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The Effects of Temperature Room on Micropipette Calibration Using Linear Regression Analysis

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ABSTRACT

A micropipette is a type of pipette that takes a liquid substance with a tiny volume on a microliter scale. Micropipette calibration is needed to find out if there is a deviation value and ensure accuracy so that it can be ascertained whether the equipment is still suitable for use or not. When calibrating micropipettes in the laboratory, the laboratory temperature must be stable. However, the facts in the field indicate that there is a possibility that the temperature is not stable following the requirements of micropipette calibration, which is at a temperature of 20ºC, which can influence the calibration results. This study aimed to determine the effect of room temperature on micropipette calibration. This study uses the linear regression analysis method by taking data thirty times at each room temperature of 10ºC, 20ºC, 25ºC, and 30ºC with micropipette sizes of 1000 µl and 50µl. The data taken and tested using the SPSS application found that the significance value (sig.) at each point was smaller than the error rate used, which was 5%. The most significant temperature effect on 1000µl micropipette size is at 15°C by 68.20%, and at 50µl micropipette size is at 20°C by 67.90%. The linear equation of each micropipette size and room temperature obtained a positive constant value showing the independent variable's positive effect (temperature). If the independent variable (temperature) increases or is greater, the dependent variable (micropipette calibration results) will decrease/be smaller in value. The overall data shows that temperature affects micropipette calibration, so H0 is rejected, and H1 is accepted.

KEYWORD: Micropipette, calibration, temperature, relation, linear regression

I. INTRODUCTION

A micropipette is a type of pipette that takes a liquid substance with a minimal volume on a microliter scale with high accuracy and precision[1]. Based on [2], there are two types of micropipettes, there are : fix volume and variable volume. Fix volume is designed by the manufacturer to move a volume of liquid of one nominal capacity. Examples : 5 µl, 10 µl, and 100 µl. Variable volume is designed by the manufacturer to move a volume that can be selected by the user in one nominal capacity. Examples: 5-50 µl, 100-1000 µl. In health services, micropipettes with different scale values are needed in the laboratory. Micropipette calibration is required to determine if there is a deviation value and ensure accuracy so that it can be ascertained whether the tool is still suitable for use. According to ISO/IEC Guide 17025:2005 and Vocabulary of International Metrology (VIM), calibration is a set of activities that establishes a relationship between the values indicated by a measuring instrument or measurement system, or the values represented by measuring materials, and the corresponding known values of the measured quantities under specified conditions[3]. In previous research[4], researchers tested electronic scales using the CSIRO (Commonwealth Scientific and Industrial Research Organization) to determine the correction value and the difference in uncertainty values at temperature variations of 20 ° C, 22 ° C, 25 ° C, and 27 ° C. In his research, the researcher concluded that temperature affects the reading value on electronic scales, the greater the room temperature, the greater the uncertainty value. In his research, the researcher concluded that temperature affects the reading value on electronic scales; the more significant the room temperature, the greater the uncertainty value.

In a previous research [5], the results of his study stated that volume and mass values were lost due to evaporation. His research also noted that the apparatus temperature and air density contribute fairly dominant components; this warns calibration operators that stable environmental conditions must be maintained during the calibration process, and fluctuations in temperature and humidity must be paid attention to. The room temperature will affect the air density value, and the water temperature will affect the water density value. The higher the water temperature, the smaller the water density, this makes it likely that there is an evaporation process when calibrating micropipettes.

Several things need to be considered when using micropipette calibration activities, including the mass of water, air density, water density, the density of the scales, water temperature, and the standard (measuring instrument) used[6].

In addition to the value of the uncertainty factor, the room temperature must be considered. In the Micropipette calibration guide of the Directorate of National Standards for Mechanical, Radiation and Biological Measurement Units, SNSU PK.M-01: 2020, it is stated that several requirements for environmental conditions need to be considered when calibrating micropipettes, namely the room air temperature that is set is a temperature range of 10 ° C ~ 30 ° C[7].

On the other hand[6], states that the environmental conditions regulated during micropipette calibration are 20°C ± 2 ° C, with a humidity of 55%RH ± 10% RH [6]. Micropipettes often found in health care facilities have two types, variable volume type, and fixed volume type, with various sizes adjusted to user needs. Usually, in sampling, some sizes that are often used include 5 µl, 10 µl, 50 µl, 100 µl, up to 1000 µl.

In a calibration laboratory, temperature and humidity monitoring must be done to maintain the performance of instruments sensitive to changes in accommodation and environmental conditions. When performing calibration activities in the laboratory, the laboratory temperature must be stable; the operator on duty in the laboratory can also contribute heat to affect the stability of the room temperature and humidity [8]. Based on the facts in the field, there is a possibility that the laboratory temperature is not stable following the micropipette calibration requirements, namely at 20°C, which is caused by the location of the laboratory directly exposed to sunlight, a humid room, and operators on duty in the laboratory can also contribute heat so that it can affect the value of temperature and humidity stability. In this study, the authors want to know how much influence room temperature has on the results of micropipette calibration with room temperatures of 15°C, 20°C, 25°C, and 30°C using linear regression analysis.

This study wants to prove if there is an influence between room temperature and micropipette calibration.

II. RESEARCH METHODS

A. RESEARCH DESIGN

This research is quantitative, a process of finding knowledge that uses data in the form of numbers to analyze information about all that is wanted to know[9]. Using linear regression analysis, the author explores the difference in calibration results of micropipettes of 50 µl and 1000 µl sizes with ambient temperatures of 15°C, 20°C, 25°C, and 30°C. The results of this analysis are expected to explain whether there is a relationship between the influence of room temperature on the results of micropipette calibration and the impact of different temperatures so that it can be seen how much temperature affects the calibration results.

B. CONCEPTUAL FRAMEWORK

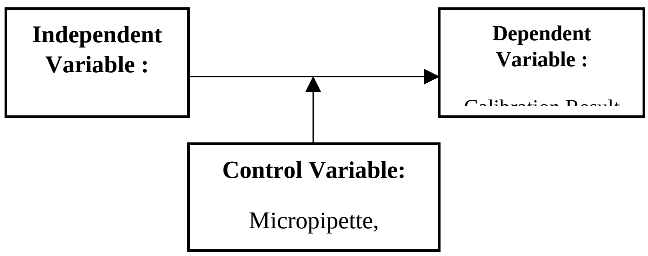

FIGURE 1. Conceptual Framework

1) *Indpendent Variable*: In this study, the independent variable is temperature. Temperature is a measure of heat or cold expressed on a scale with units of degrees Celsius (°C)[10]. The author set the room temperature at 15°C, 20°C, 25°C, and 30°C. The room temperature is conditioned using an air conditioner (AC) with a temperature acceptance limit of ±2 °C. The position of the AC is not directly related to the analytical balance table used in data collection.
2) *Dependent Variable*: In this study, the dependent is the result of micropipette calibration due to the possibility of differences in results affected by temperature. Micropipette calibration is an activity that compares the performance of micropipettes with standard tools to

ensure that the resulting volume measurements are accurate and precise. Micropipette calibration results are obtained by direct data collection.

3) *Control Variable*: The control variables in this study were micropipettes and analytical balance. A micropipette is a laboratory tool used to move or take small amounts of liquid with microliter (µl) units. The micropipettes used were 50 µl and 1000 µl. Analytical balance is a tool used to measure the mass of an object, usually in units of grams (gr). This research uses an analytical balance with a 0-220 grams capacity and a resolution of 0.0001 grams.

C. DATA COLLECTION TECHNIQUES AND RESEARCH INSTRUMENTS

1. Data Collection Techniques

The method of data collection in the research conducted by the author is primary data collection during the study. The author uses the primary data method, which is taking measurement data from micropipettes with sizes of 50µl and 1000µl thirty times, respectively, with the room temperature set in the volume laboratory, at a temperature of 15ºC, 20ºC, 25ºC, and 30ºC. Data collection was taken in the morning (8:00 A.M - 11:00 A.M) so that the room temperature could be more easily controlled. The temperature is set using an air conditioner (AC) with a temperature acceptance limit of ± 2 ºC (this refers to the Micropipette Testing and Calibration Work Method [6] that the conditioned room temperature is at 20ºC ± 2 ºC). If the actual room temperature exceeds or is less than 2ºC, then data collection will be stopped so that the AC settings will be set to the desired temperature. The position of the AC is not directly related to the analytical balance table or the author's position at the time of data collection.

2. RESEARCH INSTRUMENTS

Research instruments are all tools used to collect, examine, investigate a problem or collect process, analyze, and present data systematically and objectively to solve a problem or test a hypothesis[11]. In this study, data collection was carried out quantitatively. Quantitative testing tests the correlation between temperature and micropipette calibration results. This research uses the following research instrumentation:

1. Mikropipet 50 µl, merk DragonLab, S/N : YE218AS0296827
2. Mikropipet 1000 µl, merk JoanLab, S/N : YE218AS0296827
3. *Analytical Balance,* merk : Precisa, Model/Type : XB220A , S/N : 0801433
4. Thermohygrometer, merk: HTC Model/Type : HTC-1 , S/N : TH-32

C. DATA ANALYSIS TECHNIQUES AND METHODS OF DATA PRESENTATION

In this study, data analysis techniques and methods of data presentation carried out by the author are data normality test, correlation test, and linear regression test so that a linear regression analysis equation can be obtained.

1. Normality Test

A normality test is carried out to assess the distribution of data in a group of data or variables and whether the distribution is normally distributed or not[12]. The Kolmogorov-Smirnov normality test using the SPSS application has a basis for decision-making: if the significance value (Sig.) is more significant than 0.05, then the research data is normally distributed. Conversely, if the significance value (Sig.) is smaller than 0.05, the research data is not normally distributed [13].

2. Correlation Test

The correlation test aims to determine the degree or closeness in the relationship between two data, namely, between the effect of temperature and the results of micropipette calibration[14]. So that it will be known whether there is a relationship between the effect of temperature on micropipette calibration and how much influence it has. To determine the magnitude of the relationship between the effect of temperature on micropipette calibration using Pearson's bivariate correlation analysis [15], with the following formula[16]:

$$r = \frac{n(\sum XY)-(\sum X \sum Y)}{\sqrt{\{n\sum X^2-(\sum X)^2\}[n\sum Y^2-(\sum Y)^2]}} \quad (1)$$

With :

r = Correlation coefficient

ΣXY = The result of the Number of times the value of variable X and variable Y
ΣX = Total value of X variable
ΣY = Total value of Y variable
ΣX^2 = Sum of squared X variable values
ΣY^2 = Sum of squared Y variable values
n = Number of samples

The correlation value (r) ranges from 0 to 1, or when accompanied by its direction, the value is between 1 and +1. Furthermore, the relationship between two variables can be determined as follows,

r = 0, no linear relationship
r = -1, perfect negative linear relationship
r = +1, perfect positive linear relationship[17].

In the SPSS application, decision-making in this Pearson bivariate correlation analysis can be seen from the Sig Significance Value. (2-tailed), if the Sig value. (2-tailed) <0.05, then there is a correlation between the variables being connected. Conversely, if the Sig value. (2-tailed) > 0.05, there is no correlation [18].

According to Colton, the closeness of the relationship between the two variables can be seen as follows:

r = 0.00 - 0.25, no relationship / weak relationship

r = 0.26 - 0.50, moderate relationship

r = 0.51 - 0.75, close relationship

r = 0.76 - 1.00, a very close/perfect relationship [15]

3. Regression Linier Test

After knowing the relationship between the two variables, the author will create a model of the relationship between the two variables using linear regression analysis. Simple linear analysis aims to determine the effect of one variable on another. With linear regression analysis, a mathematical relationship pattern will be obtained from variable X and variable Y to determine the magnitude of changes in variable X to variable Y and predict variable Y if the value of variable X is known [19].

In this study, variable X results from micropipette calibration, and variable Y is temperature. In simple linear regression, the data used usually has an interval or ratio scale. The simple linear regression formula is as follows[20]:

$$Y = a + bX \quad (2)$$

Description :

Y = Dependent variable

X = Independent variable

a = Constanta (value of Y, if X = 0)

b = regression coefficient (positive or negative influence)

III. RESULT AND EXPLANATION

Based on the test results using Simple Linear Regression carried out at temperatures of 15°C, 20°C, 25°C, and 30°C at each micropipette measuring 50µl and 1000µl, the test results are shon in table 1.

TABLE I

NORMALITY AND CORRELATION TEST RESULTS

Micropipette Sizes (µl)	Temperatures (°C)	Normality Test	Correlation Test
50	15	Normal	Close Relationship
	20	Normal	Close Relationship
	25	Normal	Close Relationship
	30	Normal	Close Relationship
1000	15	Normal	Very close relationship
	20	Normal	Close Relationship
	25	Normal	Moderate Relationship
	30	Normal	Close Relationship

TABLE 2

LINEAR REGRESSION TEST RESULTS AND LINEAR REGRESSION EQUATION

Micropipette Sizes (µl)	Influence (%)	Linear Regression Model
50	45,50	Y = 100,877 + (-3,164)X
	67,90	Y = 104,309 + (-2,765)X
	53,70	Y = 111,281 + (-2,535)X
	26,40	Y = 90,584 + (-1,561)X
1000	68,30	Y = 1109,360 + (-5,982)X
	36,80	Y = 1081,671 + (-3,448)X
	22,40	Y = 1.153,474+ (-5,975X
	35,70	Y = 1.389,579 + (-14,713)X

From Table 2 above, it can be seen that the results of the normality test on the entire data obtained that the data is normally distributed. The correlation test shows a relationship between room temperature and micropipette calibration. A very close relationship was received at 15 ° C for the size of 1000 µl, a moderate relationship was obtained at 25 ° C, and other data obtained a close relationship.

The most significant temperature effect is at 15°C with a linear regression test result of 68.30% on a micropipette size of 1000 µl, which can be interpreted that the impact of room temperature variables on micropipette calibration is 68.20%, while the remaining 31.7% is influenced by other factors not studied.

At a micropipette size of 50 µl, the most significant temperature effect was obtained at a temperature of 20 ° C with a linear regression test result of 67.90%, which can be interpreted that the impact of the 20 ° C room temperature variable on the calibration of a 50 µl micropipette is 67.90%. In contrast, the remaining 32.1% is influenced by other factors not studied. There is a difference in the effect on the temperature point obtained from the two micropipette sizes; this may occur due to different influences, such as the value received having a significant deviation, the impact of human error, or an influence from the actual temperature obtained is different.

Based on the results of micropipette calibration, at a micropipette size of 50 µl at 15 ° C, the average result is 51.70 µl. At a temperature of 20 ° C, an average of 49.98 µl was obtained. At 25 ° C, an average of 49.51 µl was obtained, and at 30 ° C, an average of 49.31 µl was obtained. The maximum permissible error at 50µl is 1.4% or 0.7 µl, so the calibration results are still within the tolerance range of the maximum permitted error. Then, the average calibration result of 1000µl micropipettes at 15°C is 1011.167 µl. At a temperature of 20 ° C, an average of 1010.5 µl was obtained. At 25 ° C, an average of 1003.3 µl was obtained, and at 30 ° C, an average of 988.26 µl was obtained. In the SNSU PK.M-01:2020 micropipette calibration guide[7], it is stated that the maximum permissible error on micropipettes is 1.2% or 12µl at a micropipette size of 1000 µl, so the results of calibrating a 1000 µl micropipette at temperatures of 15°C, 20°C, 25°C, and 30°C obtained values that are still within the maximum permissible error tolerance range.

The linear equation obtained from each micropipette size and room temperature obtained a positive constant value showing the independent variable's positive effect (temperature). If the independent variable (temperature) increases or is greater, the dependent variable (micropipette calibration results) will decrease in value, this can be seen in the graphs shown in Figure 2 and Figure 3.

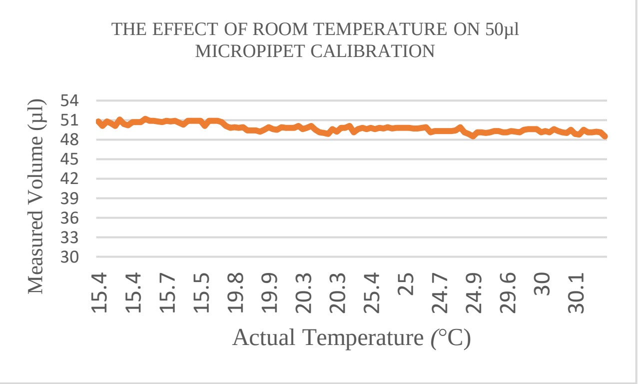

FIGURE 2. **Graph of the Effect of Room Temperature on 50µl Micropipette Calibration**

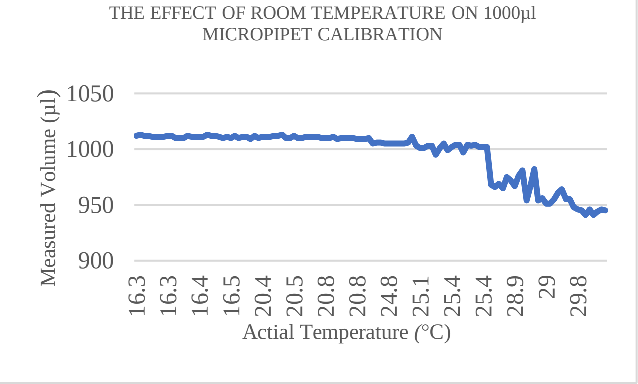

FIGURE 3. **Graph of the Effect of Room Temperature on 50µl Micropipette Calibration**

From Figure 3, the measured volume results tend to look stable judging from the graph, but there is still an influence between the actual temperature and the measured volume results. While in 4.2 above, it can be seen that the greater the exact temperature, the smaller the measured volume results. The drastic change in measured volume results was obtained at 28.7°C; this is likely to occur because the deviation value obtained in size 50 micropipettes tends to be smaller, so the value obtained tends to be stable.

V. CONCLUSIONS

This study wants to prove whether there is an influence between room temperature and micropipette calibration. Results found that room temperatures of 15 °C, 20°C, 25 °C, and 30 ° C influence the results of micropipette calibration. Also, the most significant effect of temperature on the size of 1000µl micropipettes is a temperature of 15 ° C by 68.30%. Another important result is that the temperature's most significant effect on the size of 50µl micropipettes is the temperature of 20 ° C by 67.90%. If the temperature variable increases or is more important, then the micropipette calibration results will decrease in value.

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