N. Ferry, July 16, 2004

Screen Methods

The Screen is used to determine *in vitro* activity of experimental compounds. This test utilizes a 96-well micrometer plate (8 rows by 12 columns).

Six species of test organisms were tested in the Screen and evaluated in growth inhibition tests. One species was tested in each assay. Compounds were diluted and dispensed into 96 well microtiter assay plates. The assay plates were then inoculated with a mixture of test organism and minimal media for growth. After an incubation period the plates were evaluated for percent growth inhibition by optical density (OD) readings from a spectrophotometer.

Check Data (i.e., Negative Controls)
Check or untreated wells have only the growth media, the carrier solution and the test organism.

Standards Data (i.e., Positive Controls)
Five positive standards were run on each of the assays. Standard wells had the growth media, the rate of the standard compound formulated in the carrier solution and the test organism.
Each standard was tested at 8 rates, with 4 replicates per rate. Below is the plate map for the standards. Two standard plates were tested each week with a total of n=4 replicates per rate for each standard.

	1	2	3	4	5	6	7	8	9	10	11	12
Contents of Column	Check	Std1	Std2	Std3	Std4	Std5	Check	Std1	Std2	Std3	Std4	Std5
A	Check	17	6.8	17	6.8	17	Check	17	6.8	17	6.8	17
B	Check	5.67	2.27	5.67	2.27	5.67	Check	5.67	2.27	5.67	2.27	5.67
C	Check	1.89	0.76	1.89	0.76	1.89	Check	1.89	0.76	1.89	0.76	1.89
D	Check	0.63	0.25	0.63	0.25	0.63	Check	0.63	0.25	0.63	0.25	0.63
E	Check	0.21	0.084	0.21	0.084	0.21	Check	0.21	0.084	0.21	0.084	0.21
F	Check	0.07	0.028	0.07	0.028	0.07	Check	0.07	0.028	0.07	0.028	0.07
G	Check	0.023	0.009	0.023	0.009	0.023	Check	0.023	0.009	0.023	0.009	0.023
H	Check	0.008	0.003	0.008	0.003	0.008	Check	0.008	0.003	0.008	0.003	0.008

The data is all OD values. For analysis, we took the median OD value for the checks for a specific application date and assay and use that median value to create percent control for each response for a standard/rate well where:

$$\% \text{ control} = \frac{(\text{OD for std}) - \text{median OD for check}}{\text{median OD for check}}$$

High rates of a standard that controls a test organism will have low OD values relative to the check and therefore high percent control values. Low rates of a standard that does not control the pathogen will have high OD values, similar to the check wells, and therefore low percent control values.