LID Statistical Web Tools

Thank you for using the LID Statistical Web Tools. This web page contains three statistical tools designed to estimate titer levels from neutralization assays, compute Student's T-tests from summarized data or compute contingency table analyses for 2 x 2 tables. These tools were designed by Dr. Stephen Whitehead of NIAID Laboratory of Infectious Diseases (LID) and the NIAID Bioinformatics and Computational Biosciences Branch (BCBB).

PlaqueReduction

The PlaqueReduction tab is used to estimate 50 - 90% titer levels and confidence intervals from neutralization assay data (Figure 1). Users will need to enter three constants: the initial dilution of the assay, the fold change value of the serial dilution, and the average number of plaques from all the negative control wells, followed by the plaque counts for each well. After the data is entered, the calculate button will compute 50, 60, 70, 80, and 90% titer levels and confidence intervals using simple linear regression techniques. Statistical results will be displayed underneath the main menus (Figure 1).

Data for the PlaqueReduction tool should be taken from a plaque reduction worksheet with plaque counts for test samples and media-only control samples. The PlaqueReduction Web Tool first requires that users specify the initial dilution (e.g. 1:10) and the fold change of serial dilution (e.g. 4 fold dilution). Second, users should compute and enter the average number of plaques in the serial dilution of the media-only group. Finally, researchers should enter the plaque counts from each well of each sample. As described in Figure 2, do not enter plaque counts for more than a single dilution above the appropriate cutoff value. See Figure 2 for several examples of how to determine the valid range of values. Once the appropriate data has been entered, click Calculate to view the results (Figure 1).

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1. Dilution Information

Initial serum dilution (*Format: 1:10*)): 1:10

Serial dilution fold value: 4

2. Number of plaques in each media-only control well +

1: 23.1

The average of the control is 23.1

The cutoff value is:

50%	60%	70%	80%	90%
11.6	9.2	6.9	4.6	2.3

3. Number of plaques in each serum dilution well +

Index	Dilution	Well 1	Well 2
1:	1:10	0	0
2:	1:40	0	1
3:	1:160	6	5
4:	1:640	14	13
5:	1:2560		
6:	1:10240		

Clear Sample Data After Calculation

Calculate

Dilution	Ave. #Plaques	Exp. Average
1:40.0	0.5	0.6
1:160.0	5.5	4.1
1:640.0	13.5	14.5

Level	Titer	Log2(Titer)
50%	448.8	8.8
60%	342.3	8.4
70%	254.9	8.0
80%	177.8	7.5
90%	103.5	6.7

Figure 1. Results from the Plaque Reduction Tool.

Plaque reduction neutralization calculations December 1, 2010
Whitehead, LID, 33/3W10A

Materials:

Plaque counts from neutralization assay

Assumptions:

1. Initial serum dilution does not include the 1:1 dilution that occurs during the assay when equal volumes of serum and virus are mixed.

2. Plaque counts in each well should generally be in the range of 20 and 40 per well, depending on plaque size and countability.

Process for determining valid data points:

3. Calculate and enter the mean number of plaques for the media-only control wells.

4. The program will generate the cutoff values for the various levels of reduction. This cutoff value is used to determine which data points are valid for inclusion in the calculation.

5. Enter plaque counts for each pair of wells as instructed by the program.

6. Do not enter plaque counts for more than a single dilution above the appropriate cutoff value.

Example 1:

60% PRNT
Initial dilution: 1:5
Mean number of plaques: 39
Cutoff value (for 60% level) = 15.6 (for 60% reduction in plaques, only 40% of the plaques remain)

Dilution	1:5		1:20		1:80		1:320		1:1280		1:5120	
Plaques	0	0	1	0	3	2	10	21	30	33	39	30

↑ 60% plaque reduction (15.6)

Only shaded values should be used in the calculation
$PRNT_{60}$ = 347 ($PRNT_{60}$ using all 6 dilutions = 440)

Example 2:

60% PRNT
Initial dilution: 1:10
Mean number of plaques: 24
Cutoff value (for 60% level) = 9.6 (for 60% reduction in plaques, only 40% of the plaques remain)

Dilution	1:10		1:40		1:160		1:640		1:2560		1:10240	
Plaques	0	1	5	8	16	12	21	19	20	22	21	25

↑ 60% plaque reduction (9.6)

Only shaded values should be used in the calculation
$PRNT_{60}$ = 83 ($PRNT_{60}$ using all 6 dilutions = 140)

7. Record PRNT values as generated by program.

Figure 2. Plaque reduction worksheet.

Student's T-test

The Student's T-test tab is used to compute a two-sample Student's T-test from summarized data, which could be computed in MS Excel or possibly found in an existing publication. To compute the Student's T-test, simply enter the sample means, the sample standard deviations and the sample sizes from two samples or groups into the appropriate blanks of the web page and click calculate (Figure 3). Results include the test statistic T, the degrees of freedom and the p-value.

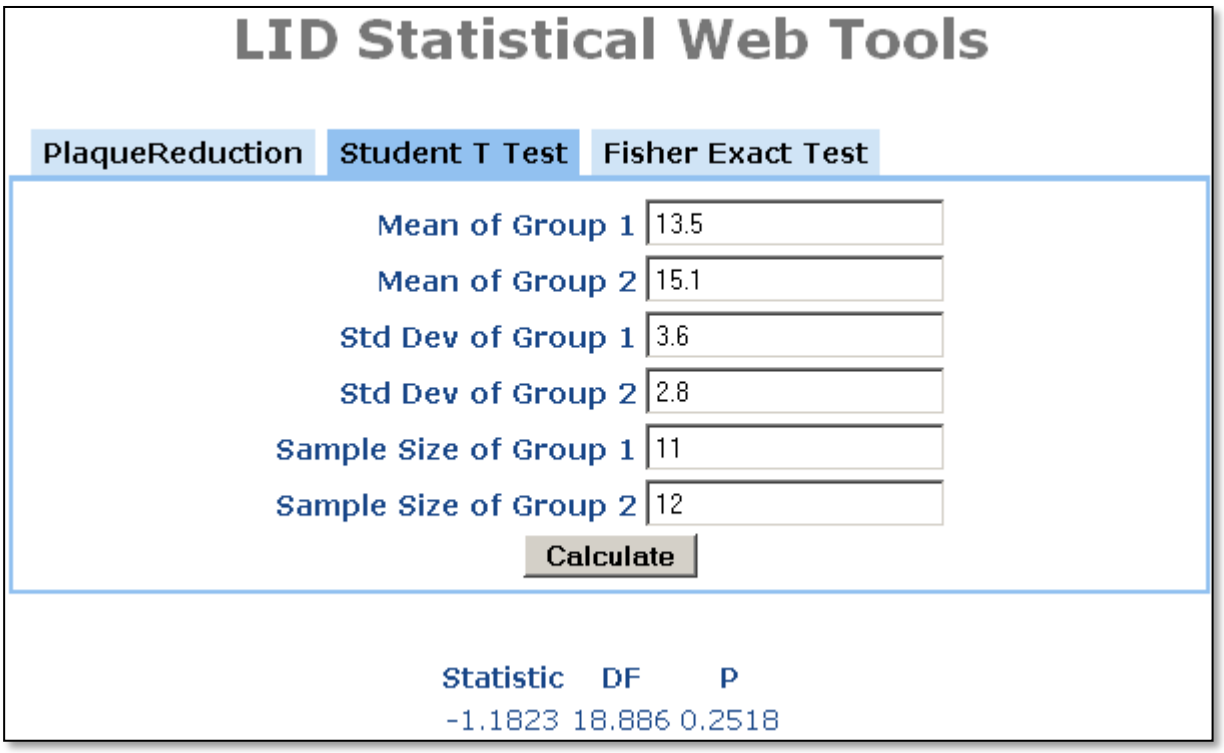

Figure 3. Results from the Student's T-test tool.

Fisher's Exact Test

The Fisher's Exact Test tab is used to compute a Fisher's exact tests and Pearson Chi-Square tests for 2 x 2 contingency tables. To compute the tests, enter data from a 2 x 2 contingency table into the four cells appropriate cells representing the two columns and two rows of the 2 x 2 table and click calculate (Figure 4). Statistical results include one-tailed and two-tailed p-values from the Fisher's exact test, odds ratios computed for the Fisher's exact tests and a two-tailed p-value for the Pearson's Chi-Square test. Results for the Fisher's exact test are more reliable when tables include small cell counts or zero cell counts, but Fisher's exact test assumes that the column and row totals from the table are known and fixed constants (e.g. the 2 x 2 contingency table data comes from a designed experiment). Results for Pearson's Chi-Square test require larger cell counts, but make no assumptions about the row and column totals of the table.

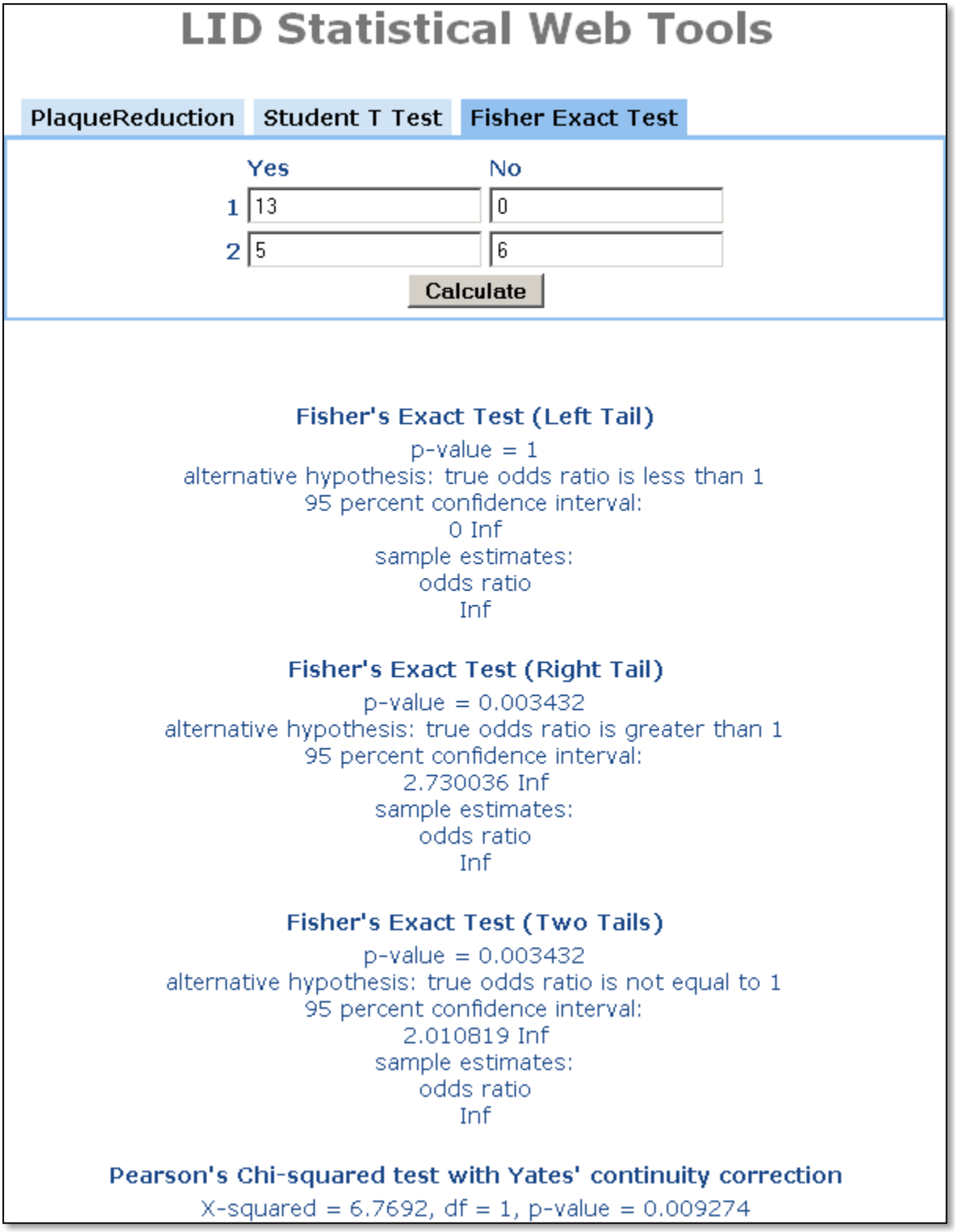

Figure 4. Results from the Fisher's Exact Test tool.