

3-20-18 Calculations using 7-day ddPCR data

Purpose: Gail calculated outliers and normalized the data to the UniSp5 spike-in. I observed there was no correlation between target expression and UniSp5 measures, suggesting that whatever is affecting the measurement of the spike-in is likely not affecting measurements in the target miRNA. Therefore I am re-doing the analysis on raw target data.

Tests:

The identification of outliers suggests that the data is not normally distributed. I will use a Shapiro-Wilk Normality Test for each group for each target and test for normality. The webtool located here was used: <http://sdittami.altervista.org/shapirotest/ShapiroTest.html>

Raw data for miR-378a is below and used to calculate normality

miR-378					
C	0.75K	1.5K	3K	6K	
1.695	0.81	6.3	2.3	5.8	
5.3	5.75	3.3	11.4	9.65	
10.45	4.8	6.45	4.4	4.5	
3.15	0.805	12.75	3.5	7.2	
1.685	9	3.3	2	4.75	
4.95	5.6	52.65	2.4	6	
4.15	7.5		4.1	18.85	
9.85			5.85	5.15	

Control group:

Results:

n = 8

Mean = 5.15375

SD = 3.3633664576687785

W = 0.8709130432637512

Threshold (p=0.01) = 0.7490000128746033 --> HO accepted

Threshold (p=0.05) = 0.8180000185966492 --> HO accepted

Threshold (p=0.10) = 0.8510000109672546 --> HO accepted

--> Your data seems normal

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

0.75K group:

Results:

n = 7
Mean = 4.895
SD = 3.1168747702359383
W = 0.9064269312945878
Threshold (p=0.01) = 0.7300000190734863 --> HO accepted
Threshold (p=0.05) = 0.8029999732971191 --> HO accepted
Threshold (p=0.10) = 0.8379999995231628 --> HO accepted
--> Your data seems normal

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

1.5K group:
n = 6
Mean = 14.125
SD = 19.1862646182106
W = 0.6430000338445044
Threshold for (p=0.01) = 0.7129999995231628 --> HO rejected
Threshold (p=0.05) = 0.7879999876022339 --> HO rejected
Threshold (p=0.10) = 0.8259999752044678 --> HO rejected
--> Your data is not normally distributed p<0.01

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

3.0K group:

Results:

n = 8
Mean = 4.49375
SD = 3.0729740712959397
W = 0.7830121262215891
Threshold (p=0.01) = 0.7490000128746033 --> HO accepted
Threshold (p=0.05) = 0.8180000185966492 --> HO rejected
Threshold (p=0.10) = 0.8510000109672546 --> HO rejected
--> Your data is not normally distributed p<0.05

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

6K group:

Results:

n = 8
Mean = 7.7375
SD = 4.785525049563527
W = 0.7013623850247308

Threshold for ($p=0.01$) = 0.7490000128746033 --> H_0 rejected

Threshold ($p=0.05$) = 0.8180000185966492 --> H_0 rejected

Threshold ($p=0.10$) = 0.8510000109672546 --> H_0 rejected

--> Your data is not normally distributed $p < 0.01$

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

Results for miR-378a: They indicate that 3 out of the 5 treatment groups are not normally distributed. Therefore a non-parametric test must be used to examine the data.

A Mann-Whitney U Test was used to compare control to the treatment groups for miR-378a. The webtool is located here: <http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>

Control vs. 0.75K

The U-value is 28. The critical value of U at $p < .05$ is 10. Therefore, the result is *not* significant at $p < .05$.

The Z-Score is 0.05786. The p -value is .95216. The result is *not* significant at $p < .05$.
Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Control vs. 1.5K

The U-value is 14. The critical value of U at $p < .05$ is 8. Therefore, the result is *not* significant at $p < .05$.

The Z-Score is -1.22644. The p -value is .2187. The result is *not* significant at $p < .05$.
Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Control vs. 3K

The U-value is 30. The critical value of U at $p < .05$ is 13. Therefore, the result is *not* significant at $p < .05$.

The Z-Score is 0.15753. The p -value is .87288. The result is *not* significant at $p < .05$.
Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

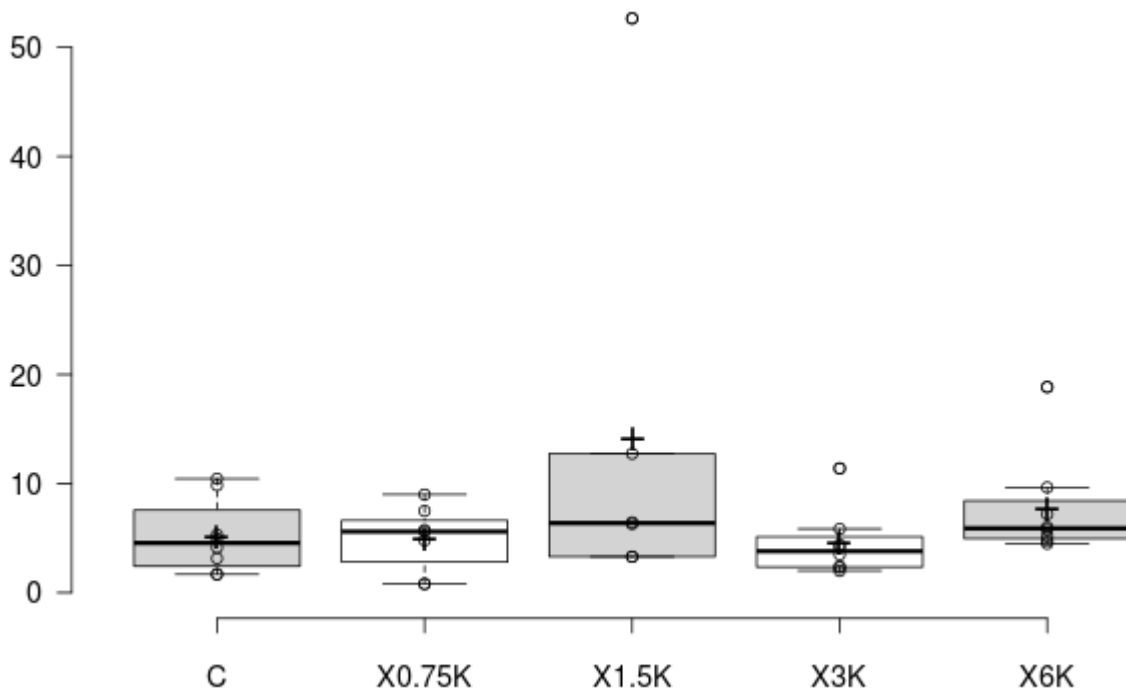
Control vs. 6K:

The U -value is 19. The critical value of U at $p < .05$ is 13. Therefore, the result is not significant at $p < .05$.

The Z -Score is -1.31276. The p -value is .1902. The result is not significant at $p < .05$. Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z -value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

No significance is therefore observed with any DEHP treatment groups for miR-378a measurements in the serum. Boxplot of data is below using the webtool <http://shiny.chemgrid.org/boxplotr/>



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Box plot statistics

	C	X0.75K	X1.5K	X3K	X6K
Upper whisker	10.45	9.00	12.75	5.85	9.65
3rd quartile	7.57	6.62	12.75	5.12	8.43
Median	4.55	5.60	6.38	3.80	5.90
1st quartile	2.42	2.80	3.30	2.35	4.95
Lower whisker	1.69	0.81	3.30	2.00	4.50
Nr. of data points	8.00	7.00	6.00	8.00	8.00
Mean	5.15	4.89	14.12	4.49	7.74

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<<180320_mir378a_DEHP_7day_serum.pdf>>

Raw data for miR-182 is below and used to calculate normality

miR-182				
C	0.75K	1.5K	3K	6K
3.8	3.2	1.8	1.25	1.65
2.75	2.45	1.65	1.55	1.255
2.65	29	1.75	0.97	1.425
3.5	1.145	1.9	1	1.65
1.81	2.71	2.65	1.3	1.84
2	5.35	8.7	1.95	2.3
1.45	2.35		2.4	2.6
2.5			2.45	1.8

Control group:

Results:

n = 8
Mean = 2.5574999999999997
SD = 0.8086275851271533
W = 0.9598843090861647
Threshold (p=0.01) = 0.7490000128746033 --> HO accepted
Threshold (p=0.05) = 0.8180000185966492 --> HO accepted
Threshold (p=0.10) = 0.8510000109672546 --> HO accepted
--> Your data seems normal

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

0.75K group:

Results:

n = 7
Mean = 6.600714285714285
SD = 9.958719976554645
W = 0.5674955145740516
Threshold for (p=0.01) = 0.7300000190734863 --> HO rejected
Threshold (p=0.05) = 0.8029999732971191 --> HO rejected
Threshold (p=0.10) = 0.837999995231628 --> HO rejected
--> Your data is not normally distributed p<0.01

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

1.5K group:

Results:

n = 6
Mean = 3.075
SD = 2.7789836271557986
W = 0.5954722667662936
Threshold for (p=0.01) = 0.712999995231628 --> HO rejected
Threshold (p=0.05) = 0.7879999876022339 --> HO rejected
Threshold (p=0.10) = 0.8259999752044678 --> HO rejected
--> Your data is not normally distributed p<0.01

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

3.0K group:

Results:

n = 8
Mean = 1.6087500000000001

SD = 0.5923666215155226
W = 0.885366016113166
Threshold (p=0.01) = 0.7490000128746033 --> HO accepted
Threshold (p=0.05) = 0.8180000185966492 --> HO accepted
Threshold (p=0.10) = 0.8510000109672546 --> HO accepted
--> Your data seems normal

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

6.0K group:

Results:

n = 8
Mean = 1.8150000000000002
SD = 0.4427914697849155
W = 0.9342965981386935
Threshold (p=0.01) = 0.7490000128746033 --> HO accepted
Threshold (p=0.05) = 0.8180000185966492 --> HO accepted
Threshold (p=0.10) = 0.8510000109672546 --> HO accepted
--> Your data seems normal

From <<http://sdittami.altervista.org/shapirotest/ShapiroTest.html>>

Results for miR-182: They indicate that 2 out of the 5 treatment groups are not normally distributed. Therefore a non-parametric test must be used to examine the data.

A Mann-Whitney U Test was used to compare control to the treatment groups for miR-378a. The webtool is located here: <http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>

Control vs. 0.75K

The U-value is 23. The critical value of U at $p < .05$ is 10. Therefore, the result is *not* significant at $p < .05$.

The Z-Score is -0.52077. The p -value is .60306. The result is *not* significant at $p < .05$.
Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Control vs. 1.5K:

The U-value is 17.5. The critical value of U at $p < .05$ is 8. Therefore, the result is *not* significant at $p < .05$.

The Z-Score is 0.7746. The p -value is .4413. The result is *not* significant at $p < .05$.

Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Control vs. 3K:

The *U*-value is 9. The critical value of *U* at $p < .05$ is 13. Therefore, the result is significant at $p < .05$.

The *Z*-Score is 2.36297. The *p*-value is .01828. The result is significant at $p < .05$.

Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Control vs. 6K:

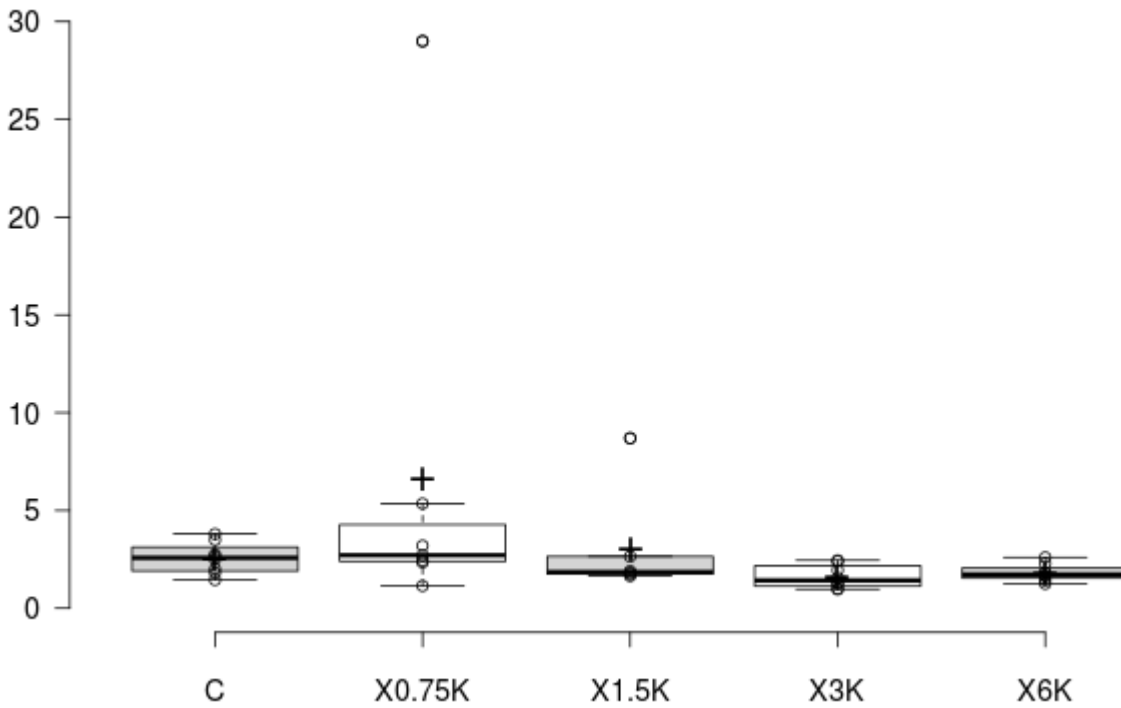
The *U*-value is 12. The critical value of *U* at $p < .05$ is 13. Therefore, the result is significant at $p < .05$.

The *Z*-Score is 2.04791. The *p*-value is .04036. The result is significant at $p < .05$.

Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10, so caution is appropriate here in making use the Z-value calculation.

From <<http://www.socscistatistics.com/tests/mannwhitney/Default2.aspx>>

Significance is therefore observed with 3K and 6K DEHP treatment groups for miR-182 measurements in the serum. Boxplot of data is below using the webtool <http://shiny.chemgrid.org/boxplotr/>



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Box plot statistics

	C	X0.75K	X1.5K	X3K	X6K
Upper whisker	3.80	5.35	2.65	2.45	2.60
3rd quartile	3.12	4.28	2.65	2.17	2.07
Median	2.58	2.71	1.85	1.43	1.73
1st quartile	1.91	2.40	1.75	1.12	1.54
Lower whisker	1.45	1.15	1.65	0.97	1.25
Nr. of data points	8.00	7.00	6.00	8.00	8.00
Mean	2.56	6.60	3.07	1.61	1.81

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