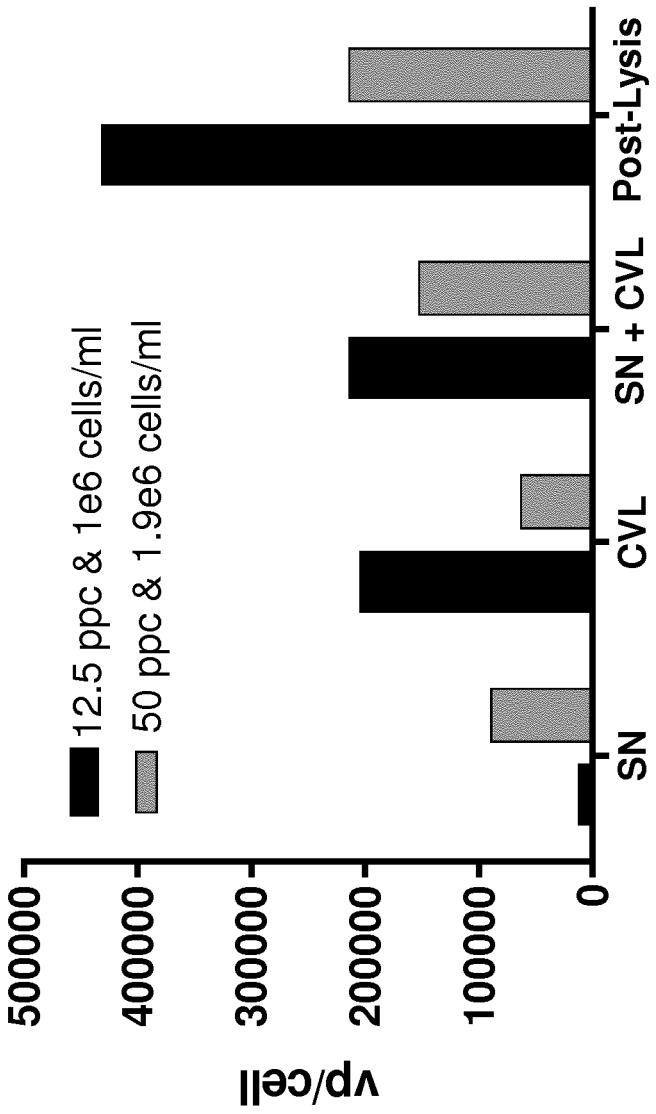

Bioreactor data
12.5 ppc & 1e6 cells/ml
50 ppc & 1.9e6 cells/ml
vp/cell
500000
400000
300000
200000
100000
0
SN
CVL
SN + CVL
Post-Lysis

Figure 16:
2.2 x 10^6 cells/mL and 50 ppc

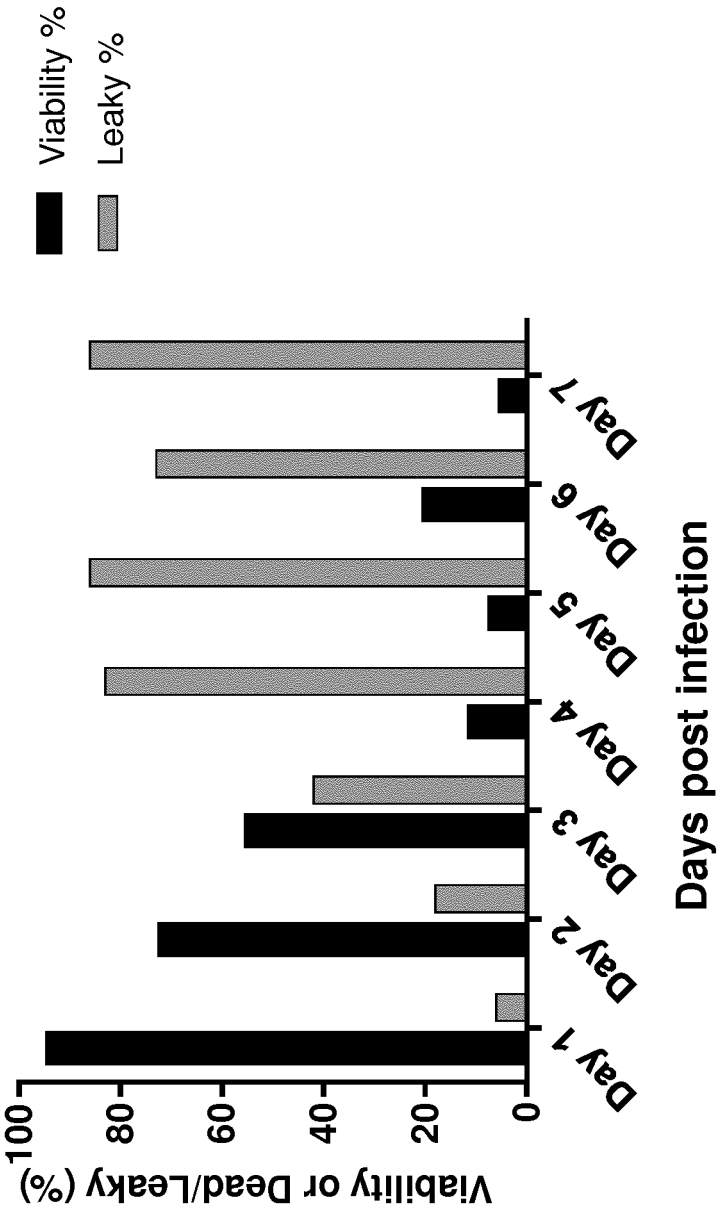


Figure 17:
2.2 x 10^6 cells/mL and 50 ppc

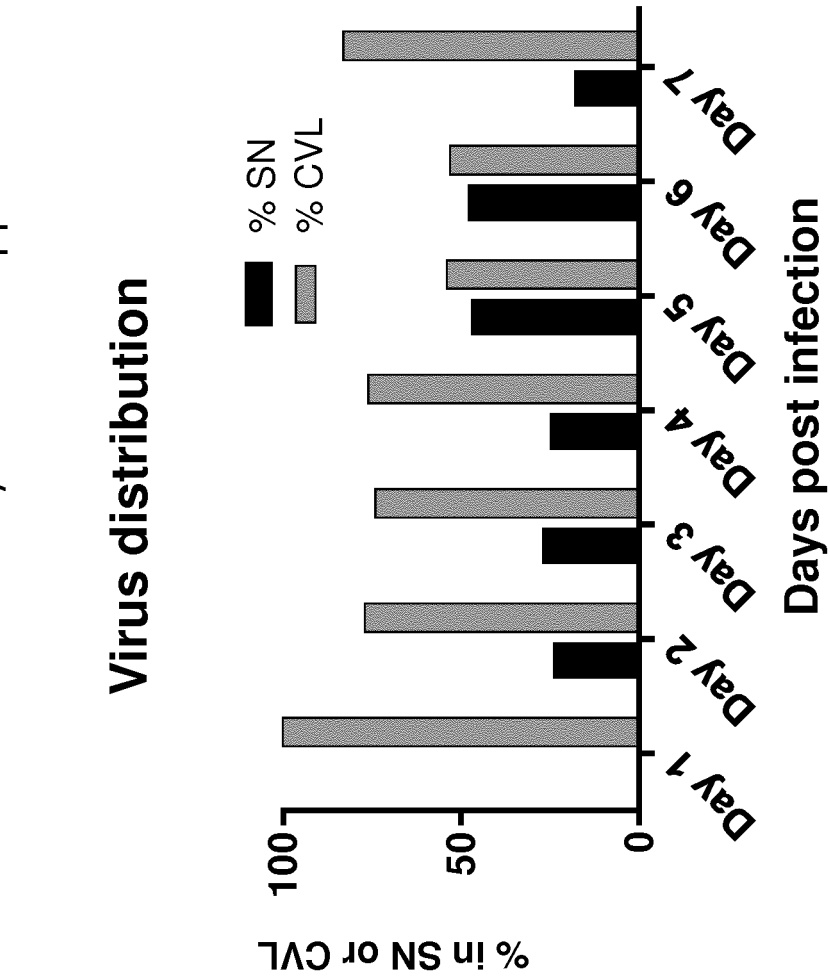


Figure 18:
2.2 x 10^6 viable cells/mL and infected with 50 ppc.
No cell suspension removal or addition of fresh cells and medium

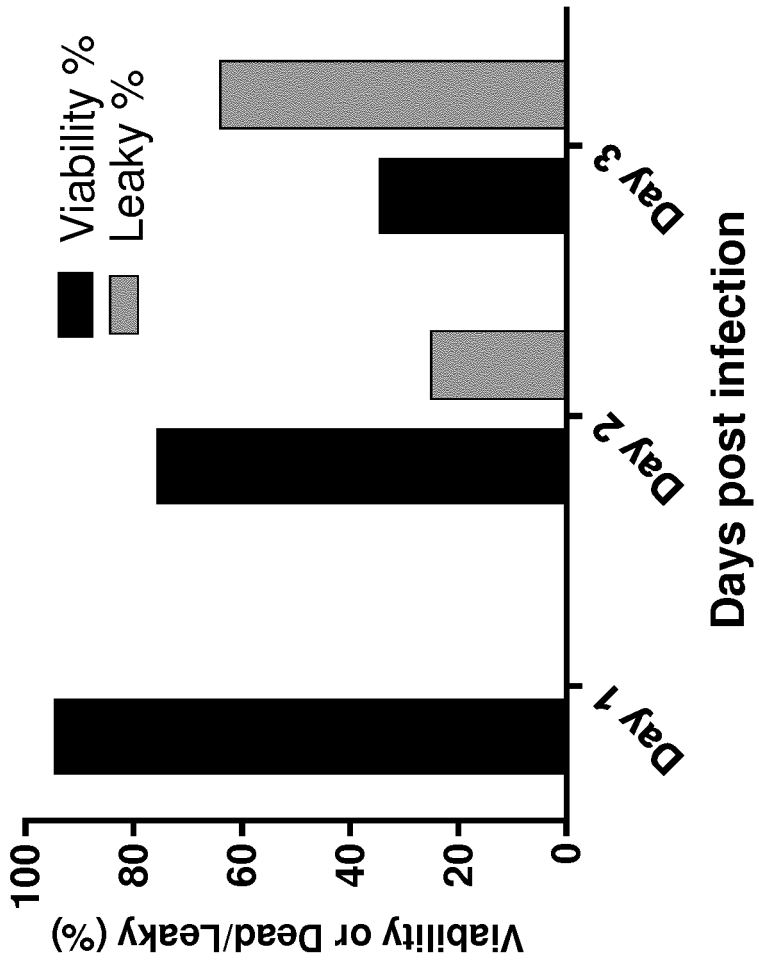


Figure 19:
2.2 x 10^6 viable cells/mL and infected with 50 ppc
No cell suspension removal /no addition of fresh cells and medium

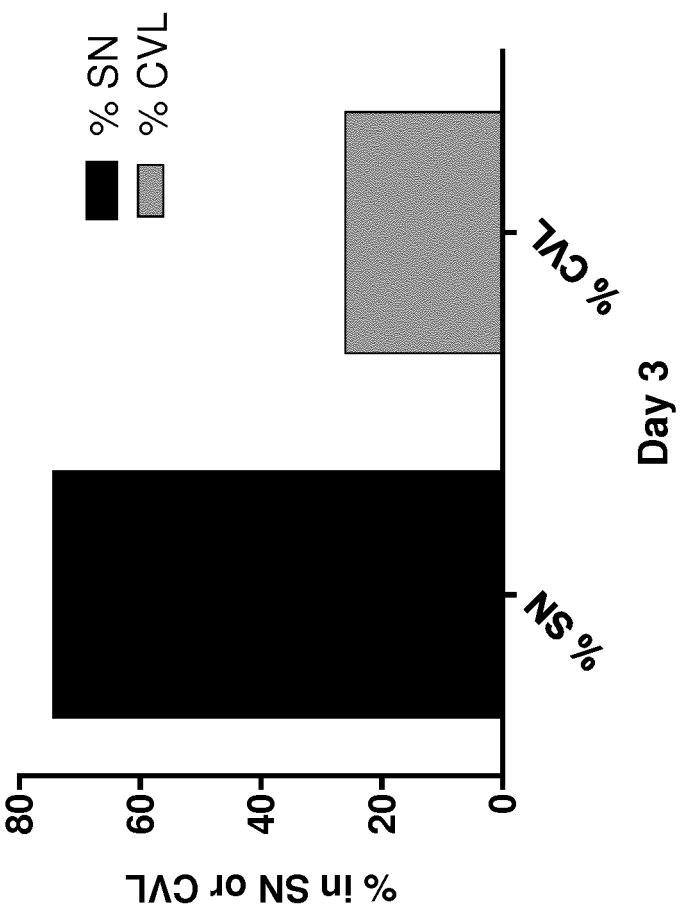


Figure 20:
2.2 x 10^6 viable cells/mL and infected with 50 ppc

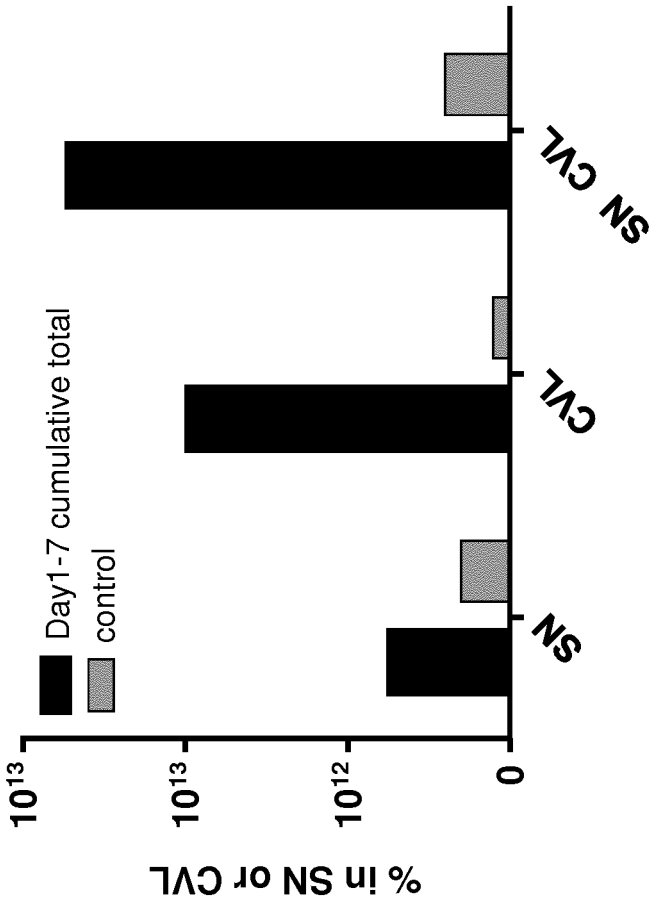


Figure 21:
1.0 x 10^6 viable cells/mL and 12.5 ppc infection

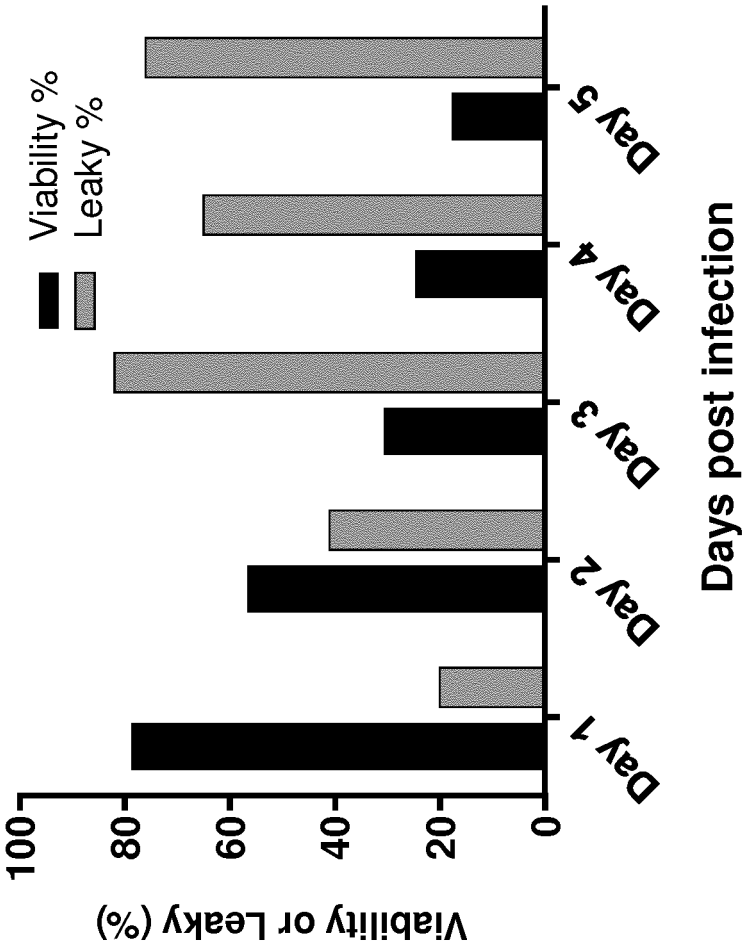


Figure 22:
1.0 x 10^6 viable cells/mL and 12.5 ppc infection

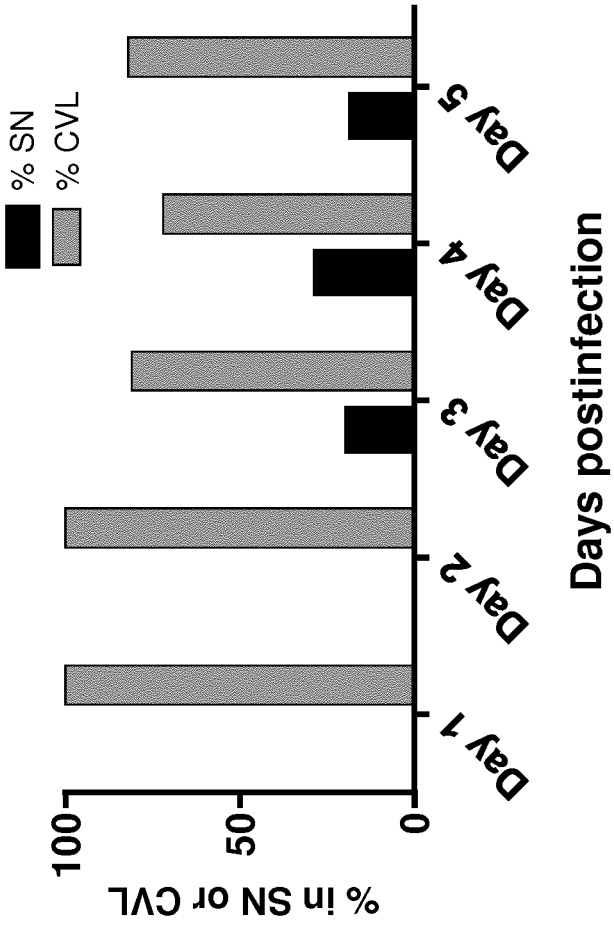


Figure 23:
1.0 x 10^6 viable cells/mL and 12.5 ppc infection
No cell suspension removal /no addition of fresh cells and medium

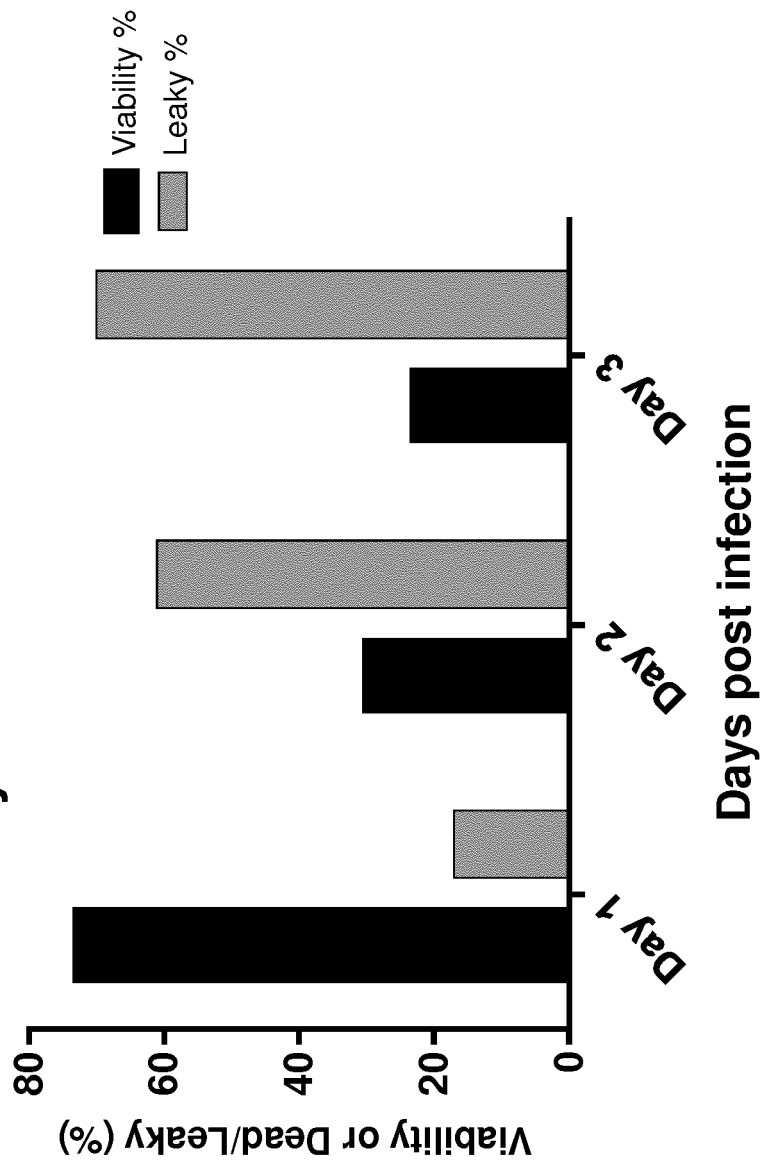


Figure 24:
1.0 x 10^6 viable cells/mL and 12.5 ppc infection
No cell suspension removal /no addition of fresh cells and medium

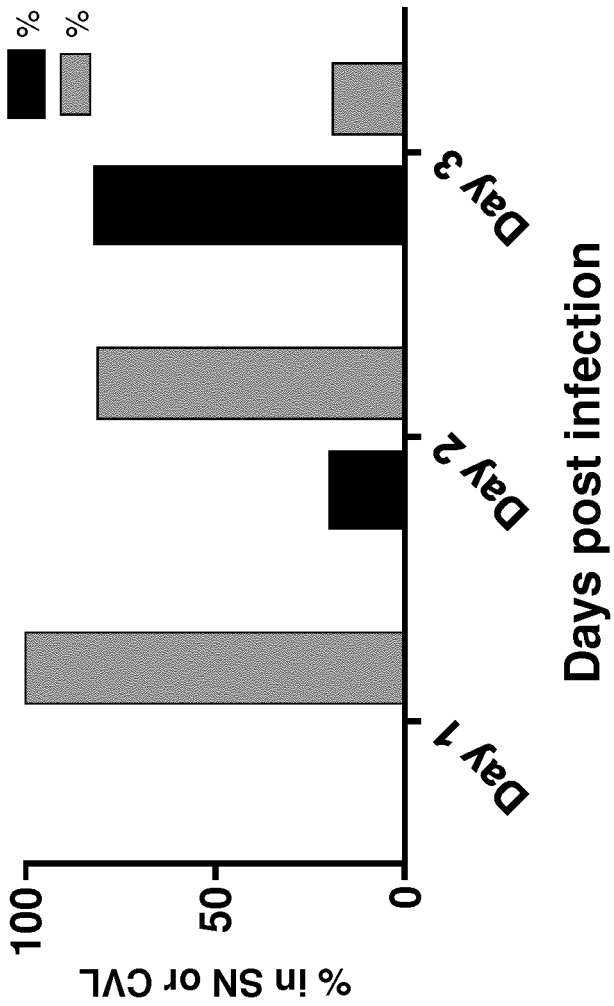


Figure 25:

1.0 x 10^6 viable cells/mL and 12.5 ppc infection

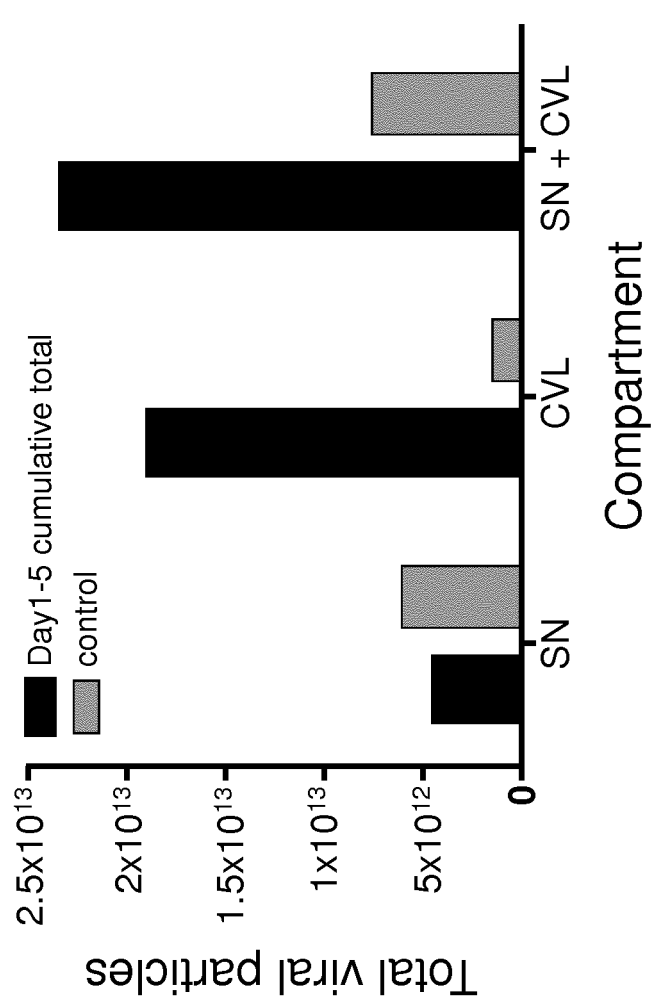


Figure 26:

1.0 x 10^6 viable cells/mL and 12.5 ppc infection
95% suspension removed & replaced with 47.5mL uninfected cells in fresh medium cell density of 1.0 x 10^6 viable cells/ml

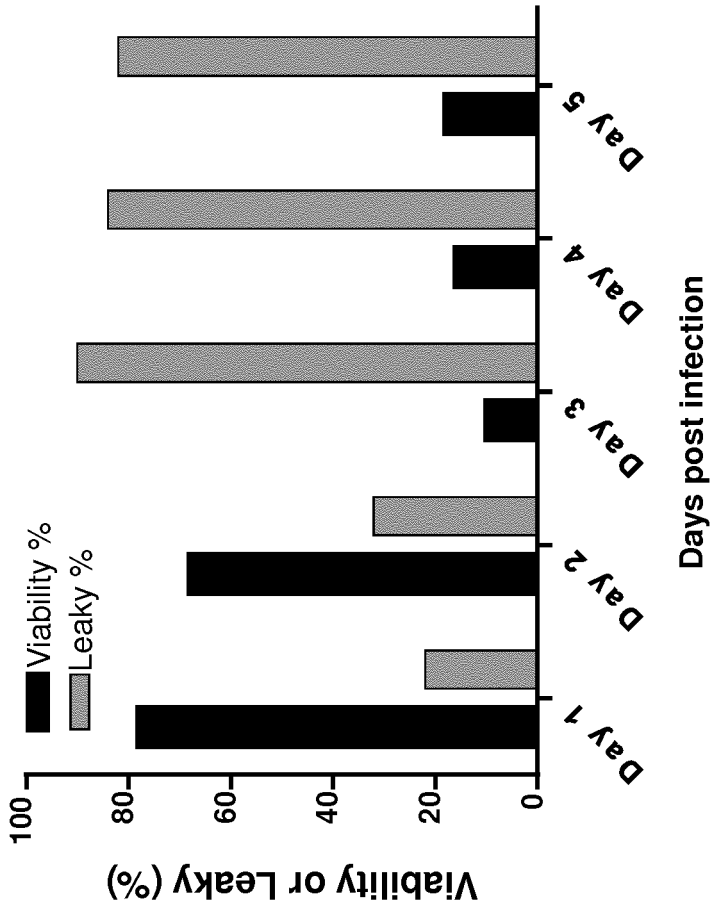


Figure 27:

1.0 x 10^6 viable cells/mL and 12.5 ppc infection
95% suspension removed & replaced with 47.5mL uninfected cells
in fresh medium cell density of 1.0 x 10^6 viable cells/ml

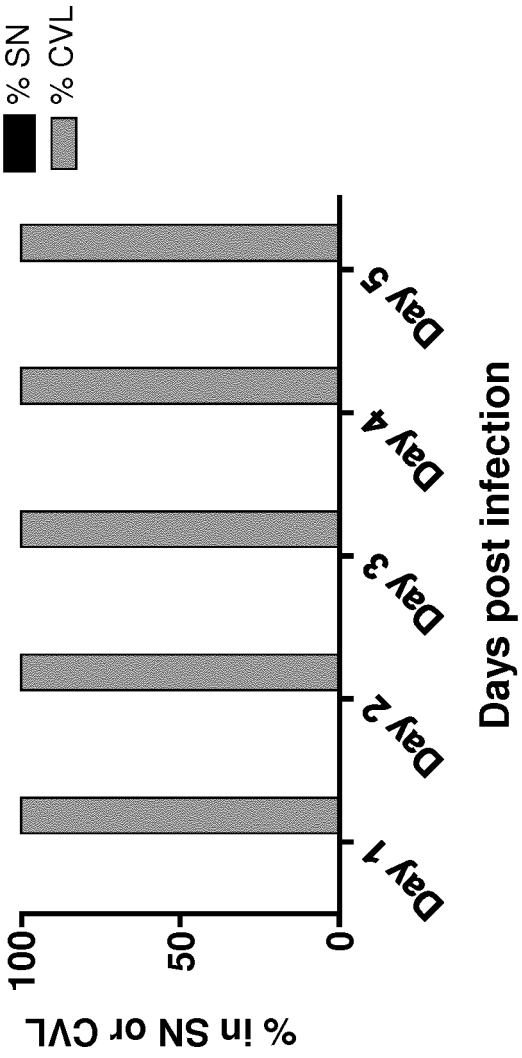


Figure 28:

1.0 x 10^6 viable cells/mL and 12.5 ppc infection
95% suspension removed & replaced with 47.5mL uninfected cells in fresh medium cell density of 1.0 x 10^6 viable cells/ml

