Calculation of neutralization capacity as a percentage of inhibition for inhibitory assay with AAV responsive reporter Cells

For research and professional use only. Not for use in diagnostic procedures.

This technical note is written to describe a suggested calculation method of neutralizing capacity of individual samples as a % of inhibition based on reference samples - other methods can be followed. Each laboratory must set up their own method and perform a relevant evaluation.

Background

The combination of *iLite*® AAV Packaging Assay Ready Cells and *iLite*® AAV Responsive Reporter Assay Ready Cells can be utilized for the detection and quantification of neutralizing antibodies against AAV vectors.

This technical note aims to instruct how to calculate the neutralizing capacity of individual samples as a percentage of inhibition based on a human normal immunoglobulin (IVIG) reference exemplified by analysis of human serum samples tested in final 2 % dilution using the combination of *iLite* AAV2 Packaging cells (BM6002) and *iLite* AAV Responsive Reporter cells (BM6100). The calculation principles illustrated here can be extrapolated to the other combinations of *iLite* AAV Responsive Reporter cells and *iLite* AAV Packaging cells. Reference to detailed assay instruction is to be found in the specific *iLite* Assay Ready Cells product specification.

Protocol - inhibitory assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	10 000	2 000	400	133	44	15	4.9	1.6	0.55	0.18	0	0	IVIG [µg/ml]
B													
C													
D	Sample 1			Sample 6			Sample 11			Sample 16			Sera samples
E	Sample 2			Sample 7			Sample 12			Sample 17			
F	Sample 3			Sample 8			Sample 13			Sample 18			
G	Sample 4			Sample 9			Sample 14			Sample 19			
H	Sample 5			Sample 10			Sample 15			Sample 20			

Table 1: Plate layout (IVIG reference together with 20 individual test samples in one dilution)

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Stock concentration for the IVIG reference is given by manufacture as 100 mg/ml. Dilution of reference is performed in diluent supplemented with IgG depleted serum or AAV-NAb negative sera to ensure matrix consistency with test samples, ex. if test samples are diluted as serum 1:25 in pure diluent (eg. final 4% human serum), then reference ought to be diluted in diluent supplemented with 4 % IgG depleted serum or AAV-NAb negative sera.

Column	Total Vol [µl]	Vol of media [µl]	Vol of IVIG [µl]		Dilution Factor	Solution conc [µg/ml]	In-assay conc [µg/ml]	Qty Left [µl]
1	750	600	150	**IVIG 100 mg/ml**	5	20000	**10000**	600
2	750	600	150	1	5	4000	**2000**	600
3	750	600	150	2	5	800	**400**	450
4	900	600	300	3	3	267	**133**	600
5	900	600	300	4	3	89	**44**	600
6	900	600	300	5	3	30	**15**	600
7	900	600	300	6	3	9.9	**4.9**	600
8	900	600	300	7	3	3.3	**1.6**	600
9	900	600	300	8	3	1.1	**0.55**	600
10	900	600	300	9	3	0.37	**0.18**	900
11-12	600	600				0	**0**	600

Table 2: Dilution scheme for IVIG, 40 µL cell solution per well

Analysis of neutralization capacity as a percentage of inhibition based upon IgG reference

Perform an XY scatter plot of serial diluted IVIG reference with the in-assay cell concentration on the X-axis (log scale) and the luminometer reading values on the Y-axis (linear scale). Perform a four-parameter logistic (4PL) curve fit to characterize the sigmoidal curve obtained by the serially diluted reference.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2662	3767	83450	452300	1251000	1516000	2320000	2378000	2575000	2446000	2947000	2106000
B	2697	6097	81170	439000	1272000	1828000	2592000	2418000	2496000	2615000	2670000	2753000
C	1045	3172	74320	533600	1183000	1851000	2230000	2620000	2711000	2583000	3065000	3029000
D	69780	116500	69100	1509000	1183000	1225000	394500	405600	315500	1098000	1006000	851600
E	1259000	1275000	1576000	1263000	1225000	1221000	907000	907700	892600	1220000	1191000	1107000
F	1096000	1150000	919900	149700	148500	97230	160400	157800	191500	18230	15910	13750
G	1339000	1578000	1229000	1615000	1251000	1102000	184900	169000	120700	2034000	1829000	1961000
H	1383000	1632000	1258000	647400	646800	433600	319700	179600	164600	554900	478800	486700

Table 3: Luminometer reading values (firefly luciferase relative light units)

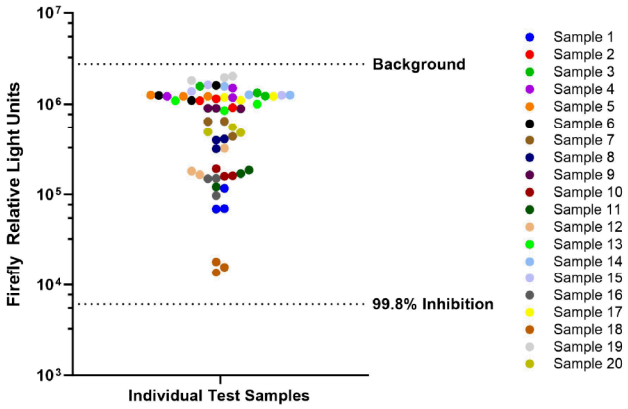

Figure 1. Example of the neutralizing capacity of individual samples containing human sera. Values are shown as triplicate values of firefly relative light units.

How background level is defined for the neutralization assay is to be established for each individual application and after relevant validation, here the background is defined as the top part of the sigmoid curve (fitting top value) obtained using the IVIG dose-response curve as a reference.

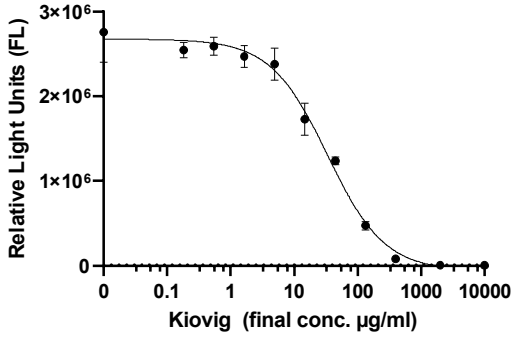

Figure 2. **XY scatter plot** of IVIG reference. Values are shown as the mean of triplicate ± SD.

Sigmoidal, 4PL, X is log(conc)	
Best-fit values	**Kiovig**
Top	2677707
Bottom	-62362
HillSlope	-0.9662
IC50	33.61
R squared	0.9763

Table 4. Fitting values of the curve using a 4-parameter logistic model.

Once the top values of the fitting is obtained, we can calculate the neutralizing capacity as a percentage of inhibition can be calculated as:

$$Percentage\ of\ inhibition = \frac{Fitting\ top\ value - sample}{Fitting\ top\ value} * 100$$

	1	2	3	4	5	6	7	8	9	10	11	12
A	99,9%	99,9%	96,9%	83,1%	53,3%	43,4%	13,4%	11,2%	3,8%	8,7%	-10,1%	21,4%
B	99,9%	99,8%	97,0%	83,6%	52,5%	31,7%	3,2%	9,7%	6,8%	2,3%	0,3%	-2,8%
C	100,0%	99,9%	97,2%	80,1%	55,8%	30,9%	16,7%	2,2%	-1,2%	3,5%	-14,5%	-13,1%
D	97,4%	95,6%	97,4%	43,6%	55,8%	54,3%	85,3%	84,9%	88,2%	59,0%	62,4%	68,2%
E	53,0%	52,4%	41,1%	52,8%	54,3%	54,4%	66,1%	66,1%	66,7%	54,4%	55,5%	58,7%
F	59,1%	57,1%	65,6%	94,4%	94,5%	96,4%	94,0%	94,1%	92,8%	99,3%	99,4%	99,5%
G	50,0%	41,1%	54,1%	39,7%	53,3%	58,8%	93,1%	93,7%	95,5%	24,0%	31,7%	26,8%
H	48,4%	39,1%	53,0%	75,8%	75,8%	83,8%	88,1%	93,3%	93,9%	79,3%	82,1%	81,8%

Table 5: Percentage of inhibition based upon reading values found in Table 3

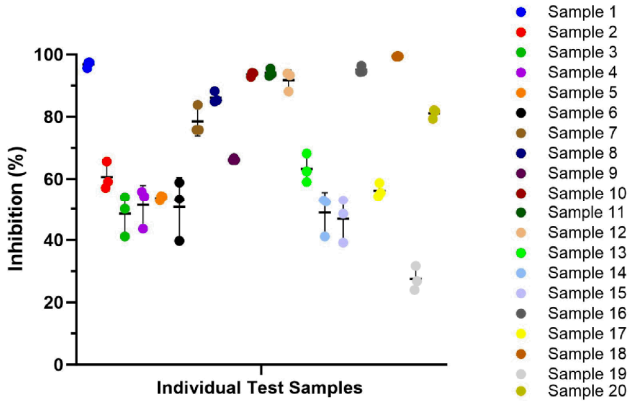

Figure 3. **Samples plotted as % of inhibition** by using the IVIG dose-response curve as reference. Values are shown as the mean of triplicate ± SD.

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com