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RESEARCH ARTICLE

Impact of Multi-Enzyme Machine Cleaning, Multi-Enzyme Soaking, and Traditional Tap Water Cleaning on the Cleanliness of Blood-Contaminated Hemostatic Forceps

Li Kang[1], Qingmin Gao[2], Yi Feng[3]*

[1]Hebei Province Pharmaceutical Professional Inspectors Team, Shijiazhuang, China
[2]Hebei Medical University Second Hospital, Shijiazhuang, China
[3]Hebei Provincial Institute of Drug and Medical Device Inspection, Shijiazhuang, China

*Corresponding Author: 13832157975@163.com

Abstract: Effective cleaning of surgical instruments is critical for infection prevention in healthcare settings. This study compared the cleaning efficacy, efficiency, and resource consumption of three methods, multi-enzyme machine cleaning (Group S1), multi-enzyme soaking (Group S2), and traditional tap water cleaning (Group S3), on blood-contaminated hemostatic forceps. A total of 600 blood-contaminated hemostatic forceps collected from the disinfection supply room of Hebei Medical University Second Hospital between May and October 2024 were randomly allocated equally across the three groups (n = 200 per group). Cleanliness was assessed using ATP bioluminescence, protein residue quantification, hemoglobin colorimetric analysis, and occult blood test strips. Cleaning time and water consumption were also recorded. Group S1 achieved significantly higher cleanliness qualification rates and protein residue qualification rates than both Group S2 and Group S3 ($p < 0.01$), with Group S2 also significantly outperforming Group S3 ($p < 0.01$). No significant differences in residual blood rates were observed among groups. Notably, Group S2 showed a significantly higher occult blood positivity rate (15.5%) compared to Group S1 (3%) and Group S3 (3.5%; $p < 0.01$), suggesting that multi-enzyme soaking may mobilize but not fully remove occult blood residues. Group S1 demonstrated significantly shorter cleaning time (10.56 ± 1.75 min) and lower water consumption (17.14 ± 2.29 L) compared to both Group S2 and Group S3 ($p < 0.001$). Multi-enzyme machine cleaning demonstrated superior performance across most cleanliness indicators while consuming less time and water, supporting its recommendation as the preferred method for hemostatic forceps reprocessing in hospital disinfection supply departments.

Keywords: Hemostatic forceps, Instrument cleaning, Multi-enzyme cleaning, ATP bioluminescence, Protein residue, Disinfection supply, Surgical instrument reprocessing

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Introduction

The cleaning and disinfection of medical devices hold a crucial position in the medical field, directly impacting the safety and effectiveness of every medical procedure and closely related to patient outcomes [1]. With the rapid development of modern medical technology and the emergence of increasingly complex and delicate medical devices, the quality of device cleaning has been subjected to unprecedentedly stringent demands [2]. Hemostatic forceps, as one of the most used medical devices in operating rooms and various diagnostic and treatment departments, frequently encounter patients' blood and tissues. During use, their surfaces are highly susceptible to contamination, especially with blood. In addition to blood, hemostatic forceps may also become contaminated with tissue debris, mucus, fat, carbohydrates, and other organic matter during use, as well as other pollutants such as drug residues and human secretions (e.g., sweat, body fluids) that may come into contact during surgical procedures. Failure to thoroughly remove these contaminants can similarly increase the risk of cross-infection and compromise subsequent sterilization efficacy. If not thoroughly cleaned, residual organic matter can increase the risk of cross-infection, but also form biofilms during subsequent high-temperature and high-pressure sterilization processes, reducing sterilization efficacy and leading to sterilization failure, thereby exposing patients to significant health risks [3, 4].

Traditional tap water cleaning, with its simplicity and low cost, has been widely used for a long time. However, in the face of increasingly complex contamination, tap water, relying solely on physical flushing, often fails to thoroughly remove dried blood on the surfaces, crevices, and joints of instruments, highlighting its limitations [5]. In recent years, with the integration of biotechnology and materials science, multi-enzyme cleaning technology has emerged [6]. Multi-enzyme formulations contain various specific enzymes that can precisely target and break down proteins, fats, and carbohydrates in blood, effectively removing stubborn stains from instrument surfaces. Multi-enzyme soaking, a key component of multi-enzyme cleaning technology, allows instruments to fully contact the multi-enzyme solution at an appropriate concentration, enabling enzyme molecules to penetrate into every detail of the instruments and significantly enhancing cleaning thoroughness [7, 8]. Multi-enzyme machine cleaning further optimizes the cleaning process through precise control and efficient operation of automated equipment. It can strictly regulate parameters such as the temperature, concentration, flow rate, and cleaning time of the cleaning solution, ensuring that each hemostatic forceps is cleaned under optimal conditions. This not only improves cleaning efficiency but also ensures the stability and consistency of cleaning quality [9].

Based on the efficient degradation capacity of multi-enzyme detergents for organic pollutants and the precise control advantages of automated equipment, this study hypothesizes that the multi-enzyme automated washing method, by combining automated processes with specialized multi-enzyme detergents, will achieve significantly superior cleaning outcomes (including cleaning qualification rate and protein residue control) compared to multi-enzyme soaking and traditional tap water cleaning methods. The study aims to compare the cleaning efficacy of multi-enzyme automated washing, multi-enzyme soaking, and traditional tap water cleaning methods for blood-contaminated hemostatic forceps, evaluating indicators such as cleaning qualification rate, protein residue rate, and residual blood rate, to clarify the advantages and disadvantages of different cleaning methods and provide experimental evidence for medical institutions in selecting efficient and economical cleaning protocols for hemostatic forceps.

Related Work

In the healthcare system, the disinfection, sterilization, and reprocessing of medical devices are key components in preventing and controlling hospital-acquired infections, directly affecting patient safety and treatment outcomes. In recent years, research in this field has continued to advance. Rutala et al. comprehensively discussed the basic concepts, methods, and importance of disinfection, sterilization, and antisepsis, establishing a solid theoretical framework for the entire research domain [10]. They emphasized the characteristics and applicability of different disinfection and sterilization techniques, providing foundational guidance for subsequent research and practice. Rowan et al. re-examined the Spaulding classification system [11]. This classic system categorizes medical devices into critical, semi-critical, and non-critical based on the nature and risk level of contact with patients, and establishes corresponding processing standards for each category. In the modern healthcare environment, this system not only guides the processing of traditional reusable medical devices but also provides insights for adapting to sustainable development needs, such as in resource utilization and environmental protection, by helping to develop more rational processing procedures and reducing unnecessary resource waste.

The impact of biofilms on the disinfection and sterilization of medical devices can't be overlooked. Weber et al. investigated the interference of biofilms (surface-attached communities composed of bacteria and their extracellular matrices) with the

reprocessing of medical devices and surface disinfection [10]. The study found that biofilms are prevalent on hospital environmental surfaces and reusable medical devices, reducing bacterial susceptibility to antibiotics and disinfectants, thereby compromising cleaning and disinfection efficacy. The research specifically highlighted that critical healthcare-associated pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, and Candida auris readily form biofilms on environmental surfaces, which significantly impede cleaning and disinfection. The authors advocate for future research to focus on developing novel methods to reduce or eliminate biofilm formation, particularly on implantable medical devices, reusable medical equipment, and hospital environmental surfaces. As a special form of microbial existence, biofilms exhibit strong resistance to antimicrobial agents, reducing the efficacy of disinfectants and increasing the risk of infection. Understanding these mechanisms can aid in the development of more effective methods for biofilm removal and improvements in disinfection processes. Regarding practical issues in medical device reprocessing, Whelan and Ling conducted in-depth analyses [12, 13]. Current reprocessing processes face numerous challenges, including limitations of disinfection and sterilization technologies, variations in operator adherence to protocols, and the complexity of processing different types of devices. These issues can lead to incomplete disinfection and sterilization, potentially causing cross-infections. They proposed measures such as enhanced personnel training, improved operational standards, and the introduction of new technologies to enhance reprocessing quality. In addition, Tyski et al. provided a detailed review of the application of regulatory documents for disinfectants and antiseptics in the medical field [14]. Proper understanding and application of these documents are crucial for ensuring the efficacy and safety of disinfection products. Different disinfectants and antiseptics are suitable for various scenarios, and adherence to regulations can guide healthcare workers in making rational choices and correct usage to achieve optimal disinfection outcomes. Francis et al. addressed the challenge of unstable medical supply in harsh environments, such as battlefield settings, by developing a rapid and efficient disinfection method for 3D-printed surgical instruments [15]. The research team evaluated different cleaning and disinfection protocols (isopropanol + chlorhexidine pretreatment followed by immersion in Cidex OPA solution) to assess their sterilization efficacy on 3D-printed devices. They successfully established a high-level disinfection protocol that could be completed within 48 hours. The study innovatively resolved the sterilization challenges of 3D-printed medical devices in resource-limited settings, demonstrating that a specific disinfection protocol (isopropanol + chlorhexidine cleaning followed by Cidex immersion) effectively eliminated both known and unknown bacteria on instrument surfaces. This provides a feasible solution for instrument supply in battlefield medicine and global health emergencies.

These publications have investigated the disinfection, sterilization, and reprocessing of medical devices from multiple dimensions, including theoretical foundations, classification systems, biofilm impacts, practical issues, regulatory applications of disinfection products, and special scenario applications. They complement each other, offering comprehensive theoretical and practical support for optimizing medical device processing procedures and reducing infection risks. These studies drive the continuous development of the field of healthcare infection control to better safeguard patient health and safety. Future research can build on this foundation to further explore new technologies and methods to enhance the quality and efficiency of medical device processing, adapting to the evolving needs of the healthcare sector.

Methods

Study Subjects

A total of 600 blood-contaminated hemostatic forceps, cleaned in the disinfection supply room of Hebei Medical University Second Hospital from May to October 2024, were selected as study samples. These forceps originated from various clinical departments of the hospital, including surgery, obstetrics and gynecology, and emergency departments, where they were frequently used in diverse surgical and emergency treatment procedures, resulting in complex and varied blood and tissue contamination. The hemostatic forceps included various models such as straight vascular forceps, curved vascular forceps. Differences in structural design, such as the shape and density of the teeth and the flexibility of the joints, may affect the ease and effectiveness of cleaning.

To ensure the scientific and reliable nature of the study, the selected hemostatic forceps met the following criteria: they were all delivered to the disinfection supply room within the specified time (generally within 2 hours after use) to ensure that the blood and other contaminants had not overly dried, avoiding increased cleaning difficulty due to prolonged time, which could interfere with the study results; they had no obvious damage in appearance, such as severe deformation or breakage of the forceps head, or loose or jammed joints, to ensure that the cleaning effect was not influenced by the device's own damage; the department of use and duration of use for each forceps were recorded to analyze the impact of different usage

scenarios on cleaning effectiveness. The exclusion criteria consisted of hemostats exhibiting severe rust, missing components, or impaired clamping function due to impact.

To minimize operator-related variability, this study implemented standardized training for all operators (to ensure uniformity in cleaning procedures and reagent preparation), utilized a fixed water source from the same hospital (to guarantee consistent water quality across all groups), and strictly controlled key operational parameters (such as water temperature and enzyme concentration) to minimize the influence of variables such as water quality and operator proficiency, thereby enhancing the methodological rigor of the research.

(1) Standardized training: all three operators underwent uniform training, including: preparation of multi-enzyme detergent (strict 1:200 dilution), ultrasonic cleaner settings (40 kHz, 50°C, 30 min), ATP swab sampling technique (200 g pressure, uniform swabbing over 20 cm2), and culture plate inoculation (100 μL per sample, three-zone streaking). Only operators passing post-training assessments participated. (2) Randomization and blinding: 600 medical devices were randomly allocated to three operators (200 each), with no disclosure of group assignments (S1/S2/S3). Two independent assessors, blinded to groupings, evaluated cleaning efficacy using coded samples, recorded data separately, and cross-verified results to prevent bias. (3) Quality control documentation: each procedure recorded operator ID, time, and critical parameters (e.g., temperature, ultrasonic duration). Post-trial, SPSS 25.0 analyzed inter-operator data for statistical differences.

Main Reagents and Instruments

Multi-enzyme detergent (Shenzhen Antaifu Disinfection Hi-Tech Co., Ltd.; main components: protease, lipase, amylase; working concentration: 1:200 dilution. This study utilized the following reagents and instruments: a multi-enzyme detergent (containing proteases, lipases, amylases, and cellulases to degrade proteins, lipids, carbohydrates, and cellulose in tissue debris, respectively, enabling synergistic removal of multiple contaminants); an ATP bioluminescence assay kit (Beyotime Biotechnology Co., Ltd.; detection range: 0–1000 RLUs); a YT-ATP bioluminescence detector (Shandong Yuntang Intelligent Technology Co., Ltd.); a protein residue detection kit (Beyotime Biotechnology Co., Ltd.; detection limit: 0.05 mg/cm2); an FB-100 fully automated fecal occult blood analyzer (Changchun Dirui Medical Technology Co., Ltd.); a Xinhua Medical Super6000 high-efficiency automated washer-disinfector; a protein residue test kit (Shanghai Tongwei Industrial Co., Ltd.); a spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd., China); a hemoglobin detection kit (Shanghai Jingkang Biological Engineering Co., Ltd., China); a biuret reagent (Shanghai Zhenzhun Biotechnology Co., Ltd., China); a fully automated fecal occult blood analyzer (Beijing Bionuokang Biotechnology Co., Ltd., China); fecal occult blood test strips (Nanjing Shenji Pharmaceutical Technology Co., Ltd., China); and LINKWELL® multi-enzyme detergent (Shanghai Likang Biological High-Tech Co., Ltd., China).

Grouping and Cleaning Methods

The 600 hemostatic forceps were randomly and evenly divided into three groups, with 200 forceps in each group: Group S1 (multi-enzyme machine cleaning), Group S2 (multi-enzyme soaking), and Group S3 (traditional tap water).

Group S1 (Multi-enzyme machine cleaning): The Xinhua Medical Super6000 high-efficiency automated washer-disinfector was employed. The standard operating conditions for the cleaning equipment were as follows: water temperature was maintained at 50 ± 2°C during the main washing phase, multi-enzyme detergent concentration was strictly prepared at a 1:200 dilution, and the flow rate of the cleaning solution was set at 3 L/min. The cleaning program employed was the “General Instrument Cleaning Mode,” which consisted of four phases: pre-rinsing (2 min, water temperature 30–35°C), main washing (10 min, water temperature 50 ± 2°C, with mechanical arm rotation and high-pressure spraying), enzymatic immersion (5 min, maintaining the temperature and concentration of the main washing phase), and final rinsing (3 min, water temperature 60–65°C, using purified water). All parameters were automatically controlled by the equipment throughout the process to ensure cleaning consistency (total duration: 20 minutes). Before cleaning, the hemostatic forceps were pre-treated with running water to remove most of the visible contaminants from the surface. The forceps were then placed into the automated washer-disinfector. According to the hemostat forceps’ contamination level and type, an appropriate amount of multi-enzyme detergent compliant with national standards (consistent with the 1:200 concentration specified in the operating conditions) was added. The detergent contained multiple enzymatic components capable of specifically degrading various organic contaminants including proteins, lipids, and carbohydrates. The cleaning device simulated manual cleaning actions through the movement and rotation of mechanical arms and the spraying of high-pressure water jets (as part of the main cleaning stage) to thoroughly clean all parts of the forceps. After the completion of the above cleaning program, the device’s thermal disinfection function was used to heat the water to 90°C for 5 minutes to achieve preliminary disinfection and reduce microbial residues. Finally,

the forceps were rinsed with pure water at least three times (integrated into the final flushing stage) to remove any residual cleaner and impurities, ensuring no cleaner residue on the surface of the cleaned forceps.

Group S2 (Multi-enzyme soaking): The hemostatic forceps was initially also rinsed with running water to remove large surface contaminants. A dedicated soaking container was prepared, and a multi-enzyme cleaning solution of appropriate concentration was configured according to the product instructions. The hemostats were completely immersed in the multi-enzyme cleaning solution for a duration of 30 minutes to ensure optimal contact between the enzymatic detergent and contaminants for effective decomposition. During soaking, every 10 minutes, a specialized instrument brush was used to gently scrub hard-to-clean areas such as the teeth and joints of the forceps, ensuring that the cleaning solution penetrated all crevices. After soaking, the forceps were thoroughly rinsed with running water for no less than 5 minutes to remove the multi-enzyme solution and decomposed contaminants. Subsequently, the forceps were placed in an ultrasonic cleaner with an adequate amount of pure water and cleaned for 10 minutes using the cavitation effect of ultrasonic waves to further remove tiny particles and hidden contaminants. Finally, the forceps were rinsed with pure water three times to ensure no cleaner residue.

Group S3 (Traditional tap water): This group used the hospital's traditional tap water cleaning method. The hemostatic forceps were placed under running tap water, and a soft-bristle brush was used to carefully scrub all parts of the forceps, including the surface, teeth, and joints, for approximately 15 minutes. The cleaning water was continuously changed during scrubbing to maintain its cleanliness. After scrubbing, the forceps were soaked in tap water for 10 minutes to further remove any residual contaminants. Finally, the forceps were rinsed with running tap water for 5 minutes to ensure no visible contaminants on the surface. The entire cleaning process used only tap water without any cleaning agents, serving as a control group to compare the effectiveness of the other two cleaning methods.

Evaluation Indicators

The detection time points were set at 12 h, 24 h, and 48 h. These intervals were selected based on clinical practice patterns for instrument storage and turnover: 12 h represents the routine pre-use inspection point after short-term storage; 24 h and 48 h correspond to the maximum potential storage durations for daily and alternate-day instrument turnover respectively, allowing evaluation of cleaning efficacy persistence. The cleaning standard adopted in the study was an ATP bioluminescence value of ≤200 RLUs (relative light units). Samples with ATP values at or below this threshold were considered to meet the cleaning criterion. At 12 hours, all 200 instruments in the Group S1 met the standard, compared to 190 in the Group S2 and 142 in the Group S3. At 24 hours, 193 instruments in the Group S1 met the standard, versus 180 in the Group S2 and 118 in the Group S3. At 48 hours, 175 instruments in the Group S1 met the standard, compared to 155 in the Group S2 and 85 in the Group S3.

Immediately following each cleaning protocol (within three minutes), sterile swabs were used to collect surface samples from instruments. ATP testing was performed within ten minutes post-sampling. The cleanliness of hemostatic forceps in each group was detected using ATP bioluminescence technology, and the cleanliness qualification rate was calculated. Representative detection sites were selected on the hemostatic forceps, such as the teeth, joint crevices, and surface of the forceps body, which are usually areas where contaminants tend to remain and are difficult to clean. A dedicated ATP detection swab was used to wipe and sample these selected sites. During wiping, the swab was ensured to make full contact with the detection site and was operated according to the specified wiping path and pressure to ensure that the collected sample was representative. The wiping area and time for each detection site needed to be consistent, for example, wiping each site for 10 - 15 seconds with an area of about 1 - 2 square centimeters. After sampling, the swab was quickly placed into a detection tube containing lysis solution, and fully shaken to break the microbial cells on the swab and release ATP. ATP reacted with luciferin-luciferase in the detection reagent to produce a fluorescent signal under specific conditions. The detection tube was placed into an YT-ATP bioluminescence detector (Detection wavelength set to 560 nm), which measured the ATP content in the sample based on the intensity of the fluorescent signal and automatically converted it into Relative Light Units (RLU).

After testing randomly selected samples of hemostatic forceps from each group, the number of hemostatic forceps that met cleanliness standards was counted, and the cleanliness qualification rate was calculated. The cleanliness qualification rate was calculated: (Number of hemostatic forceps that met cleanliness standards ÷ Total number of tested hemostatic forceps) × 100%.

Protein residue qualification rate. Several hemostatic forceps were randomly selected from each group. Concurrent with ATP detection, absorbance measurements were completed within 20 minutes post-sampling. In areas prone to protein

residue, such as the teeth, joint movement areas, and instrument surfaces, a dedicated sampling cotton swab moistened with deionized water was used to wipe 3 - 5 times, with a wiping area of about 1 - 2 square centimeters. The sampled cotton swab was placed into a test tube containing a specified amount of buffer solution and fully shaken to dissolve the protein. Following the instructions of the protein residue kit, the corresponding reagent (no self-preparation required) was added to the cuvette containing the sample buffer solution and gently mixed. The biuret reagent reacted with the peptide bonds in the protein to form a purple complex, which was left to stand for 10 - 15 minutes to allow the color reaction to fully proceed. A spectrophotometer was used to measure the absorbance of the sample solution at a wavelength of 540 - 560nm. First, the blank control solution (containing only buffer solution) was used to calibrate and zero the instrument, and then the absorbance of the sample solution was measured. The number of hemostatic forceps with qualified protein residue in each group was counted, and the qualification rate was calculated: Protein residue qualification rate=(Number of hemostatic forceps with qualified protein residue ÷ Total number of tested hemostatic forceps) × 100%. A qualified protein residue was defined as ≤0.1 mg/cm^2 of residual protein on the instrument surface.

Residual blood rate. Immediately after cleaning, the sampling swab eluate was inoculated onto culture media. Colony counting was performed after 48 hours of incubation for all groups, with counting procedures completed within an hour. A certain number of hemostatic forceps were randomly selected from each group. The hemostatic forceps were completely immersed in a fixed amount of saline and soaked for 5 - 10 minutes, with occasional shaking to fully dissolve any residual blood in the saline. Then, an appropriate amount of the soaking solution was taken and tested using the hemoglobin colorimetric method. Using a specific hemoglobin detection kit, the soaking solution was reacted with the reagent in the kit. The absorbance was measured using a spectrophotometer at a specific wavelength, and the hemoglobin content in the soaking solution was calculated using a standard curve. Based on the volume of saline before soaking the hemostatic forceps, the residual blood volume of each hemostatic forceps was calculated, and the residual blood rate was computed: Residual blood rate=(Residual blood volume of hemostatic forceps ÷ Theoretical maximum blood volume) × 100%; the theoretical maximum blood volume can be estimated based on the specifications of the hemostatic forceps). The theoretical maximum blood capacity was estimated based on hemostat structural parameters. For different hemostat types (straight or curved), the total clamping surface area (including grooves) was measured. Combined with the maximum clinically possible blood contact thickness (standardized at 0.1 mm) and blood density (1.05 g/cm^3), the theoretical maximum blood capacity (mL) per hemostat was calculated as: Theoretical maximum blood capacity (mL)=Total clamping area (cm^2) × 0.01 cm × 1.05 g/cm^3 ÷ 1 g/mL. Specifically, straight hemostats (16 cm length) has a clamping area ≈ 4.2 cm^2 and a theoretical maximum blood capacity ≈ 0.044 mL; curved hemostats (16 cm length) has a clamping area ≈ 4.5 cm^2 and a theoretical maximum blood capacity ≈ 0.047 mL.

Occult blood positivity was defined as a test strip color intensity meeting or exceeding the manufacturer-specified positive threshold (corresponding to a hemoglobin concentration ≥0.1 μg/mL) when assessed using fecal occult blood test strips. Several hemostatic forceps were randomly selected from each group. A dedicated occult blood detection strip was used to wipe and sample areas such as the teeth, joints, and surfaces of the hemostatic forceps. The sampled test strips were inserted into the matching FB-100 fully automated fecal occult blood analyzer for analysis, and the test result was observed. If the strip color changed and reached or exceeded the specified positive threshold, the hemostatic forceps were judged as occult blood positive; otherwise, it was negative. The number of forceps testing positive for occult bloody in each group was counted, and the occult blood positivity rate was calculated: Occult blood positivity rate = (Number of hemostatic forceps with occult blood positivity ÷ Total number of tested hemostatic forceps) × 100%.

The cleaning time and water consumption of the three groups of hemostatic forceps were recorded.

Statistical Methods

SPSS 25.0 was used to process the data. Descriptive statistical analysis was performed on the basic demographic information of the three groups, with continuous variables expressed as mean ± standard deviation and categorical variables presented as frequency and percentage. When comparing the body mass index and other indicator data before and after intervention, the Shapiro-Wilk test was first used to assess normality. For normally distributed data, independent samples *t*-tests and paired samples *t*-tests were used to analyze baseline differences before intervention and group differences after intervention, respectively. For non-normally distributed data, the Mann-Whitney U test and Wilcoxon signed-rank test were selected. Repeated measures data were analyzed using repeated measures ANOVA. $P<0.05$ was considered statistically significant.

Results

Analysis of ATP values from samples processed by three operators (using Group S1 as an example) showed means of 128±15 RLUs, 132±18 RLUs, and 125±12 RLUs, respectively. One-way ANOVA revealed F = 0.32, P=0.73 (P>0.05), indicating no significant bias from operator technique and demonstrating data consistency.

In Table 1, Group S1 (multi-enzyme cleaning + manual wiping) exhibited ATP qualification rates of 100%, 96.5%, and 87.5% at 12 h, 24 h, and 48 h post-cleaning, respectively. These rates were significantly higher than those of Group S2 (multi-enzyme cleaning + ultrasonic cleaning: 95%, 90%, 77.5%) and Group S3 (manual wiping only: 71%, 59%, 42.5%) (P<0.05).

Table 1: Comparison of cleanliness qualification rate among three groups

Time point	Group	Cleaning pass rate (%
12 h	S1	100.0
	S2	95.0
	S3	71.0
24 h	S1	96.5
	S2	90.0
	S3	59.0
48 h	S1	87.5
	S2	77.5
	S3	42.5

Note: Data were analyzed using the chi-square test (12 h and 48 h) and Fisher's exact test (24 h). Significant differences were observed among groups at all time points (P < 0.05). Pairwise comparisons indicated that Group S3 was significantly lower than Groups S1 and S2 at all time points (P < 0.01), while Group S2 was lower than Group S1 (P < 0.05).

In Table 2, the three protocols demonstrated significant differences in qualified protein residue rates for hemostats: Group S1 (multi-enzyme cleaning + manual wiping): 158 qualified (79%); Group S2 (multi-enzyme cleaning + ultrasonic cleaning): 129 qualified (64.5%); Group S3 (manual wiping only): 44 qualified (22%). Statistical analysis revealed that Group S1 had significantly higher qualification rates than Group S2 (P<0.05) and Group S3 (P<0.01). Protein residue quantification showed average 48h residual levels of 0.12 mg/cm2 (Group S1), 0.25 mg/cm2 (Group S2), and 0.48 mg/cm2 (Group S3), exhibiting strong concordance with ATP detection trends.

Table 2: Comparison of protein residue qualification rate among three groups

Group	Qualified protein residue rate (%)	Comparison with Group S1 (*P*-value)	Comparison with Group S2 (*P*-value)	Statistical Method	Main Statistic
S1	79	-	<0.05		
S2	64.5	<0.05	-	Chi-square test	χ^2=45.67
S3	22	<0.01	<0.01		

Note: The chi-square test was employed as the data consisted of dichotomous categorical variables, all groups had balanced sample sizes (n=200), and all expected frequencies exceeded 5, thus satisfying the application conditions for the chi-square test

Table 3 demonstrates no significant differences in residual blood rates among the three protocols (P>0.05), with Group S1 (multi-enzyme cleaning + manual wiping) showing 12 positive cases (6%), Group S2 (multi-enzyme cleaning + ultrasonic cleaning) 12 cases (6%), and Group S3 (manual wiping only) 13 cases (6.5%).

Table 3: Comparison of residual blood rate among three groups

Group	Residual blood rate (%)	Statistical method	Intergroup comparison results	Main statistic
S1	6	Fisher's exact probability	*P*>0.05	*P*=0.983
S2	6			
S3	6.5			

Note: Fisher's exact test was employed because the number of positive cases in some groups was less than 5, which did not meet the conditions for the chi-square test. Therefore, Fisher's exact test was used to directly calculate the P-value

Table 4 shows significant differences in occult blood positivity rates among the three protocols: Group S1 (multi-enzyme cleaning + manual wiping) had 6 positive cases (3%), Group S2 (multi-enzyme cleaning + ultrasonic cleaning) 31 cases (15.5%), and Group S3 (manual wiping only) 7 cases (3.5%). Statistical analysis revealed that Group S2 had significantly higher positivity rates than both Group S1 (P<0.05) and Group S3 (P<0.05), while no significant difference was observed between Group S1 and Group S3 (P>0.05).

Table 4: Comparison of occult blood positivity rate among three groups

Group	Positive rate of occult blood (%)	Comparison with Group S1 (*P*-value)	Comparison with Group S2 (*P*-value)	Statistical method	Main statistic
S1	3	-	<0.01	Fisher's exact probability	
S2	15.5	<0.01	-		*P*<0.001
S3	3.5	0.832	<0.01		

Note: Fisher's exact test was employed due to the low number of positive cases (e.g., only six cases in the Group S1), which resulted in non-normal distribution of the data, making Fisher's exact test the appropriate method

Table 5 demonstrates significant differences in cleaning time among the three groups: Group S1 (multi-enzyme cleaning + manual wiping) showed statistically significantly shorter cleaning time compared to both Group S2 (multi-enzyme cleaning + ultrasonic cleaning) and Group S3 (manual wiping only), with these differences being statistically significant (*P*<0.05).

Table 5: Comparison of cleaning time among three groups

Group	Cleaning time	Comparison with Group S1 (*P*-value)	Comparison with Group S2 (*P*-value)	Statistical method	Main statistic
S1	10.56±1.75	-	<0.001	ANOVA	*F*=287.4
S2	36.48±4.82	<0.001	-	+Tukey test	*P*<0.001
S3	41.05±5.11	<0.001	<0.05		

Note: One-way analysis of variance (ANOVA) with Tukey's multiple comparisons test was applied. The data were confirmed to follow a normal distribution by the Shapiro-Wilk test (P>0.05), and homogeneity of variances was verified by Levene's test (P>0.05), thus satisfying the assumptions for parametric testing

Table 6 reveals significant differences in water consumption during the cleaning process among the three groups: Group S1 (multi-enzyme cleaning + manual wiping) demonstrated significantly lower water consumption compared to both Group S2 (multi-enzyme cleaning + ultrasonic cleaning) and Group S3 (manual wiping only), with these differences being statistically significant (P<0.05). However, no statistically significant difference was observed between Group S2 and Group S3 (P>0.05).

Table 6: Comparison of water consumption among three groups

Group	Water consumption	Comparison with Group S1 (*P*-value)	Comparison with Group S2 (*P*-value)	Statistical method	Main statistic
S1	17.14±2.29	-	<0.001	ANOVA	*F*=492.6
S2	108.45±9.33	<0.001	-	+Tukey test	*P*<0.001
S3	115.57±11.15	<0.001	0.063		

Note: One-way analysis of variance (ANOVA) with Tukey's post hoc test was employed. Normality was confirmed by Shapiro-Wilk test (P>0.05), and homogeneity of variances was verified by Levene's test (P>0.05), thus meeting the assumptions for parametric analysis

Discussion

This study systematically evaluated the cleaning efficacy of three protocols for surgical hemostats, demonstrating that the multi-enzyme cleaning combined with manual wiping (Group S1) showed superior comprehensive performance, providing a reference for clinical practice. Analysis of ATP values from Group S1 samples processed by three operators yielded means of 128±15 RLUs, 132±18 RLUs, and 125±12 RLUs respectively, with one-way ANOVA showing F=0.32 and P=0.73 (P>0.05), confirming operator technique did not affect results and data consistency. Regarding ATP qualification rates, Group S1 achieved 100%, 96.5%, and 87.5% at 12h, 24h, and 48h post-cleaning respectively, significantly higher than Group S2 (95%, 90%, 77.5%) and Group S3 (71%, 59%, 42.5%) (P<0.05). These findings align with existing clinical understanding of synergistic enhancement of immediate decontamination efficiency through enzymatic action combined with mechanical force. The multi-enzyme detergent, containing protease and lipase, rapidly degrades organic matter such as blood and mucus, while manual wiping (200 g pressure) removes residual debris from crevices, achieving thorough decontamination through their synergistic action. Group S2's slightly inferior ultrasonic cleaning performance may stem from limited penetration of cavitation effects into complex lumens or narrow gaps, necessitating supplementary wiping for instruments with crevices. Group S3's manual wiping alone proved inadequate for removing denatured organic residues, particularly coagulated proteins, explaining its higher cross-contamination risk [16]. The Group S3 (traditional tap water cleaning) exhibited the lowest cleaning qualification rate, primarily due to its reliance solely on physical wiping and water flushing, lacking the synergistic effect of chemical agents such as enzymes. Once outside the physiological environment, proteins in blood undergo denaturation and coagulation in response to temperature changes and pH fluctuations, forming stubborn residues that are difficult to remove through mechanical force alone. These residues tend to accumulate in structurally complex areas of hemostats, such as the serrations and joint gaps. Furthermore, tap water contains no components capable of degrading organic pollutants or breaking the chemical bonds between protein molecules, and can only remove loosely adhered surface contaminants. For coagulated blood stains, repeated wiping may further embed the pollutants into the instrument crevices, thereby reducing cleaning efficacy. Additionally, manual wiping is inconsistent in force and coverage, making it difficult to ensure all contaminated areas are effectively treated. These factors collectively contributed to the significantly lower cleaning qualification rate in the Group S3 compared to the Group S1 and Group S2 at all time points. Protein residue serves as a critical indicator of cleaning efficacy due to its strong correlation with biofilm formation. Significant differences were observed in qualified protein residue rates among the three groups: 79% for Group S1, 64.5% for Group S2, and 22% for Group S3 (P<0.05 or P<0.01), with overall residual levels following the trend Group S1 (0.12 mg/cm2) < Group S2 (0.25 mg/cm2) <Group S3 (0.48 mg/cm2). Hemostats' hinge gaps and serrations are particularly prone to protein accumulation. Group S1's superior performance stems from enzymatic degradation of collagen and fibrin by specific enzymes combined with physical removal of crevice residues through manual wiping, demonstrating synergistic enhancement in cleaning complex structures [17]. Group S2 showed incomplete residue removal due to ultrasonic energy attenuation in gaps ≤0.5 mm, while Group S3 could only eliminate superficial loose proteins without decomposing denatured fibrin clots, potentially resulting in "visually clean but protein-positive" instruments that pose postoperative infection risks [18].

No significant differences were observed in residual blood rates among the three groups (Group S1: 6%, Group S2: 6%, Group S3: 6.5%, P>0.05), indicating that all protocols effectively removed visible surface blood stains, meeting the basic requirement of "visual cleanliness." However, it should be noted that dried residual blood proteins can denature and coagulate, forming stubborn residues (as shown in Table 2 protein residue differences), making residual blood rate an insufficient standalone evaluation criterion [19]. Occult blood positivity rates revealed Group S2 (15.5%) was significantly higher than both Group S1 (3%) and Group S3 (3.5%) (P<0.05), while no significant difference existed between Group S1 and Group S3 (P>0.05). The occult blood test demonstrated a sensitivity of 0.1 μg/mL, capable of detecting trace blood residues invisible to the naked eye, particularly in hinge gaps and serrations. Group S1 achieved low positivity rates through enzymatic hemoglobin degradation combined with manual wiping to eliminate trace residues in crevices, effectively reducing transmission risks of pathogens like HBV and HIV. Group S2 showed higher positivity rates due to insufficient removal of trace blood in complex structures caused by ultrasonic energy attenuation, suggesting potential hidden residue risks with ultrasound alone. It is noteworthy that the residual blood rate detection primarily reflects substantial blood residue (based on the quantitative threshold of hemoglobin colorimetric assay), while the occult blood test exhibits high sensitivity (0.1 μg/mL), enabling the detection of trace amounts of uncleared hemoglobin in complex structures. This likely explains the core discrepancy wherein the Group S2 demonstrated a high occult blood positive rate despite a residual blood rate comparable to other groups. The two methods differ in sensitivity and detection thresholds for blood residue: the former is suitable for evaluating obvious blood residue, while the latter is more adept at identifying trace residues. The ultrasonic cleaning employed in the Group S2 exhibited

insufficient efficacy in removing trace blood from complex structures, resulting in a higher positive rate under high-sensitivity detection. Although Group S3 exhibited comparable positivity rates to Group S1, the absence of enzymatic action may only achieve surface-level cleaning while leaving deeper denatured proteins, warranting vigilance against superficial compliance with underlying residual contamination [20]. Regarding cleaning time, Group S1 demonstrated significantly shorter durations compared to Groups S2 and S3 ($P<0.05$). The efficiency of Group S1 stems from enzymatic reduction of repetitive wiping and targeted cleaning that minimizes ineffective friction, potentially enhancing instrument turnover in high-volume surgical departments. Group S2, constrained by fixed ultrasonic duration (30 minutes in this study) and subsequent wiping requirements, follows a more rigid protocol better suited for batch processing. Group S3, lacking enzymatic assistance, requires prolonged manual scrubbing of stubborn stains, resulting in the longest processing time and potential instrument damage, making it suitable only for emergency situations. In terms of water consumption, Group S1 showed significantly lower usage than Groups S2 and S3 ($P<0.05$), while no significant difference existed between Groups S2 and S3 ($P>0.05$). Group S1's enzymatic approach reduces water dependency through targeted cleaning that avoids indiscriminate rinsing, aligning with sustainable operational trends. Group S2's continuous water injection and multiple water changes during ultrasonic cleaning result in high consumption. Group S3's need for frequent rinsing to remove contaminants and maintain instrument moisture leads to consistently high water usage, demonstrating inferior economic and environmental performance compared to Group S1. The activity of enzymes in the multi-enzyme detergent is closely related to the operating temperature and incubation time. As proteins, enzymes are susceptible to denaturation due to alterations in their spatial structure caused by deviations in these two parameters. In this study, the main washing phase for the Group S1 was set at 50 ± 2°C, which represents the optimal temperature range for composite enzymes such as proteases and lipases. At temperatures below this range, the molecular motion of enzymes slows down, reducing their efficiency in degrading pollutants like proteins and lipids. Conversely, temperatures exceeding 60°C may disrupt secondary bonds (e.g., hydrogen bonds) within the enzyme structure, leading to irreversible denaturation and loss of catalytic activity. Additionally, the enzymatic immersion time in the Group S1 was strictly controlled to 5 minutes. This duration was determined based on enzyme kinetic characteristics: at the optimal temperature, the binding efficiency between enzymes and substrates (pollutants) peaks within 5 minutes. Prolonging this period may reduce degradation efficiency due to gradual substrate depletion and natural enzyme inactivation. This also explains why the Group S1 demonstrated a significantly higher protein residue qualification rate (79%) compared to the Group S2 (64.5%): The Group S1 maintained efficient degradation of blood proteins by precisely controlling the temperature (preventing enzyme denaturation) and incubation time (aligning with the period of stable enzyme activity). In contrast, although the Group S2 used the same enzyme formulation, it failed to strictly regulate the enzyme reaction temperature during the ultrasonic cleaning phase (where localized overheating may have occurred due to ultrasonic heating) and did not terminate the reaction promptly after immersion. Partial enzyme denaturation occurred due to prolonged exposure to suboptimal conditions, resulting in incomplete protein degradation and ultimately higher protein residue rates.

In summary, the combined multi-enzyme cleaning with manual wiping demonstrates significant comprehensive advantages for hemostat cleaning and is recommended as the standard clinical protocol. Ultrasonic cleaning remains suitable for batch processing of smooth-surfaced instruments but requires supplemental wiping to address cleaning deficiencies in complex structures. Manual wiping alone should be reserved for emergency situations and necessitates enhanced post-cleaning monitoring.

Conclusion

In this article, the aim was to compare the cleaning effects of multi-enzyme machine cleaning, multi-enzyme soaking, and traditional tap water cleaning on blood-contaminated hemostatic forceps, in order to explore the optimal pathway for clinical instrument processing. The multi-enzyme automated washing group demonstrated significantly higher cleaning qualification rates and protein residue qualification rates at all time points compared to the multi-enzyme soaking group ($P<0.05$) and the traditional tap water cleaning group ($P<0.01$). Its cleaning time and water consumption were significantly shorter/lower than those of the multi-enzyme soaking group and the traditional tap water cleaning group ($P<0.001$). The multi-enzyme soaking group exhibited a significantly higher occult blood positive rate than both the multi-enzyme automated washing group and the traditional tap water cleaning group ($P<0.01$), while no statistically significant difference was observed in residual blood rate among the three groups ($P>0.05$). The advantages of multi-enzyme machine cleaning were remarkable. Prior to the experiment, it was hypothesized that its automated process combined with professional multi-enzyme cleaning agent could achieve efficient cleaning, and the results confirmed this. Pre-treatment quickly removed large surface contaminants, while the multi-enzyme precisely broke down various organic impurities. The mechanical arms and high-pressure water jets

provided comprehensive flushing, and the thermal disinfection and multiple rinses ensured thorough cleaning. It performed exceptionally well in terms of cleanliness qualification rate, protein residue control, as well as cleaning efficiency and water conservation, strongly arguing for its rationality as the clinical first choice. This can significantly improve instrument turnover and usage safety. Regarding multi-enzyme soaking, it was initially thought that the combination of multi-enzyme breakdown and ultrasonic cleaning of hidden areas could achieve good results. However, practical experience revealed some shortcomings despite the effectiveness. The combination of soaking and manual brushing lacked coherence in the process and uneven cleaning in complex structures, resulting in lower cleanliness durability, protein and occult blood removal compared to multi-enzyme machine cleaning. However, with optimization, such as fine-tuning the soaking and ultrasonic parameters, it can serve as an auxiliary method in specific scenarios to meet diverse needs. Traditional tap water cleaning, used as a control, as expected, lacked targeted decontamination components and relied solely on simple brushing, which proved inadequate against complex contaminants. It resulted in poor cleanliness and high resource consumption, making it suitable only for very specific emergency situations. This highlights the indispensability of modern cleaning technologies.

However, this study focused solely on hemostats, which feature a relatively simple structure (primarily consisting of clamping jaws and joints). In contrast, complex instruments such as laparoscopic devices and staplers contain lumens and precision components, where the cleaning efficacy of multi-enzyme automated washing may be influenced by structural differences. Future research should involve stratified studies targeting different types of instruments to clarify the applicability of the current findings and enhance the rigor of extrapolation. Although the experimental conditions were idealized, the implementation of standardized operating procedures and uniform experimental parameters minimized the impact of variables such as water quality and operator technique, thereby providing a reasonable basis for the generalizability of the results. Moreover, the experimental environment was idealized. In real hospital settings, variables such as water quality and operator proficiency can affect the results, limiting the generalizability of the findings. Nevertheless, overall, this article provides key guidance for instrument cleaning decisions and helps enhance medical quality.

This study focused exclusively on hemostats, which feature a relatively simple structure. Future research could expand the scope to include medical instruments with complex lumens and precision components, such as laparoscopic devices and staplers, to investigate the differential cleaning efficacy of multi-enzyme automated washing, multi-enzyme soaking, and traditional tap water cleaning across various structural regions. It would be valuable to systematically evaluate the influence of cleaning parameters (e.g., temperature, enzyme concentration, cleaning duration) on the cleaning outcomes of complex instruments. Additionally, further studies could explore the targeted mechanisms of enzyme types and formulations against specific contaminants (e.g., high-viscosity tissue fluids, drug residues) to optimize the composition of multi-enzyme detergents. Long-term follow-up data could also be incorporated to analyze the impact of different cleaning methods on the functional longevity of medical devices, thereby providing a more comprehensive evidence base to support cleaning protocol selection in healthcare settings.

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Author’s Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Li Kang and Yi Feng. The first draft of the manuscript was written by Li Kang, Qingmin Gao assisted in the revision of the article and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics

The authors have no relevant financial or non-financial interests to disclose. The study protocol was submitted to the hospital Institutional Review Board (IRB) for review. As the research solely evaluated medical device cleaning efficacy without involving human subjects, animal experiments, or biological samples, ethical committee approval was not required.

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