ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

Description of differences in ELISA results under different temperature conditions

Baoqin Jiang, Guoyan Li*, Dejie Feng, Chenming Liu, Yingtong Liu, Kai Wang, Qin Huang, Li Zhang, Rui Wang, Jiangmin Wu, Hai Zhu

Lanzhou Institute of Biological Products Co. Ltd., Lanzhou, 730046, China
**Corresponding author*

Abstract: *The significance test for paired sample differences in biological testing is the most commonly used statistical method in biological testing data analysis. Before conducting the test, it is necessary to check whether there is any difference between groups and calculate the degree of difference. This article takes the results of enzyme-linked immunosorbent assay (ELISA) under different temperature conditions as an example, and describes the differences from the aspects of descriptive analysis, correlation, variance, and normality analysis. Finally, non-parametric testing methods are used for significance testing. The results indicate that the analysis method is more applicable.*

Keywords: *Significance of differences, Descriptive analysis, Normality analysis, Correlation analysis, Variance analysis, Nonparametric test*

1. Introduction

In the process of researching new drugs and vaccines, biological testing plays a crucial role. Two sets of samples that are interrelated belong to paired samples, for example, the results of three enzyme-linked immunosorbent assay (ELISA) tests at 36 ℃ and the results at 37 ℃ for a certain sample, which belong to self paired samples. The significance test for paired samples is the most commonly used statistical method in biological testing data analysis. Before conducting the test, it is necessary to check whether there is any difference between groups and calculate the degree of difference [1-3].

This article describes the differences in enzyme-linked immunosorbent assay (ELISA) results under different temperature conditions through descriptive analysis, correlation analysis, variance analysis, and normality analysis. Finally, non parametric testing methods are used for significance testing[4].

2. Descriptive analysis

Descriptive analysis is used to study the overall situation of quantitative data and how the overall average score is. The main indicators include minimum, maximum, mean, standard deviation, median, quantile, skewness, kurtosis, and coefficient of variation. The completed content is as follows:

① Overall description of the average score of the analysis items;

② Emphasize the analysis items with higher or significantly lower average values;

③ If the standard deviation is large, consider using the median to represent the overall scoring situation;

④ Summarize the analysis.

Table 1: Basic indicators of enzyme-linked immunosorbent assay results under different temperature conditions

name	sample size	minimum value	maximum value	average value	standard deviation	median
36℃	8	5.223	9.382	6.524	1.308	6.549
37℃	8	5.042	6.439	5.652	0.464	5.494
38℃	8	5.155	10.045	7.108	1.475	7.055

ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

Table 2: Depth indicators of enzyme-linked immunosorbent assay results under different temperature conditions

name	average value±standard deviation	variance	25th percentile	median	75th percentile	standard error
36℃	6.524±1.308	1.712	5.471	6.549	6.714	0.463
37℃	5.652±0.464	0.215	5.339	5.494	6.066	0.164
38℃	7.108±1.475	2.175	6.008	7.055	7.717	0.521
name	average value95% CI(LL)	average value95% CI(UL)	IQR	kurtosis	skewness	coefficient of variation(CV)
36℃	5.617	7.43	1.244	3.497	1.628	14.054%
37℃	5.33	5.974	0.727	-0.534	0.6	8.213%
38℃	6.086	8.13	1.709	1.742	0.952	14.750%

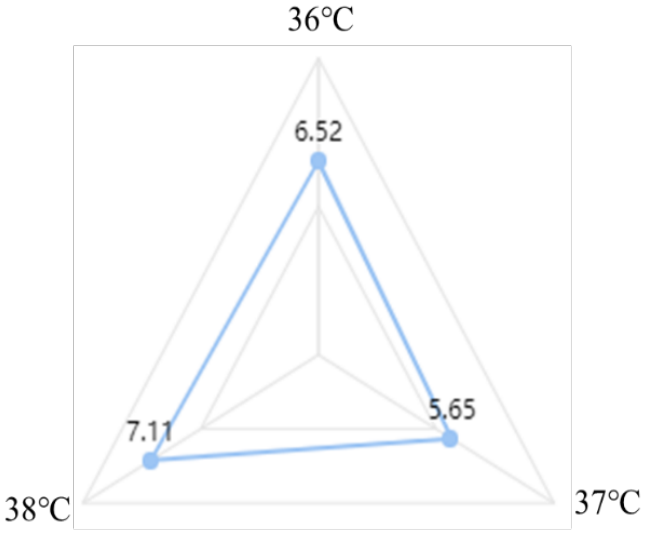

Figure 1: Comparison of average values of enzyme-linked immunosorbent assay results under different temperature conditions

Descriptive analysis describes the overall situation of data through mean or median values. From Tables 1, 2, and Figure 1, it can be seen that there are no outliers in the current data, so it is recommended to directly describe and analyze the average value. In summary, it can be concluded that there are no outliers in the data, and the average value can be directly described and analyzed.

3. Normality analysis

Normality analysis studies whether quantitative data analysis has the characteristic of a normal distribution; It is recommended to use the Shapiro-Wilk test for small samples (less than 50), and the Kolmogorov-Smirnov test for large samples (greater than 50). The achievable tasks are as follows:

① Determine whether the statistical values show significance (p-value less than 0.05 or 0.01);

② If it shows significance, it indicates that the item does not have a normal distribution characteristic. If it is necessary to compare the differences in data between different groups, non parametric tests can be considered;

③ If there is no significant difference (p>0.05), it indicates that the item has a normal distribution characteristic;

④ The requirements for normality testing are strict and difficult to meet. If the absolute value of kurtosis is less than 10 and the absolute value of skewness is less than 3, it indicates that although the data is not absolutely normal, it is generally acceptable to have a normal distribution;

ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

⑤ Summarize the analysis.

Table 3: Normality Analysis Results

name	Kolmogorov-Smirnov test		Shapiro-Wilk test	
	Statistics D value	p	Statistics W value	p
36℃	0.316	0.018*	0.819	0.045*
37℃	0.216	0.334	0.945	0.663
38℃	0.205	0.416	0.939	0.605
* p<0.05 ** p<0.01				

The results of normality test analysis are shown in Table 3. The sample size of the research data is all less than or equal to 50, therefore S-W test is used. Specifically, the results of the enzyme-linked immunosorbent assay (ELISA) under 36 ℃ temperature showed significant ($p<0.05$), indicating that the ELISA results under 36 ℃ temperature conditions did not exhibit normality. In addition, the results of enzyme-linked immunosorbent assay (ELISA) at 37 ° C and 38 ° C did not show significant differences ($p>0.05$), indicating that the ELISA results at 37 ° C and 38 ° C exhibited normality. In summary, it can be concluded that the enzyme-linked immunosorbent assay results under 36 ℃ temperature conditions do not exhibit normality characteristics. In addition, the enzyme-linked immunosorbent assay (ELISA) results under 37 ℃ temperature conditions and 38 ℃ temperature conditions exhibit normality characteristics. In general, strict requirements for normality testing cannot be met. If the absolute kurtosis value is less than 10 and the absolute skewness value is less than 3, it indicates that although the data is not absolutely normal, it is generally acceptable to have a normal distribution.

When conducting normality tests, S-W tests can be used for small samples, K-S tests can be used for large samples, and in addition, Jarque Bera tests can also be used for large sample data; Can complete the following tasks:

① The Jarque Bera test principle combines skewness coefficient and kurtosis coefficient for normality testing;

② Determine whether the Jarque Bera test chi square statistical value shows significance. If it shows significance ($p<0.05$), it indicates that the item does not have a normal distribution characteristic. If it does not show significance ($p>0.05$), it indicates that the item has a normal distribution characteristic;

③ Thirdly, for large samples, either K-S test or Jarque Bera test can be used. It should be noted that the results of the two tests may not be the same;

④ Summarize the analysis.

Table 4: Jarque-Bera test results

name	sample size	$\chi 2$	*df*	p
36℃	8	2.603	2	0.272
37℃	8	0.591	2	0.744
38℃	8	0.785	2	0.675

Table 4 shows the results of the Jarque Bera test. From Table 4, it can be seen that the Jarque Bera test results for the data showed that there was no significant difference ($p>0.05$) in the results of the enzyme-linked immunosorbent assay (ELISA) at 36 ℃, 37 ℃, and 38 ℃, indicating that the null hypothesis (null hypothesis: normal distribution of data) was accepted. The ELISA results at 36 ℃, 37 ℃, and 38 ℃ all exhibited normality.

4. Correlation analysis

Correlation analysis is used to study the relationship between quantitative data, whether there is a relationship, and the degree of closeness of the relationship. Can complete the following tasks:

1) Firstly, check if there is a significant relationship between Y and X;

2) Next, analyze whether the correlation is positive or negative, and the degree of closeness of the relationship can also be indicated by the magnitude of the correlation coefficient;

3) Summarize the analysis.

ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

Table 5: Spearman correlation

		36℃	37℃	38℃
36℃	correlation coefficient	1		
	p	-		
37℃	correlation coefficient	-0.476	1	
	p	0.233	-	
38℃	correlation coefficient	0.786*	-0.595	1
	p	0.021	0.12	-
* p<0.05 ** p<0.01				

From Table 5 above, it can be seen that using correlation analysis to study the correlation between the results of enzyme-linked immunosorbent assay (ELISA) at 36 ℃ and 37 ℃, as well as the correlation between ELISA results at 38 ℃, Spearman correlation coefficient is used to represent the strength of the correlation. Specific analysis shows that the correlation coefficient between the enzyme-linked immunosorbent assay (ELISA) results at 36 ℃ and 37 ℃ is -0.476, close to 0, and the p-value is 0.233>0.05. Therefore, it indicates that there is no correlation between the ELISA results at 36 ℃ and 37 ℃. The correlation coefficient between the results of enzyme-linked immunosorbent assay (ELISA) under 36 ℃ and 38 ℃ temperature conditions is 0.786, and shows significance at the 0.05 level, indicating a significant positive correlation between the ELISA results under 36 ℃ and 38 ℃ temperature conditions.

5. Variance analysis

Analysis of variance studies the differences between X (categorical) and Y (quantitative), such as the relationship between the differences in enzyme-linked immunosorbent assay results under different temperature conditions. Can complete the following tasks:

① Analyze whether there is a significant difference (p-value less than 0.05 or 0.01) between X and Y;

② If significant; Describe the specific differences by comparing the average values;

③ If there is no significant difference; There is no difference in Y among different groups of X;

④ Summarize the analysis.

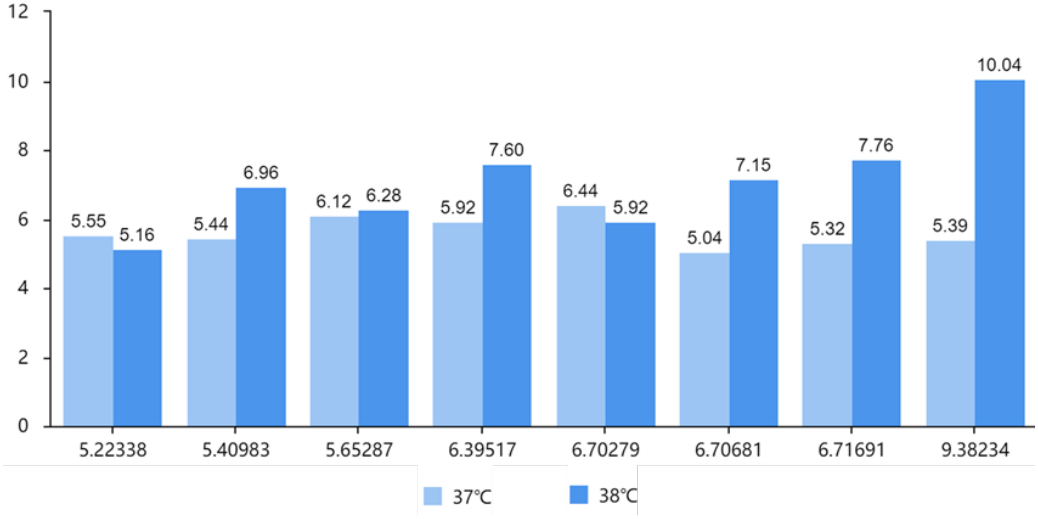

Figure 2: Comparison of Enzyme Labeling Results with Other Results under 36 ℃ Temperature Conditions

As shown in Figure 2, the results of enzyme-linked immunosorbent assay under 36 ℃ temperature conditions are compared with other results. Use one-way analysis of variance (ANOVA) to study the differences between the results of enzyme-linked immunosorbent assay (ELISA) at 36 ℃ and at 37 ℃ and 38 ℃, respectively. It can be seen that the results of enzyme-linked immunosorbent assay (ELISA) under different 36 ℃ temperature conditions showed no significant difference (p>0.05) compared to the

ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

ELISA results under 37 ℃ temperature conditions. This means that the ELISA results under different 36 ℃ temperature conditions showed consistency and no difference in the ELISA results under 37 ℃ temperature conditions. In summary, it can be concluded that there is no significant difference in the enzyme-linked immunosorbent assay (ELISA) results between samples under different 36 ℃ temperature conditions and those under 37 ℃ and 38 ℃ temperature conditions.

6. Nonparametric test

If the data belongs to a special distribution or has certain characteristics, it can be transformed into normality or homogeneity of variance after certain transformations, and then tested using paired t-test. If the data conforms to the Poisson distribution, it can be transformed by square root; The data that conforms to the binomial distribution can be transformed using the square root inverse sine function; It can also be converted through logarithmic transformation. However, when the overall distribution of two sets of data cannot be determined or there is no appropriate conversion method, non parametric statistical methods can be used. Non parametric statistical methods compare distributions rather than parameters. It does not consider the distribution type of the data, but directly compares it using the symbol, size order number, comprehensive judgment of the ranking, severity, or quality level of the sample data. The level data that is difficult to process by the parametric method can be analyzed by the non parametric method, so its application range is wide[5-7]。

Non parametric rank sum test is used to study the differences in Y between different groups of X, and to compare the differences in data with uneven variance or non normality (Y) (MannWhitney test is used when X is two groups, Kruskal Wallis test is used when X exceeds two groups). Can complete the following tasks:

① Analyze whether there is a significant difference (p-value less than 0.05 or 0.01) between X and Y;

② If significant; Describe the specific differences by comparing the median size in detail;

③ Summarize the analysis.

Table 6: Kruskal Wallis test results

37℃	36℃	38℃
5.04235(n=1)	6.707	7.15
5.32146(n=1)	6.717	7.758
5.39185(n=1)	9.382	10.045
5.44244(n=1)	5.41	6.96
5.54558(n=1)	5.223	5.155
5.91751(n=1)	6.395	7.596
6.11553(n=1)	5.653	6.279
6.43886(n=1)	6.703	5.918
H	0.111	1.778
p	0.739	0.182
* p<0.05 ** p<0.01		

As shown in Table 6. The results of enzyme-linked immunosorbent assay (ELISA) at 37 ℃ exceeded the composition of two groups, so Kruskal Wallis test statistic was used for analysis. The enzyme-linked immunosorbent assay (ELISA) results of samples under different 37 ℃ temperature conditions showed no significant difference (p>0.05) compared to the ELISA results under 36 ℃ temperature conditions. This means that the ELISA results of samples under different 37 ℃ temperature conditions showed consistency and no difference in the ELISA results under 38 ℃ temperature conditions.

7. Conclusion

The significance test for differences is the most commonly used statistical method in the analysis of biological testing data, but its usage conditions need to be noted. Before conducting the test, it is necessary to test whether there is any difference between groups and calculate the degree of difference. This article describes the differences in enzyme-linked immunosorbent assay (ELISA) results under different temperature conditions through descriptive analysis, correlation, variance, and normality analysis. Finally, non-parametric testing methods are used for significance testing, which is more

ISSN 2616-5910 Vol. 5, Issue 1: 57-62, DOI: 10.25236/AJALS.2024.050109

applicable.

References

[1] Theodorssonnorheim E. Kruskal-Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. [J]. Computer Methods & Programs in Biomedicine, (1986), 23(1): 57-62.
[2] Elliott A C, Hynan L S. A SAS (®) macro implementation of a multiple comparison post hoc test for a Kruskal-Wallis analysis.[J]. Computer Methods & Programs in Biomedicine, (2011), 102(1): 75-80.
[3] West RM. Best practice in statistics: Use the Welcht-test when testing the difference between two groups[J]. Annals of Clinical Biochemistry. (2021), 58(4): 267-9.
[4] The SPSSAU project. SPSSAU. (Version 24.0) [Online Application Software]. (2024) Retrieved from https://www.spssau.com.
[5] Hussein,Nili,Sumeet, et al. Memristors: Nanoscale Resistive Switching in Amorphous Perovskite Oxide (a-SrTiO3) Memristors (Adv. Funct. Mater. 43/2014)[J].Advanced Functional Materials, 2014, 24(43):6733-6733.DOI:10.1002/adfm.201470279.
[6] Mehmood K .Serosurveillance of Canine Leptospirosis under Different Climatic Conditions in and Around Lahore, Pakistan.[J].Pakistan Veterinary Journal, 2013, 33(2):240-242. DOI:10.4067/S0301-732X2013000300015.
[7] Tong C , Changqing W , Xiaofan L I ,et al.Determination of aflatoxin B_1 in peanut under different storage conditions by indirect ELISA[J].China Oils and Fats, 2014.