## **ORIGINAL ARTICLES**



# Assessment of Blood Volume in Liposuction Fluids Using Colorimetry

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#### Abstract

*Objective* The possibility of using a color contrast method to evaluate blood loss during liposuction was assessed. A color chart of blood–lipid content associated with different blood volumes was developed.

Methods Three color cards with different concentrations of blood were developed based on clinical parameters. The color cards were used to evaluate the volume of blood present in liposuction solutions obtained from 60 clinical liposuction patients. The red blood cell count also was evaluated for each patient. The data obtained using each evaluation method were compared and statistically analyzed to determine the most accurate calculation formula. Results The red blood cell counts were compared to the color card results. The paired t test results for the calculated values for the 3:1, 2:1, and 1:1 color cards and the red blood cell count values were comparable (44.3  $\pm$  22.1 ml vs. 53.6  $\pm$  25.0 ml, t = 10.5; 45.4  $\pm$  19.0 ml vs. 55.2  $\pm$ 20.7 ml, t = 18.1; 41.9  $\pm$  25.6 ml vs. 52.8  $\pm$  28.3 ml, t =14.0). The P values were < 0.05, and the difference between the two groups was statistically significant. The average standard error of the mean was 0.90, 0.54, and 0.77, respectively. Sixty samples were evaluated in a scatter diagram using the two detection methods. Trend analysis revealed that the two results demonstrated a linear increase (y = 5.6 + 1.1x),  $R^2 = 0.989$ , indicating that the

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*Conclusion* The colorimetric card protocol developed in this study could quickly, accurately, and conveniently calculate blood volumes in liposuction fluids, which has considerable clinical significance.

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**Keywords** Liposuction · Blood loss · Color chart · Continuous color contrast

## Introduction

Liposuction is often accompanied by varying degrees of blood loss that can result in a range of postoperative complications [1], directly affecting patient safety and recovery post-surgery. Accurate assessment of intraoperative blood loss is essential in guiding postoperative fluid replacement and ensuring patient safety. In clinical practice, blood loss is often estimated based on changes in red blood cell (RBC) counts obtained during routine assessments [2]. However, this method is relatively cumbersome and does not directly reflect the actual blood loss because bleeding during liposuction includes apparent and occult blood loss. Bleeding in the interstitial space is considered occult blood loss and is the predominant form of blood loss

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during liposuction. Generally, occult or hidden blood loss and apparent or overt blood loss are positively correlated, and occult blood loss occurs more than overt blood loss [2]. It is challenging to accurately measure the blood loss volume that occurs with interstitial and drainage exudation. The volume of blood present in liposuction fluids reflects actual blood loss, knowing that volume is essential for accurate estimation of total blood loss. This study developed a protocol to accurately calculate blood loss volume by establishing a color chart that could be used to assess liposuction fluids. The study did not require review by an ethical committee.

## **Materials and Methods**

## Materials

- 1.1 Liposuction bottle: 2500-ml glass liposuction bottle, Beijing Yanshan Medical
- 1.2 Technology Co., Ltd., Beijing, China.
- Contrast color card: National Standard GSB05-1426-2001 No. 62, color No. R03, bright red/red.
- 1.4 Camera and usage parameters: Canon EOS R6; ISO 5000; 0 ev; f 4.0; 1/100 s.
- Centrifuges: (1) Beijing Yanshan Medical Technology Co., Ltd., Beijing, China; model XYQ-2 (operating room centrifuge). (2) Xiangyi Medical Technology Co., Ltd., Beijing, China; model TDZ4-WS desktop low-speed centrifuge (laboratory centrifuge).
- 1.6 Pure blood samples: Venous blood was obtained from healthy adults using sodium citrate as the anticoagulant. Additional specimens were obtained from blood samples obtained from patients seen in our hospital.
- 1.7 Microscope: OLYMPUS  $10 \times 40$
- 1.8 Assessment of RBC counts: Sysmex XS-1000i, which uses fluorescence flow cytometry for high-quality hematology analysis.

### **Color Card Production**

### **Obtaining Images**

The color card was composed of color photos of liposuction bottles with different amounts of blood in the fluid. The lipid, blood, and water in the liposuction bottle were shaken to form uniform, red mixtures of different degrees. A crimson film was used to prevent chromatic aberrations from occurring when the images were taken. The liposuction bottle was photographed at the same location each time, using natural light and a white cloth as a background.

## Extraction of Lipid Devoid of Blood or Water

The liposuction fluid was obtained from a clinical patient (Fig. 1A) and allowed to stand for approximately one hour. After the blood and water had fully settled to the bottom of the container, a tube was used to remove the blood and water mixture at the bottom of the vessel (Fig. 1B). Then, physiological saline was added to a volume equivalent to 70% of the lipid volume. The resulting mixture was shaken well and allowed to stand for approximately 15 min. The liquid at the bottom of the container was removed. The lipid was rinsed three times to remove any remaining blood. Then the lipid was aliquoted into 50 ml test tubes and centrifuged (1000 rpm, 1 min) to remove any residual water to obtain lipid with essentially no blood or water (Fig. 1C).

# Development of a Continuous Color Card with a Lipid– Water Ratio of 3:1

Approximately 1400 ml of lipid without blood or water was obtained, to which 450 ml of normal saline was added to create a lipid–water mixture with a ratio of 3:1. Then, 40 units of pure blood were added to the mixture in 5ml aliquots. After each addition of blood, the mixture was thoroughly shaken. Forty solutions with different blood volumes were obtained. Photographs of each solution were taken under the same conditions, and the photographs were combined into a continuous color card based on the total blood volume from low to high. Thus, the color cards represented blood volumes of 5, 10, 15 ml, and so on, until 200 ml (Fig. 2).

## Development of a Continuous Color Card with a Lipid– Water Ratio of 2:1

One thousand ml of lipid without blood and water was obtained. Then, 500 ml of physiological saline was added to obtain a lipid–water ratio of 2:1. Using the same method described above, pure blood was added to the mixture in 40 aliquots of 5ml each. The mixture was thoroughly shaken after each addition of blood to obtain the 40 mixtures with different blood volumes. Photographs were taken using the same conditions as described above. The 40 photographs were combined into a continuous color card based on the blood volume from low to high representing a blood content of 5ml, 10ml, 15ml, and so on until the blood volume of 200ml was obtained (Fig. 3).



Fig. 2 The continuous color chart with a lipid-water ratio of 3:1

Development of a Continuous Color Card with a Lipid– Water Ratio of 1:1

One thousand ml of lipid without blood and water was obtained, and 1000 ml of physiological saline was added to obtain a lipid–water ratio of 1:1. Using the same protocol described above, 5 ml aliquots of pure blood were added to the mixture 40 times. The solution was shaken to adequately mix the solution after each aliquot of blood was added. Photographs were taken of each mixture using the same conditions as described above. The 40 photographs were combined into a continuous color card based on the



Fig. 3 The continuous color chart with a lipid-water ratio of 2:1

blood volume from low to high, representing the blood volumes of 5, 10, 15 ml, and so on until the blood volume of 200 ml was obtained (Fig. 4).

#### **Protocol Implementation**

Color Charts Were Used to Calculate the Blood Volume in the Liposuction Fluid

The continuous color charts were used to calculate the blood volume contained in the liposuction solutions obtained from 60 clinical liposuction patients. First, the lipid-to-water ratio of the liposuction solution was determined and matched to one of the three possible lipid-water ratios, as shown in Fig. 5. As seen in Fig. 5A, a patient's liposuction bottle was determined to have a lipid-water ratio of 3:1 and the 3:1 color colorimetric chart was used for the assessment. Next, the container was shaken well (Fig. 5B), and five physicians without color blindness compared the solution to the colorimetric color chart at the same time. The color card that most individuals selected was considered to be the match. After the comparison, the color of the mixture (Fig. 5B) was determined to be closest to the color image obtained with a blood volume of 60ml (Fig. 5D). Therefore, the blood concentration of the two fluid containers could be considered the same. Based on volume conversion, we determined that the volume of blood in bottle B was 42.3 ml (60 ml  $\times$  1350/1960).

### Laboratory Tests

First, each mixture obtained after liposuction was shaken well before being tested. Five ml of the mixture were observed under a microscope. It was noted that RBCs were not agglutinated, but most RBCs were shrunken, with only a few normal RBCs present. No ruptured RBCs were observed (Fig. 6), but the shrunken RBCs were detected using routine laboratory tests, indicating that RBCs could be detected immediately after liposuction.

Second, five ml of the mixture was centrifuged twice (3000 rpm, 5 min each) (Fig. 7A) to remove lipids and other impurities from the upper layer (Fig. 7B). The blood–water mixture in the lower layer was analyzed, and the RBC counts were determined.

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Blood \ content \ in \ the \ bottle = \frac{(units/liter) \ \times \ the \ volume \ of \ the \ mixture \ (liters)}{The \ number \ of \ RBCs \ per \ unit \ in \ the \ patient's} \\ blood \ before \ surgery \ (units/liter)
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#### Comparison of the Two Methods

After repeating the steps described above, statistical analysis was performed, and the calculation formula was revised. Twenty mixtures with lipid–water ratios close to 3:1, 2:1, and 1:1 were compared to the colorimetric cards, and the blood content was determined. Then the results obtained from the two groups were compared.

SPSS 24.0 software was used for analysis and paired *t* tests were used for data analysis. The counts were expressed as means  $\pm$  standard deviation ( $\pm$  SD). *P* < 0.05 indicated that the difference was statistically significant.

# Results

1. Color chart observations. Three groups of continuous color cards with lipid–water ratios of 3:1, 2:1, and 1:1 were developed. The colors of the continuous color



Fig. 4 The continuous color chart with a lipid–water ratio of 1:1

**Fig. 5** Selection of the appropriate continuous color card for comparison





Fig. 6 Microscopic field of view of the mixture that was tested



Fig. 7 Sample after laboratory centrifugation

cards gradually deepened with increased blood volumes. The increases in blood content were visible with the naked eye using aliquots of 100 ml. As shown in the last row of Figs. 2, 3, and 4, the largest blood volume that could be discerned with the naked eye was 120 ml. The blood volume of 20 samples of liposuction fluids with a lipid–water ratio of 3:1 was 44.3  $\pm$  22.1 ml. The blood volume of 20 samples of liposuction fluids with a lipid–water ratio of 2:1 was 45.4  $\pm$  19.0 ml. Finally, with a lipid–water ratio of 1:1, the blood volume of 20 samples of liposuction fluids was 41.9  $\pm$  25.6 ml.

2. Laboratory tests. Laboratory tests revealed that the blood volume of 20 samples of liposuction fluids with a lipid-water ratio of 3:1 was 53.6  $\pm$  25.0 ml. The

blood volume of 20 samples of liposuction fluids with a lipid–water ratio of 2:1 was  $55.2 \pm 20.7$  ml. The blood volume of 20 samples of liposuction fluids with a lipid–water ratio of 1:1 was  $52.8 \pm 28.3$  ml.

- 3. Comparison of the two methods. Thirty samples were included in each of the two groups that were compared. The paired t test indicated that when the lipid-water ratio was 3:1, the blood volume was determined to be 44.3  $\pm$  22.1 ml based on the continuous color card, and the laboratory test for RBCs calculated a volume of  $53.6 \pm 25.0$  ml (t = 10.5, P < 0.05) for the same liposuction bottle. The mean value  $(\Delta \overline{x})$  of the difference between the two groups was 9.4  $\pm$  4.0 ml, and the mean value ( $\overline{\sigma}$ ) of the standard error was 0.90. Furthermore, when the lipidwater ratio was 2:1, the results of the assessed blood volume in the same liposuction bottle were 45.4  $\pm$ 19.0 ml for the continuous color card and  $55.2 \pm 20.7$ ml for the laboratory test for RBCs (t = 18.1, P < 18.10.05). The mean value  $(\Delta \overline{x})$  of the difference between the two groups was  $9.8 \pm 2.4$  ml, and the mean value  $(\overline{\sigma})$  of the standard error was 0.54. Finally, when the lipid-water ratio was 1:1, the results of the assessed blood volume in the same liposuction bottle were 41.9  $\pm$  25.6 ml for the continuous color card and  $52.8 \pm 28.3$  ml for the laboratory test for RBCs (t = 14.0, P < 0.05). The mean value ( $\Delta \overline{x}$ ) of the difference between the two groups was  $10.8 \pm 3.4$  ml, and the mean value  $(\overline{\sigma})$  of the standard error was 0.77 (Table 1).
- 4. The results obtained from the laboratory tests were approximately 10ml more than the results obtained with the colorimetric card. Therefore, in clinical practice, the blood volume obtained with the colorimetric card is, on average, 10ml less than the blood volume determined using the laboratory test. The results of the two detection methods were placed in a scatter plot that documented the 60 samples. Trend analysis revealed that the two results increased linearly (y = 5.6 + 1.1x) with an R<sup>2</sup> = 0.989. These results

Blood-lipid ratio	Continuous color card comparison method	Laboratory test method	$\Delta \overline{x}$	$\overline{\sigma}$	t	р
3:1 (n = 20)	$44.3 \pm 22.1$	53.6 ± 25.0	$9.4 \pm 4.0$	0.90	10.5	< 0.05
2:1 $(n = 20)$	$45.4 \pm 19.0$	$55.2 \pm 20.7$	$9.8 \pm 2.4$	0.54	18.1	< 0.05
1:1 $(n = 20)$	$41.9 \pm 25.6$	$52.8\pm28.3$	$10.8\pm3.4$	0.77	14.0	< 0.05

**Table 1** Comparison of the continuous color card and laboratory test methods for blood volume assessment in the same liposuction bottle ( $\bar{x} \pm s$ ) (ml)

revealed that the two detection methods were highly correlated (Table 2).

## **Discussion and Conclusions**

Bleeding in association with liposuction is a concern for every plastic surgeon. Postoperative bleeding has been reduced due to continued research on liposuction surgery protocols and improved management of swelling [3]. However, bleeding is still common with large-volume liposuction, especially in specific body regions such as near the ribs and back [3, 4]. One study [2] reported that the average blood loss for 96 patients undergoing large-volume liposuction was more than 600 ml, and their average postoperative hemoglobin levels continued to decrease to 76.3 g/L on the third day following surgery. Furthermore, nine patients in that study became severely anemic. Due to dense adipose tissue and robust blood supply in the rib area, back, and other body regions, postoperative bleeding can be substantial. Massive bleeding severely influences patient safety and recovery and increases the risk of complications, including infections, hematomas, and seromas [5]. Therefore, careful monitoring of the degree of blood loss during liposuction is critical. The ability to assess the amount of blood loss rapidly and accurately in patients is an urgent clinical problem that needs to be addressed. Total blood loss is proportional to the amount of apparent blood loss [6]. Therefore, assessment of apparent blood loss is critical to rapid estimations of total blood loss.

The amount of overt bleeding includes the blood volume in the liposuction bottle and the amount of drainage bleeding. Thus, it is represented by the blood volume in the liposuction bottle [6]. Because different blood concentrations exhibit different colors in the liposuction bottle, a continuous color card with an isoconcentration gradient was designed to assess blood volume loss. Based on this principle, the blood volume in the liposuction bottle could be rapidly and accurately determined.

Based on clinical experience, the lipid–water ratio that occurs in liposuction containers is typically between 1:1 and 3:1. Because the lipid–water ratio is a critical factor affecting the color of the fluid in the liposuction bottle [5, 6], the experimental design used in this study focused on three typical lipid–water ratios, 1:1, 2:1, and 3:1.

The three continuous color cards were composed of 120 images obtained in this study. These images were used to estimate blood volumes in liposuction fluids accurately. First, the continuous color chart with the closest lipidwater ratio was selected. Then the liposuction bottles were compared using the same light that was present in the operating room. To reduce observational errors, five physicians carried out the comparisons at the same time. This approach improved the accuracy of the color chart comparison. Notably, the comparisons were made immediately after the procedure was completed, which provided a more accurate and timely reference for physicians to replenish patient fluids during and after the procedure. According to the continuous color chart created in this study, the blood content in the liposuction bottle can be accurately and timely compared. The main effects of intraoperative intervention are as follows: 1. If the blood content in the liposuction bottle is greater than 50ml less than half of the surgery, it is recommended to immediately use hemostatic drugs or add swelling fluid locally. Wait for at least 10 minutes before liposuction can be performed on the same area again. 2. If there is still a significant amount of bleeding at the bleeding site, it is recommended to stop the surgery in a timely manner or reduce the amount of liposuction, and wait for the second stage surgery. The main effects after surgery are reflected in the following points: 1. The blood content in the liposuction bottle, evaluate whether to open the incision or place a drainage tube after surgery. Based on our experience, it is recommended that the blood content of the liposuction bottle be less than 50ml, and the incision can be sutured directly without placing a drainage tube. If the blood content in the bottle is greater than 50ml, it is recommended to open the incision or place a drainage tube. After the drainage fluid in the bottle is less than 50ml within 24 hours or the exudate is significantly reduced, remove the drainage tube and close the incision. 2. The blood content in the liposuction bottle can serve as an important reference for postoperative fluid replacement and hospital stay. Based on our experience, if the blood content in the liposuction bottle is less than 50ml, routine hemostatic drugs can be given after surgery,



**Table 2** Chart showing the trend in change for the continuous color cards and laboratory detection methods (n = 60)

without excessive fluid replacement. After one day of observation after surgery, the patient can be discharged. If the blood content in the liposuction bottle is greater than 50ml, in addition to conventional hemostatic drugs, appropriate fluid replacement should also be given according to the patient's vital signs. It is recommended to stay in hospital for no less than 2 days. 3. Nurses can quickly calculate the amount of bleeding based on the blood content measured by the color chart, making it easier to write nursing records.

The continuous color charts provided a rapid and convenient method for evaluating blood volume loss that was carried out by observing the intensity of the color. However, no clear reference standard is used currently, and physicians vary in their assessments of the blood volume in liposuction fluids. Thus, developing a continuous color card could improve the accuracy of assessing blood loss during liposuction. After using the continuous color card multiple times, the physician will be able to rapidly determine the amount of blood in the liposuction bottle and eventually might not need to refer to the continuous color card. It was evident in this study that the colors of the three continuous color charts with different lipid-water ratios gradually intensified with increasing blood volumes. The increases in blood volumes were clearly distinguishable with the naked eye up to 100 ml. Furthermore, the highest blood concentration discernable with the naked eye was 120 ml.

The blood volume assessment obtained using colorimetry was comparable to the blood volume determined using RBC counts. The comparisons of the blood volumes and the t-values for the three groups were obtained using paired *t* tests (44.3  $\pm$  22.1 ml vs. 53.6  $\pm$  25.0 ml, *t* = 10.5, 45.4  $\pm$  19.0 ml vs. 55.2  $\pm$  20.7 ml, *t* = 18.1, and 41.9  $\pm$  25.6 ml vs. 52.8  $\pm$  28.3 ml, *t* = 14.0; *P* values were < 0.05). Thus, the difference between the two measurement methods was statistically significant, indicating that the rapid and easy colorimetry method could replace RBC counting.

The difference between the results of the two measurement methods in this study was approximately 10ml. There are several possible reasons for this observed difference. First, although the lipid was rinsed twice with saline, a few RBCs likely remained. Second, in addition to RBCs, other cells might have remained in the lipid. During the red blood cell counts, other cells in addition to RBCs might have been included, resulting in an artificially higher RBC count. It has been reported that the Tallquist hemoglobin scale method [6] accurately estimates blood volume in liposuction bottles. While the method is appropriate, implementation places stringent requirements on the experimental equipment, which can be challenging in a clinical setting. Several reports [2, 7] have indicated that the blood volume in aspirates = the volume of blood in aspirate  $\times$  the RBC count per unit volume of aspirate  $\div$  $4.5 \times 10^{12}$ . However, this formula ignores RBCs attached to fat cells and does not take into account those individuals with variations in their RBC counts.

In the calculation formula used in this study, the blood volume in the aspirates = the unit number of RBCs in the postoperative fluid × the volume of the fluid in the liposuction bottle  $\div$  the unit RBC number before surgery. This formula fully considered the two factors mentioned above, thus, providing greater accuracy. Although the difference between the laboratory measurement and colorimetric comparison was approximately 10 ml, the average standard error ( $\overline{\sigma}$ ) in the experimental data was less than 1. Furthermore, trend analysis indicated that the two results increased linearly (y = 5.6+1.1x), R<sup>2</sup> = 0.989, suggesting that the two inspection methods were highly correlated and the error was small. Therefore, the colorimetric method developed in this study should be advantageous in clinical practice.

#### Limitations

Several limitations were noted in this study. It was not possible to remove all of the RBCs that were attached to fat cells when removing RBCs from the lipid, even if the fluid was left to stand and rinsed multiple times. Furthermore, when the aliquots of blood were added, the total volume of the fluid increased each time, which could affect the volume conversion. Currently, the physician must select the specific volume for the colorimetric card conversion. Also, the upper limit of blood volume that could be assessed

using the colorimetric cards developed in this study was 200ml. Thus, the colorimetric card would not be applicable if the blood volume in the liposuction bottle was greater than 200 ml. At the same time, this fact should remind the surgeon that if the amount of intraoperative blood loss is severe, the procedure should be halted or addressed during the procedure. In addition, it must be noted that the blood added as aliquots in this study was obtained as laboratory specimens from different individuals, and hemolysis might have occurred after the samples were mixed. However, no hemolysis or agglutination was observed when the mixed blood was examined under a microscope. Even minimal hemolysis will cause hemoglobin to be released from ruptured RBCs, but this would not contribute to a color difference in the overall mixture [8]. In conclusion, the continuous colorimetric chart obtained in this study rapidly and accurately estimated the blood volume in liposuction fluid and provided a reliable basis for evaluating blood loss during liposuction.

#### Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest to disclose.

**Human or Animal Rights** This article does not contain any studies performed by any of the authors that utilized human participants or animals.

**Informed Consent** Informed consent was not required for this type of study.

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