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Protein Concentration Determination in Latex Glove Using Biocompatibility Morphological Mean Test

Chean Khim Toa*, Kok Swee Sim, and Yee Kit Chan

***Abstract*—Latex gloves are seen as an indispensable item in the healthcare field because it offers superior protection for both the medical staff and patient against harmful substances. However, latex gloves with high protein concentration have a high possibility to induce latex allergy which in the worst case can lead to a life-threatening condition. To minimize the occurrence of an allergy reaction, the computerized Biocompatibility Morphological Mean (BMM) test for protein detection is proposed. This test initially goes through the chemical process to determine the protein that resides in the glove sample. After that, the sample is electronically converted into a digital image. Finally, the image undergoes color image processing for calculating the color difference values. These values are then plotted on a standard curve. A high correlation coefficient (R^2>0.97) of the standard curve gives better accuracies. The proposed method only takes about 40 minutes to complete the test, while existing methods need at least 6 hours.**

***Keywords*—Protein, Latex Glove, Biocompatibility, Morphologic Mean Test.**

I. INTRODUCTION

Latex gloves or called as medical gloves are used during patient examinations to prevent cross-contamination between patient and healthcare staff. Those disposable gloves were made from Natural rubber latex (NRL) which is obtained from the rubber tree called Hevea Brasiliensis. Latex gloves were first introduced into the medical field in the year 1880 [1]. During that time, the glove was only used to protect the hand of nurses from exposure to blood borne pathogens and dangerous microorganism [2], [3]. Nowadays, improvement of latex glove qualities such as elasticity, barrier protection, and tear resistance can offer two-ways of protection for patient and healthcare staff [4]. Because of this, the gloves become an indispensable medical item and commonly used in the healthcare sector. However, few people still avoid using latex gloves. This is due to those people have allergic reaction [5], [6], to the latex protein present in the NRL. The latex allergy will be slowly developed after repeated exposure the gloves which contain high protein concentration. This situation has been getting serious public health concern regarding the possibility of health risk to healthcare workers (HCWs) [7].

Thus, to reduce possibility of inducing latex allergy, the protein concentration level must be estimated. In the past, conventional methods such as Rubber Research Institute of Malaya (RRIM) test [8], ASTM modified Lowry [8], maximum minimum variation (MMV) [9], and Bradford microassay [10], have been developed. Those methods detect the total protein of the glove through colorimetric analysis [11]. The process of those methods usually involved in many chemical reagent and numerous lab equipment, lead to time consumption, complicated chemical test, and high-priced.

In this project, the objective is to implement a simple yet effective protein concentration determination test. The test was focusing on reducing the chemical reagent usage by digitizing the protein determination process [12]. The proposed test named as Biocompatibility Morphological Mean (BMM) consists of two phase which are chemical process and image analysis. The first phase is to detect the protein concentration within the glove sample by using only one chemical reagent, while the second phase digitalizes the chemical binded sample to calculate delta E color difference value. Those values are then plotted in a standard curve and the protein concentration of the glove sample was calculated based on the equation of the curve.

The result of the proposed test will determine the protein level of the gloves and those with high protein

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concentration will be notified to the person in charge for further process. The test is important as it represents the biocompatibility of the gloves so that they did not bring harm to the user.

II. Review of the Conventional Protein Estimation Methods

To estimate the protein level of the glove sample, several conventional methods have been developed. Those methods were based on colorimetric analysis to determine the concentration of protein with the aid of color chemical reagents.

A. Modified Lowry Method

Lowry protein assay was a biochemical assay used in the modified Lowry method [8], [13], [14], to estimate the amount of protein in the solution. The principle behind the modified Lowry method lies in the combination of the original Lowry method [15] with the folin-ciocalteu phenol reagent. To estimate the protein level, the protein solution must be extracted from the latex glove sample. After that, the solution will be mixed with Lowry reagent and folin phenol reagent, resulting in color changes of the solution. The color will be measured using the spectrometer to estimate protein level. Figure 1 shows the process of extracting and estimation of protein using the modified Lowry method.

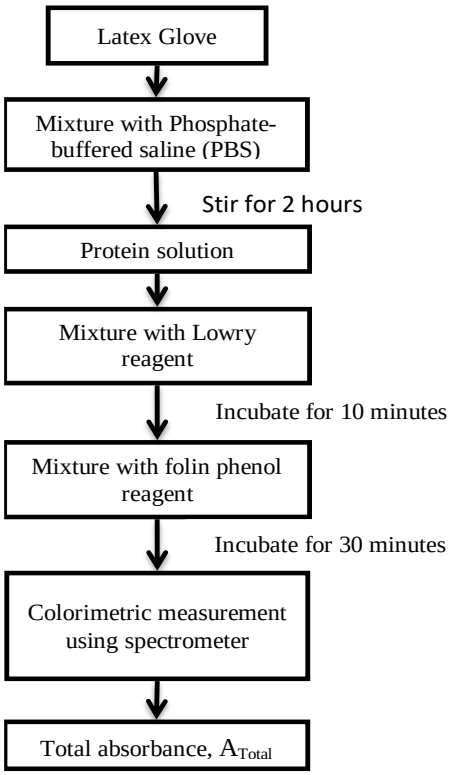

FIGURE 1. Process of modified Lowry method.

The absorbance data obtained from the spectrometer will be plotted in the graph as shown in Figure 2 to estimate the protein level of glove sample. Based on the graph, the accuracy can be achieved up to 95%.

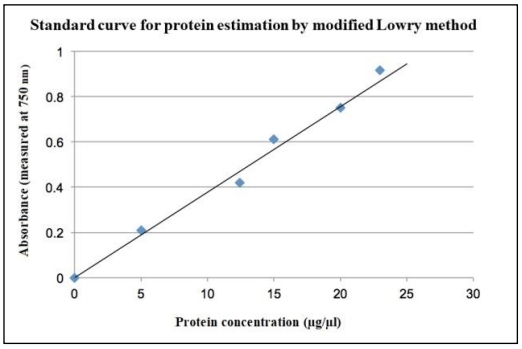

FIGURE 2. Standard curve of the modified Lowry method.

Although the modified Lowry method can be used to estimate the protein level of glove sample, it is still time-consuming since the chemical test involved many chemical reagents. Besides, an experienced technician is needed in order to perform the chemical test since some of the chemical reagents is hazardous to health.

B. Bradford protein assay method

The Bradford protein assay is the dye-binding assay which was first described by Marion M. Bradford in 1976 [10], [16], [17]. It became the preferred method for quantifying protein in many laboratories. The process of the assay is through the binding of Coomassie brilliant blue G-250 dye at acidic ph to basic amino acid residues in proteins, changing the color of the solution to blue. Figure 3 shows the flow chart for the determination of glove protein using the Bradford assay.

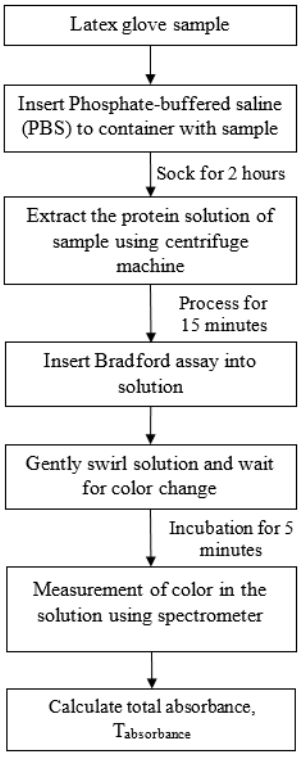

FIGURE 3. Flow chart of protein determination using Bradford assay.

First, the latex glove sample will mix with the Phosphate-buffered saline (PBS) inside the container in order to extract protein solution from the sample. After that, the Bradford assay will insert into the solution and gently swirl, waiting for the color change in the solution. Then, it will be measured using the spectrometer to calculate the total absorbance, $T_{absorbance}$. The total absorbance data will then be plotted in the standard graph as shown in Figure 4. The graph shows that the accuracy is up to 95% between the data of absorbance and concentration of BSA (protein).

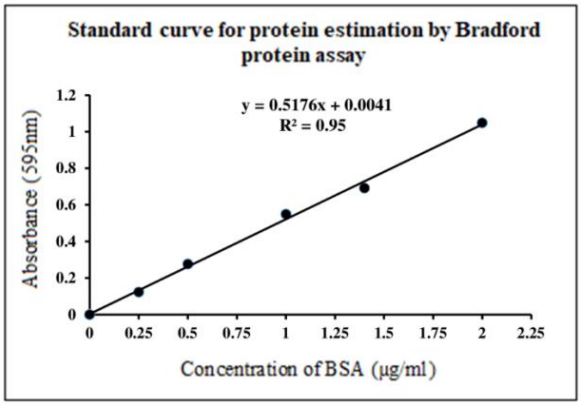

FIGURE 4. Standard graph of Bradford assay.

Even though the Bradford assay method can be used to determine the protein of glove sample, the process is still tedious since it took a long processing time to obtain the protein solution from the sample. Moreover, it also using expensive devices such as centrifuge machine, shaking machine and spectrometer in order to complete the procedure. Thus, the process of protein estimation was time-consuming and costly.

III. PROPOSED METHOD

The computerized Biocompatibility Morphological Mean (BMM) test has been proposed to determine the protein in the latex glove. This test contains three stages which are the chemical stage, scanning stage, and analysis stage. For the chemical stage, the glove sample first immerses into the chemical reagent called as Bradford assay. Its functionality is to change the color of the sample into blue upon binding with the protein that contained inside the sample. The duration of sample immerse into the Bradford was 15 minutes. It was determined by immersing the sample at varying time as shown in Figure 5. It is seen that the line in the graph start to stabilize after 15 minutes of immerse, indicating that chemical binding between sample and Bradford has reached the optimum stage. The chemical sample later must have cleaned using the distilled water and dry out before goes through the next process.

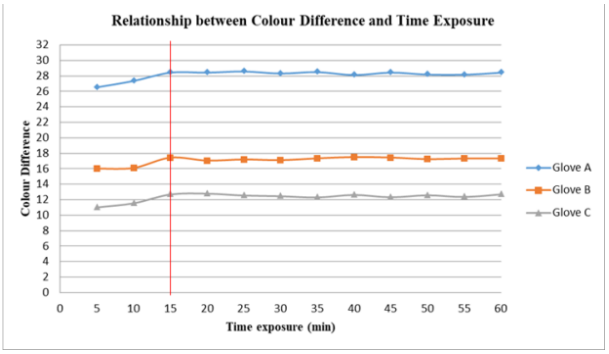

FIGURE 5. Chemical binding of the sample at varying times.

After that, the sample will go through a scanning stage to digitally acquired sample image. A robust algorithm, Morphological Color Difference (MCD) is used to perform the calculation and analysis between raw and chemical images. The result of calculation will then be used to determine the protein of glove. The detail flow diagram and flowchart of BMM test was shown in Figure 6 and Figure 7.

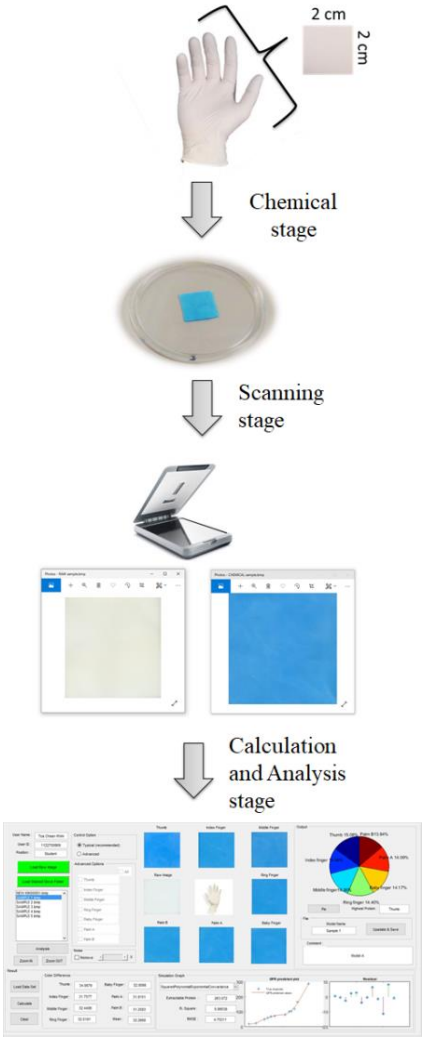

FIGURE 6. The process flow diagram of Biocompatibility Morphological Mean (BMM) test.

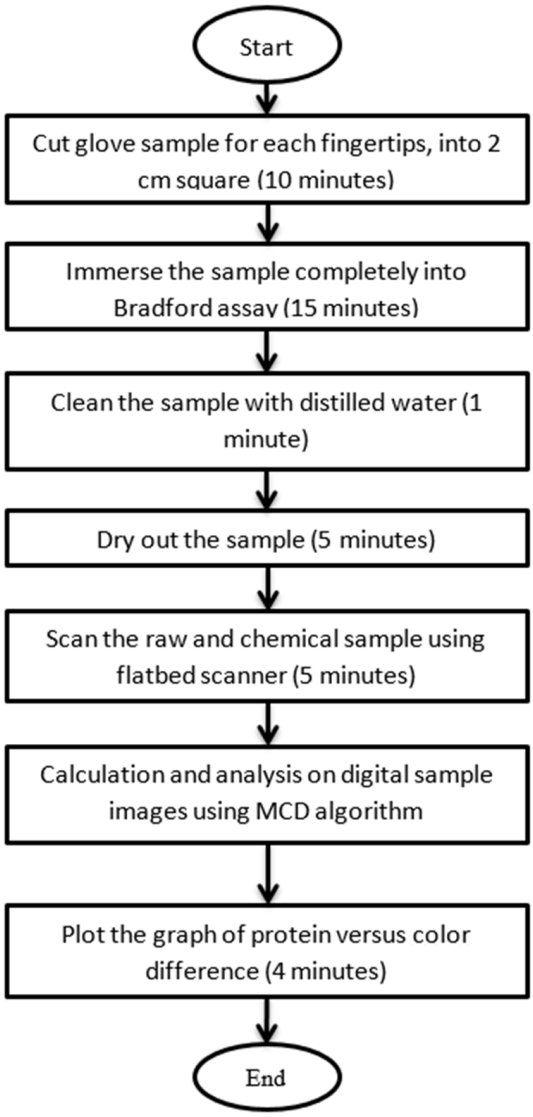

FIGURE 7. Detailed flowchart for Biocompatibility Morphological Mean (BMM) test.

After obtaining raw and chemical sample images, the calculation and analysis will be done in the GUI using the Matlab software. This GUI will apply the Morphological Color Difference (MCD) algorithm on the images in order to determine the protein value of the sample. Figure 8 shows the block diagram of the MCD algorithm.

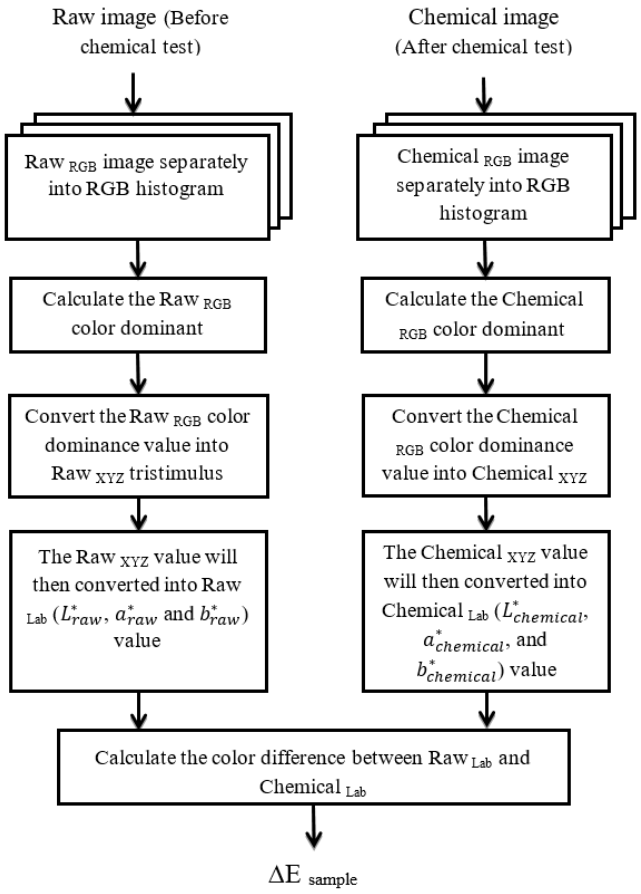

FIGURE 8. Block diagram of Morphological Color Difference (MCD) algorithm.

Generally, the sample image is constructed as m x n x 3 data array that defined as red, green, and blue components for each pixel. For color images, the color pixels are formed by a combination of red, green, and blue pixels which denotes as

$$S[x,y] = \frac{1}{3}\left(S_r(x,y) + S_g(x,y) + S_b(x,y)\right) \quad (1)$$

where $S_r(x,y)$, $S_g(x,y)$, and $S_b(x,y)$ is the red, green, and blue component value of the (m,n)th pixel. Since there has a different level of intensity for each color pixel in the image, the histogram of color dominant can be generated by consolidating the RGB component as shown in Figure 9. The consolidate process will convert the RGB component into index values, and those values will be used to calculate the color dominant in the image.

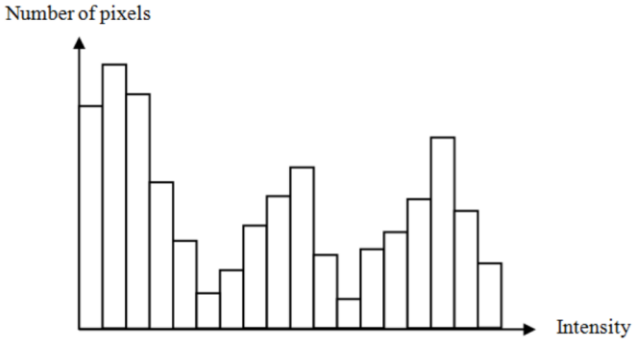

FIGURE 9. Color histogram of the sample image.

After that, the RGB color dominant value will be converted into XYZ tristimulus values by first goes through a linear transformation as shown in Equation 2:

$$S^* = \begin{cases} \left(\frac{(S+0.055)}{1.055}\right)^{2.4} & for\ S > 0.04045 \\ \frac{S}{12.92} & for\ S \leq 0.04045 \end{cases} \quad (2)$$

$$S^* \in \{R^*, G^*, B^*\} \quad (3)$$

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.41245564 & 0.3575761 & 0.1804375 \\ 0.212672 & 0.7151522 & 0.0721750 \\ 0.01933399 & 0.1191920 & 0.9503041 \end{bmatrix} \begin{bmatrix} R^* \\ G^* \\ B^* \end{bmatrix} \quad (4)$$

where 3 x 3 transformation matrix (D65 reference white) convert the R*,G*,B* value into XYZ value. Later those values will be transforming into L*, a*, and b* coordinates [18], [19] by following equations:

$$L^* = 116\left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - 16 \quad (5)$$

$$a^* = 500\left[\left(\frac{X}{X_n}\right)^{\frac{1}{3}} - \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}}\right] \quad (6)$$

$$b^* = 200\left[\left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - \left(\frac{Z}{Z_n}\right)^{\frac{1}{3}}\right] \quad (7)$$

where X_n, Y_n, Z_n are the tristimulus value of white achromatic stimulus. After that, the color difference, ΔE^* between the $L^*a^*b^*_1$ (raw sample) and $L^*a^*b^*_2$ (chemical sample) will be computed by using Equation 11 [20].

$$\Delta L^* = L_2^* - L_1^* \quad (8)$$

$$\Delta C^* = \sqrt{(a_2^{*2} + b_2^{*2})} - \sqrt{(a_1^{*2} + b_1^{*2})} \quad (9)$$

$$\Delta H^* = 2\sqrt{|(a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2 - \Delta C^{*2}|} \quad (10)$$

$$\Delta E^* = \sqrt{\Delta L^* + \Delta C^{*2} + \Delta H^{*2}} \quad (11)$$

where ΔL^*, ΔC^*, and ΔH^* is the difference between L^*(luminance), C^*(chroma) and H^*(hue) of the raw and chemical image.

IV. Result and Discussion

In this project, 11 glove models with different protein level have been carried out. Those models are varied from low to high protein level. Figure 10 shows the chemical glove samples for each fingertip after go through the chemical test.

Based on Figure 10, it seems that there has a different blue color intensity for each chemical sample. For low protein level, the samples contain low intensity of blue color, while high protein level, the samples contain high intensity of blue color. This indicates that a higher protein level led to higher binding intensity of the Bradford reagent. Table I shows the blue intensity value of chemical samples that calculated using the Morphological Color Difference (MCD).

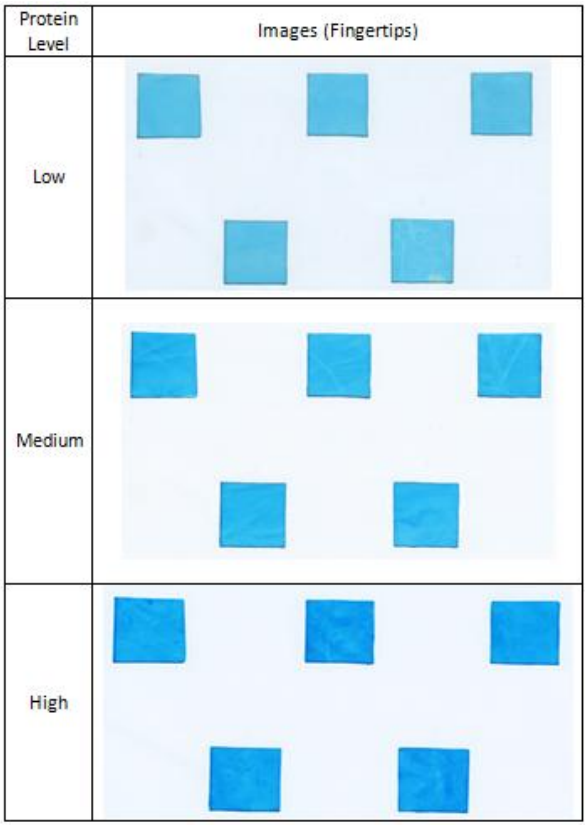

FIGURE 10. Protein Level for the chemical glove samples.

TABLE 1. Color difference and glove protein data.

Model	Thumb	Index	Middle	Ring	Pinky	Color	Proteins Content (EP),µg/g
A	38.467	39.528	37.964	36.361	41.619	38.788	22
B	36.997	40.305	38.939	40.421	40.244	39.381	24
C	43.275	41.191	39.289	38.533	40.697	40.597	38
D	36.74	40.824	42.953	41.605	43.698	41.164	41
E	44.502	49.913	42.916	46.975	41.095	45.08	92
F	49.969	46.93	49.753	43.055	49.311	47.804	118
G	50.041	50.304	49.852	49.298	48.716	49.642	140
H	49.936	51.957	54.501	50.128	49.833	51.271	162
I	53.045	54.388	52.742	49.824	52.38	52.476	220
J	53.173	53.146	51.697	54.274	53.493	53.157	274
K	55.02	55.02	58.295	56.801	56.588	56.345	350

Based on Table I, the ΔE values of the fingertips were averaged arithmetically in order to plot the graph as shown in Figure 11. The plotted graph will be used to find the protein value of the glove by referring to the ΔE data. A predefined protein value for each glove model was obtained from Malaysia Rubber Board (MRB) using the ASTM D5712 standard method [8], [21].

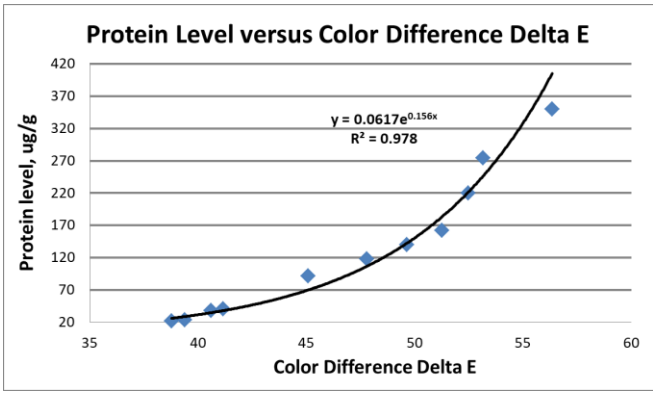

FIGURE 11. Correlation between protein level and color difference of glove model.

Figure 11 shows a good correlation with R^2> 97% between the protein data from MRB and the calculated color difference, ΔE data. This indicates that the proposed protein determination test able to show a good result with high accuracy on determining the protein level of the glove. Moreover, the equation of exponential function in which $y= 0.0617e^{0.156x}$ will be used to find the unknown protein level of new glove models.

V. CONCLUSION

This study shows that the Biocompatibility Morphological Mean (BMM) Test has successfully used to determine the protein level of latex gloves. The samples which go through chemical test able to display the significant difference of blue color intensity between the low, medium, and high protein level. Moreover, the graph shows that there was a good correlation between the BMM test and glove protein level where the calculated color difference, ΔE data using Morphological Color Difference (MCD) algorithm was exponentially increased with the accuracy more than 97%. In conclusion, the BMM test proved to be able to offer an uncomplicated protein determination on a latex glove.

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AUTHOR CONTRIBUTIONS

Chean Khim Toa: Conceptualization, Methodology, Validation, Writing – Original Draft Preparation;

Kok Swee Sim: Project Administration, Supervision, Writing – Review & Editing;

Yee Kit Chan: Validation, Writing – Review & Editing.

CONFLICT OF INTERESTS

No conflict of interests was disclosed.

ETHICS STATEMENTS

Our research work follows The Committee of Publication Ethics (COPE) guideline. https://publicationethics.org.

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