Instructions for AAV Reference Standard Characterization Report

These instructions are intended to aid in the completion of this largely automated report of assay data associated with the characterization of AAV reference material.

General Guidelines:

1. Save this file as data is entered with a name that includes your institution and your name.
2. Due to the automatic nature of this report most cells are locked and can not be selected.
3. Cells that require data are high-lighted green. Instructions for each sheet follow below.
4. Spaces for institution, technician, reviewer, and all dates are unlocked but are not high-lighted.
5. Once this report is completed save it with a name including your institution, your name, and the date submitted.

Summary Report

1. Data values on this page automatically fill as assay data is entered in its respective space.
2. Values displayed on this sheet are simply copies of the data entered on other sheets.
3. Upon review of reported data values the reviewer must enter their name and the date in the space provided below the data.
4. When all assay data is reported, enter the institution name, submitted by, and date sections.

Physical Titer by qPCR

1. Enter assay data in appropriate locations indicated by green high-light. Enter names and dates where indicated.
2. PCR efficiency, GC/ mL, and Average GC/ mL are automatically calculated and reported to the summary report.

Identity and Purity by SDS PAGE

1. Each column of each table poses a Yes/ No question. Enter Yes, No, or NA as appropriate in the green high-lighted cells. Enter names and dates where indicated.
2. Gel B SYPRO Ruby Reduced also asks for % Purity. Enter this value as a number, i.e. enter 90% as 90.
3. Following the guidelines outlined in the protocol, enter pass of fail in the indicated cell for each gel. This value is automatically entered in the summary report.

Particle Titer by ELISA

1. Enter A450, dilution, and particles/ mL, determined according to the protocol, in green high-lighted cells.
2. Enter names and dates where indicated.
3. Average Particles/ mL is automatically calculated and entered in the summary report.

Infectious Titer by GFP

1. Follow the directions to enter data in green high-lighted cells. Enter names and dates where indicated.
2. Dilutions averaging less than 20 GFU/ well are labeled "low" and dilutions averaging greater than 300 GFU/ well are labeled "High".
3. Dilutions with 20 to 300 GFU/ well are used to automatically calculate GFU/ mL. Averaged GFU/mL is automatically entered in the summary report.
4. "Input Vector Physical Titer by qPCR" automatically appears as an average GC/ mL when the Physical Titer by qPCR assay data is entered. Pt:GFU ratios are automatically calculated and entered in the summary report.

Infectious Titer by TCID50

1. Enter names and dates where indicated.
2. Paste the pre-determined input-subtracted copies per well for each replicate of each dilution into the green high-lighted cells in the same orientation they were in on the PCR plate.
3. Positive replicates automatically high-light yellow and the number of positives in each dilution is automatically entered to the right of the table
4. "Input Vector Physical Titer by qPCR" automatically appears as an average GC/ mL when the Physical Titer by qPCR assay data is entered. Input vector values are automatically calculated.
5. Log(TCID50), standard error, coefficient of variation, TCID50/ mL, and P:I ratio are automatically entered as each trial worksheet is completed.

TCID50 Trial Worksheets

1. Follow the directions to enter data in the green high-lighted cells. Enter names and dates where indicated.
2. "Vector concentration (GC/ mL)" automatically appears as an average GC/ mL when the Physical Titer by qPCR assay data is entered.
3. All calculations automatically complete, are entered in the Infectious Titer by TCID50 page and, subsequently, in the summary report.

AAV2 Reference Standard Stability Characterization Report

Institution: Gene Therapy Program, University of Pennsylvania **Date:** 10-Jan-1900

Identity and Purity Analysis by SDS-PAGE

	Silver Stain		SYPRO Ruby Stain		
	Reduced	**Native**	**Reduced**	**Native**	**% Purity**
Pass or Fail	N.D.	N.D.	Pass	Pass	99.99

Reviewed by: Martin Lock Review Date: 25-Oct-2010

Infectious Titer by TCID50

Trial	Log TCID50	SE	CV %	TCID50/ mL	P:I Ratio
1	-8.458	0.2205	47.08	5.75E+09	3.88
2	-8.167	0.1816	38.14	2.94E+09	7.60

Reviewed by: Martin Lock Review Date: 25-Oct-2010

Submitted by: Julio Sanmiguel **Report Date:** 15-Oct-2010

AAV Identity and Purity Determination by SDS-PAGE

Institution: University of Pennsylvania **Date:** 26-Oct-2010

Gel A - SYPRO Ruby Stain, Reduced

Pass or Fail Pass

Sample	Lane	VP1 Present?	VP2 Present?	VP3 Present?	VP3 darker than VP1 and VP2?	Valid?	Viral Protein Purity (%)
Protein Standard	1						
Positive Control	2	YES	YES	YES	YES	YES	100.00
Negative Control	3	NO	NO	NO			
AAV Reference Standard	4	YES	YES	YES	YES		95.40

Performed by: Elias Papaioannou **Date:** 15-Sep-2010

Gel A - SYPRO Ruby Stain, Non-Reduced

Pass or Fail Pass

Sample	Lane	Contaminating Protein Present?	Molecular Weights of Contaminating Protein	Valid?	Viral Protein Purity (%)
Protein Standard	7				
Positive Control	8	NO			
Negative Control	9	NO			
AAV Reference Standard	10	NO			

Performed by: Elias Papaioannou **Date:** 15-Sep-2010

Gel B - SYPRO Ruby Stain, Reduced

Pass or Fail Pass

Sample	Lane	VP1 Present?	VP2 Present?	VP3 Present?	VP3 darker than VP1 and VP2?	Valid?	Viral Protein Purity (%)
Protein Standard	1						
Positive Control	2	YES	YES	YES	YES	YES	99.99
Negative Control	3	NO	NO	NO			
AAV Reference Standard	4	YES	YES	YES	YES		99.99

Performed by: Elias Papaioannou **Date:** 22-Sep-2010

Gel B - SYPRO Ruby Stain, non-reduced

Pass or Fail Pass

Sample	Lane	Contaminating Protein Present?	Molecular Weights of Contaminating Protein	Valid?	Viral Protein Purity (%)
Protein Standard	7				
Positive Control	8	NO			
Negative Control	9	NO			
AAV Reference Standard	10	NO			

Performed by: Elias Papaioannou **Date:** 22-Sep-2010

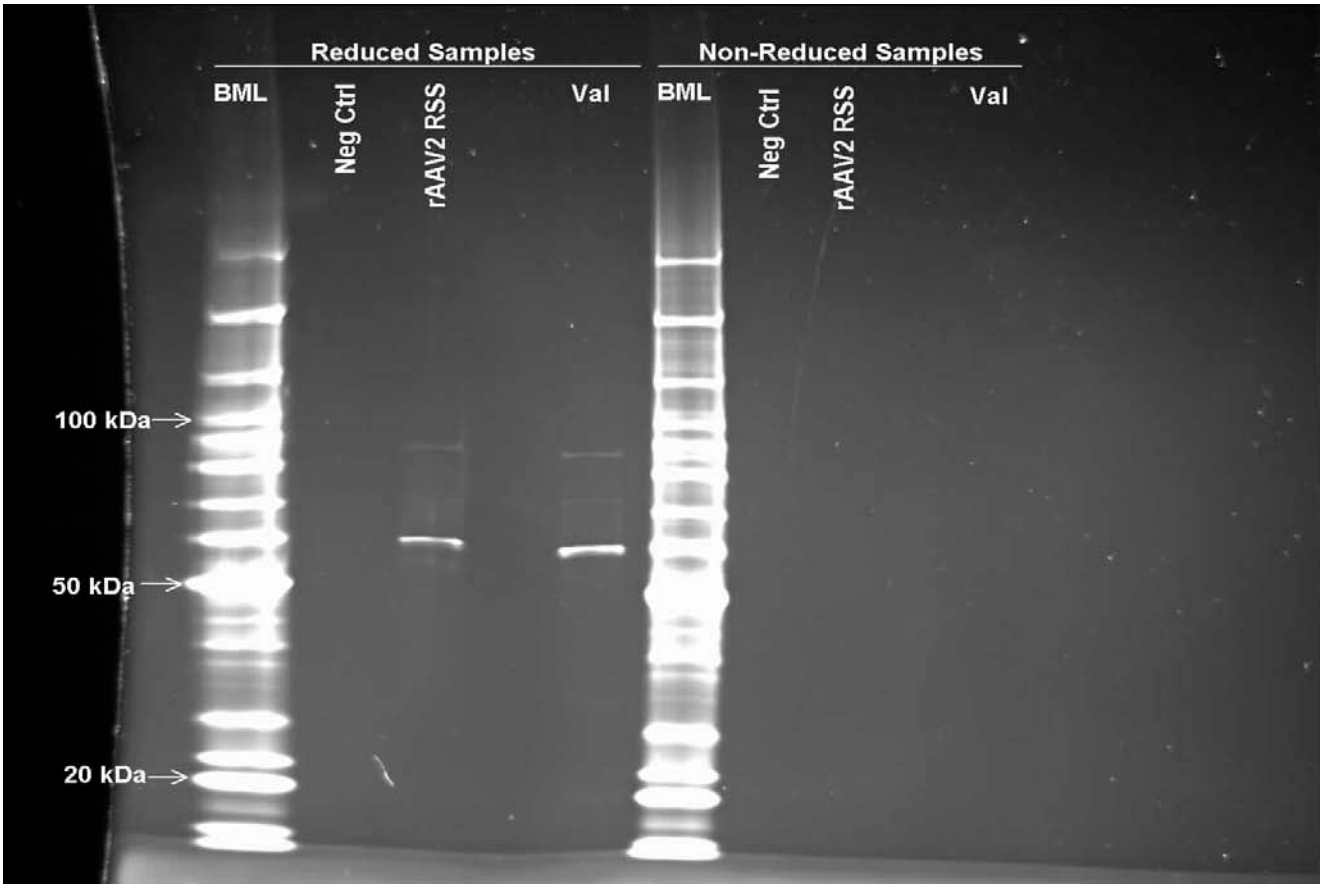
AAV Identity and Purity Determination by SDS-PAGE (Gel Pictures)

Institutuion: Gene Therapy Program **Date:** 23-Sep-2010

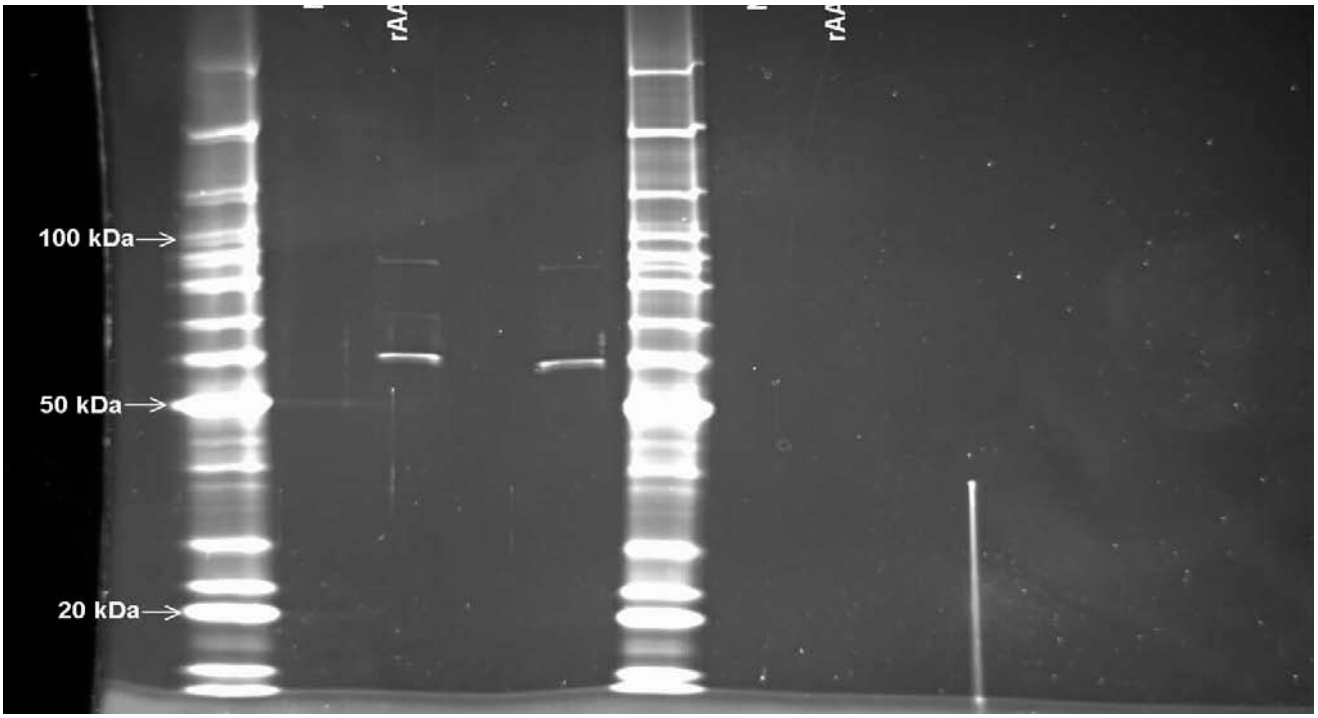
Using the "Insert" menu above, insert SDS PAGE gel pictures here from your saved image files.
This page has been left unprotected allowing you to insert, size, and format your pictures.
A suggested loading order has been entered below. Please check this loading order and make corrections as needed.

Lane	Sample
1	Benchmark Ladder - Reduced
2	Negative Control - Reduced
3	AAV Reference Material - Reduced
4	
5	Marker/ Validation - Reduced
6	Benchmark Ladder - Native
7	Negative Control - Native
8	AAV Reference Material - Native
9	
10	Marker/ Validation - Native

Aliquot 1



Aliquot 2


rAA
rAA
100 kDa
50 kDa
20 kDa

Infectious Titer Detmination by TCID50-PCR Assay Report

Institution: University of Pennsylvania **Date:** 4-Oct-2010

Trial	Log TICD50	SE	CV%	TCID50/ mL	P:I Ratio
1	-8.458	0.2205	47.08	5.75E+09	3.88
2	-8.167	0.1816	38.14	2.94E+09	7.60
3					

Input Vector Physical Titer by qPCR:	1.24E+10 GC/ mL

Trial 1

Performed by: Julio Sanmiguel **Date:** 13-Sep-2010

Dilution	Vector Input	Input subtracted copies per well												# >740
1.E-06	6.20E+02	3.22E+08	1.54E+08	2.64E+08	2.01E+08	3.03E+08	5.46E+07	1.19E+08	2.82E+08	1.33E+08	8.88E+07	1.11E+08	3.96E+06	12
1.E-07	6.20E+01	1.26E+07	2.02E+07	2.27E+07	1.28E+07	1.12E+08	9.96E+06	1.48E+07	1.89E+07	5.13E+08	2.47E+07	3.47E+07	1.37E+07	12
1.E-08	6.20E+00	-6.20E+00	-6.20E+00	8.55E+05	5.55E+04	5.93E+06	-6.20E+00	1.94E+06	1.96E+03	-6.20E+00	4.25E+05	1.29E+06	-6.20E+00	7
1.E-09	6.20E-01	-6.20E-01	-6.20E-01	-6.20E-01	-6.20E-01	2.34E+03	2.65E+02	-6.20E-01	-6.20E-01	2.72E+02	-6.20E-01	-6.20E-01	-6.20E-01	1
1.E-10	6.20E-02	1.63E+02	-6.20E-02	-6.20E-02	3.68E+02	-6.20E-02	-6.20E-02	1.08E+04	-6.20E-02	-6.20E-02	-6.20E-02	-6.20E-02	-6.20E-02	1
1.E-11	6.20E-03	-6.20E-03	-6.20E-03	-6.20E-03	-6.20E-03	-6.20E-03	1.18E+03	-6.20E-03	9.39E+03	-6.20E-03	-6.20E-03	-6.20E-03	-6.20E-03	2
Neg Ctrl	0	0.00E+00	1.10E+05	7.13E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1

logTCID50	SE	CV (%)	TCID50/mL	P:I Ratio
-8.458	0.2205	47.08	5.75E+09	3.88

Trial 2

Performed by: Julio Sanmiguel **Date:** 30-Sep-2010

Dilution	Vector Input	Input subtracted copies per well												# >740
1.E-06	1.12E+03	2.01E+08	4.68E+08	6.70E+08	4.20E+08	2.70E+08	2.03E+08	7.83E+08	1.02E+09	6.22E+08	3.94E+08	4.81E+08	2.13E+08	12
1.E-07	1.12E+02	1.66E+07	3.80E+06	2.83E+07	1.59E+07	2.46E+07	2.20E+07	1.17E+07	5.18E+07	1.40E+07	2.97E+06	4.80E+07	1.57E+07	12
1.E-08	1.12E+01	3.84E+06	-1.12E+01	2.04E+05	-1.12E+01	7.72E+05	8.03E+06	3.98E+06	-1.12E+01	-1.12E+01	-1.12E+01	7.95E+02	6.63E+06	7
1.E-09	1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	-1.12E+00	5.14E+05	1
1.E-10	1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	-1.12E-01	0
1.E-11	1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	-1.12E-02	3.12E+03	-1.12E-02	-1.12E-02	1
Neg Ctrl	0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0

logTCID50	SE	CV (%)	TCID50/mL	P:I Ratio
-8.167	0.1816	38.14	2.94E+09	7.60

Trial 3

Dilution
1.E-06
1.E-07
1.E-08
1.E-09
1.E-10
1.E-11
Neg Ctrl

Trial 1 TCID50 Calculation by Karber's Method

Institute: University of Pennsylvania **Date:** 13-Sep-2010

1. Enter data in high-lighted areas.
2. Dose #1 is the highest dilution with 100% positive response.
3. Dose entries should be subsequent dilutions until the first dose with 0% positive response.
4. Enter log (dilution) as log, i.e. enter 1e-8 dilution as -8.

Dose #	log (dilution)	# Positive	# Treated	% Positive	$(P_i + P_{i+1})$	$[P_i \times (1 - P_i)] \div n$
1	-6	12	12	1.000	2.000	0.0000
2	-7	12	12	1.000	1.583	0.0000
3	-8	7	12	0.583	0.833	0.0203
4	-9	3	12	0.250	0.333	0.0156
5	-10	1	12	0.083	0.167	0.0064
6	-11	1	12	0.083		0.0064
P = % Positive		n = # Treated		Total	4.917	0.049

Volume Inoculm (ml): 0.050

Vector Concentration (GC/ mL): 2.23E+10

X = log (dilution) X1 = log (dilution) at the first dose d = X1 − X2 ml= volume of inoculum in ml

Standard Error log ($TCID_{50}$)

SE log ($TCID_{50}$)= d x $\sqrt{\sum [Pi \times (1 - Pi)] \div n}$

SE log ($TCID_{50}$)= 1 x $\sqrt{0.049}$

SE log ($TCID_{50}$)= 0.2205

log ($TCID_{50}$) Calculation

log ($TCID_{50}$) = X_1 − 0.5 x d x $\sum (P_i + P_{i+1})$

log ($TCID_{50}$) = -6 − 0.5 x 1 x 4.917

log ($TCID_{50}$) = -8.458

$TCID_{50}$/ ml Calculation

$TCID_{50}$/ ml = 10 ^ (-log ($TCID_{50}$)) ÷ ml

$TCID_{50}$/ ml = 10 ^ 8.458 ÷ 0.050

$TCID_{50}$/ ml = 5.75E+09

P:I Ratio = Input Vector (pt/ ml) ÷ $TCID_{50}$/ ml = 3.88

Coefficient of Variation $TCID_{50}$/ ml

SE $TCID_{50}$/ ml = $TCID_{50}$/ ml x ln[-log ($TCID_{50}$)] x SE log ($TCID_{50}$)

SE $TCID_{50}$/ ml = 5.75E+09 x 2.135 x 0.220

SE $TCID_{50}$/ ml = 2.70E+09

CV $TCID_{50}$/ ml (%)= SE $TCID_{50}$/ ml ÷ $TCID_{50}$/ ml x 100

CV $TCID_{50}$/ ml (%)= 2.70E+09 ÷ 5.75E+09 x 100

CV $TCID_{50}$/ ml (%)= 47.08 %

log ($TCID_{50}$) 95% Confidence Interval

log ($TCID_{50}$) limit = log ($TCID_{50}$) ± (1.96 x SE log ($TCID_{50}$))

lower log ($TCID_{50}$) = -8.458 + (1.96 x 0.2205) = -8.026

upper log ($TCID_{50}$) = -8.458 − (1.96 x 0.2205) = -8.890

lower $TCID_{50}$/ ml = 2.12E+09 lower P:I Ratio = Input Vector (pt/ ml) ÷ upper $TCID_{50}$/ ml = 10.50

upper $TCID_{50}$/ ml = 1.55E+10 upper P:I Ratio = Input Vector (pt/ ml) ÷ lower $TCID_{50}$/ ml = 1.43

Trial 2 TCID50 Calculation by Karber's Method

Institute: University of Pennsylvania **Date:** 30-Sep-2010

1. Enter data in high-lighted areas.
2. Dose #1 is the highest dilution with 100% positive response.
3. Dose entries should be subsequent dilutions until the first dose with 0% positive response.
4. Enter log (dilution) as log, i.e. enter 1e-8 dilution as -8.

Dose #	log (dilution)	# Positive	# Treated	% Positive	$(P_i + P_{i+1})$	$[P_i \times (1 - P_i)] \div n$
1	-6	12	12	1.000	2.000	0.0000
2	-7	12	12	1.000	1.583	0.0000
3	-8	7	12	0.583	0.667	0.0203
4	-9	1	12	0.083	0.083	0.0064
5	-10	0	12	0.000	0.000	0.0000
6	-11	1	12	0.083		0.0064
P = % Positive		n = # Treated		Total	4.333	0.033

Volume Inoculm (ml): 0.050

Vector Concentration (GC/ mL): 2.23E+10

X = log (dilution) X1 = log (dilution) at the first dose d = X1 − X2 ml= volume of inoculum in ml

Standard Error log ($TCID_{50}$)

SE log ($TCID_{50}$)= d x $\sqrt{\sum [Pi \times (1 - Pi)] \div n}$

SE log ($TCID_{50}$)= 1 x $\sqrt{0.033}$

SE log ($TCID_{50}$)= 0.1816

log ($TCID_{50}$) Calculation

log ($TCID_{50}$) = X_1 − 0.5 x d x $\sum (P_i + P_{i+1})$

log ($TCID_{50}$) = -6 − 0.5 x 1 x 4.333

log ($TCID_{50}$) = -8.167

$TCID_{50}$/ ml Calculation

$TCID_{50}$/ ml = 10 ^ (-log ($TCID_{50}$)) ÷ ml

$TCID_{50}$/ ml = 10 ^ 8.167 ÷ 0.050

$TCID_{50}$/ ml = 2.94E+09

P:I Ratio = Input Vector (pt/ ml) ÷ $TCID_{50}$/ ml = 7.60

Coefficient of Variation $TCID_{50}$/ ml

SE $TCID_{50}$/ ml = $TCID_{50}$/ ml x ln[-log ($TCID_{50}$)] x SE log ($TCID_{50}$)

SE $TCID_{50}$/ ml = 2.94E+09 x 2.100 x 0.182

SE $TCID_{50}$/ ml = 1.12E+09

CV $TCID_{50}$/ ml (%)= SE $TCID_{50}$/ ml ÷ $TCID_{50}$/ ml x 100

CV $TCID_{50}$/ ml (%)= 1.12E+09 ÷ 2.94E+09 x 100

CV $TCID_{50}$/ ml (%)= 38.14 %

log ($TCID_{50}$) 95% Confidence Interval

log ($TCID_{50}$) limit = log ($TCID_{50}$) ± (1.96 x SE log ($TCID_{50}$))

lower log ($TCID_{50}$) = -8.167 + (1.96 x 0.1816) = -7.811

upper log ($TCID_{50}$) = -8.167 − (1.96 x 0.1816) = -8.523

lower $TCID_{50}$/ ml = 1.29E+09 lower P:I Ratio = Input Vector (pt/ ml) ÷ upper $TCID_{50}$/ ml = 17.24

upper $TCID_{50}$/ ml = 6.66E+09 upper P:I Ratio = Input Vector (pt/ ml) ÷ lower $TCID_{50}$/ ml = 3.35