Feasibility and efficacy of newly developed eco-friendly, automatic washer for endoscope using electrolyzed alkaline and acidic water

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Abstract

Introduction: The development of reliable and eco-friendly washing for medical equipment not only prevent nosocomial and healthcare-associated infections, but also be safe for the global environment. The aim of the study was to evaluate the efficacy of a new automatic washing system (Nano-washer) that uses electrolyzed water and ultrasonication without detergent for washing endoscopes.

Methods: Patients who underwent laparoscopic lobectomy or laparoscopic colectomy at Nagasaki University between 2018 and 2022 were included. A total of 60 cases of endoscope use were collected and classified according to endoscope washing method into the Nano washer group (n = 40) and the Manual washing group (n = 20). Protein and bacterial residues were measured before and after washing, using absorbance spectrometry and 16S rRNA PCR. The effectiveness of protein and bacterial removal and endoscope surface damage after washing were compared under specular vision between the groups.

Results: Nano washer did not use detergent unlike manual washing. There was no difference in demographic or clinical characteristics between the groups except for the presence of comorbidities in lobectomy group (Nano-washer, 85%; Manual washing, 40%, p=0.031). Compared with the Manual washing group, residual protein levels in the Nano-washer group were significantly reduced after washing (lobectomy, 0.956 mg/mL vs 0.016 mg/mL, p<0.001; colectomy, 0.144 mg/mL vs 0.002 mg/mL, p=0.008). Nano-washer group showed a significant reduction in bacteria between before and after lobectomy (9,437 copies/cm^2 vs 4,612 copies/cm^2, p=0.024).

Conclusion: Nano-washer is promising, effective, and eco-friendly automatic washing device that is safer and more efficient than manual washing.

Introduction

Minimally invasive surgery has become common in general surgery and is expected to increase in the future. It can achieve better results than open surgery in terms of reduced wound pain and infection, early improvement in organ function, and shorter hospital stay (1, 2). The endoscopes are cleaned repeatedly and re-used in multiple patients, and inadequate washing can lead to nosocomial and healthcare-associated infections with the devices acting as vectors for the transmission of infectious agents (3-6). Therefore, to prevent the transmission of pathogenic agents from contaminated equipment to patients and healthcare workers, it is necessary to strictly enforce washing and disinfection in accordance with the manufacturer's written instructions or washing guidelines (7-12).

In many hospitals, medical instruments are washed manually. Problems associated with manual washing include high labour costs, individual differences in the standard of work, and the risk of human error. There are also concerns regarding the impact of residual washing agents on the human body and on medical devices (13). In addition, precision instruments such as endoscopes cannot withstand heat or strong chemicals, which makes it difficult to efficiently remove biofilms and special proteins such as prions (14). Automated, effective, and atraumatic washing methods are therefore crucial for precision instruments; however, few studies have examined these aspects in detail (15).

In general, surfactant-containing detergents are used to wash medical devices. In addition, chemical washing for antimicrobial efficacy is of the greatest importance (16). However, these detergents produce a large amount of wastewater.

Electrolysed water is reported to be effective for removing bacteria and proteins from surgical instruments (17, 18). Electrolysed acidic water contains HCl and HOCl and has bactericidal effects (17), whereas electrolysed alkaline water is reported to have excellent

protein removal effects in addition to bactericidal effects and inactivation of infectious proteins (18). Furthermore, electrolysed water is ideal for global environmental protection because it does not require detergents for washing. There are two previous reports of an effective washing procedure that uses electrolysed water and ultrasonication (14, 19). Using stainless steel cylinders, Nakano et al. compared the effectiveness of the new procedure with the conventional washing method (Association of Official Agricultural Chemists methods) (20, 21) against *Staphylococcus aureus* (ATCC 29213 strain), *Pseudomonas aeruginosa* (ATCC 27853 strain) and *Candida albicans,* and reported that the new procedure was effective in all cases (14). Mori et al. evaluated the effect on prion protein infectivity and reported the successful inactivation of infectious proteins (19). Based on that study, a medical device decontamination washer was developed (Medical Nano-washer washer, JMDN code: 35424000, Kyowakiden Industry Co., Ltd (Nagasaki, Japan)); however, the effectiveness of the device has not yet been tested with actual surgical instruments.

In this study, we compared the abilities of the Nano-washer and manual washing to remove protein and bacteria from endoscopic instruments.

Materials and Methods

Data collection

Patients who underwent laparoscopic lobectomy or laparoscopic colectomy at Nagasaki University Department of Surgical Oncology between October 2018 and March 2022 were included. Sixty cases of endoscope use were collected, 30 each for lobectomy and colorectal resection. Of these, 40 were assigned to the Nano-washer group (lobectomy, n = 20; colectomy, n = 20) and 20 to the Manual washing group (lobectomy, n = 10;

colectomy, n = 10). The following were compared between the groups: age, sex, body mass index, performance status, comorbidity, target organ, surgical technique, operative time, blood loss, postoperative complications, and length of hospital stay.

The study protocol was reviewed and approved by the Clinical Research Review Board of our institution (No. 18082029) and was performed in line with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Endoscopes

Three specular rigid endoscopes were used during the study period: HOPKINS II telescope (K26003BA; KARL STORZ, Tuttlingen, Germany), Olympus HD laparoscope (WA53005A; Olympus Medical Systems, Tokyo, Japan) and Stryker laparoscope (SR-502-457-030, Stryker, Michigan, USA). The choice of endoscope was decided according to the surgeon's preference, according to the technique and conditions in each operative case.

Production of electrolysed water

Acidic and alkaline electrolysed water was generated from 36 L of 0.15% sodium chloride solution at room temperature and 27 V, using an electrolyser. Oxidation-reduction potential (ORP) and pH were measured using an ORP sensor (9300-10D; Horiba Ltd, Kyoto, Japan) attached to an electrometer (D-53S; Horiba Ltd). The free chlorine concentration was measured using a chlorometer (6560-10C, Horiba Ltd).

Washing protocols

The experimental processes for both systems are shown in Fig. 1. The manual washing

protocol was based on complete immersion following the endoscope manufacturer's recommendations and national guidance documents and took 75–80 min per endoscope (21). The manual washing protocol was performed as follows. After disassembling the equipment, one component was cleaned at a time using tap water and a brush to remove large contaminants. The equipment was then soaked in enzyme washing solution (NT-1; Micro-scientific, Il, USA and Medi-pole EX-1; Inui medix, Osaka, Japan) before being rinsed under running tap water, transferred to a tray, and placed in the dryer for 10 minutes. Dust was then removed with an airbrush.

The automated washing, Nano-washer, procedure uses electrolysed water and sonication, and was developed in collaboration with Kripton Co., Ltd (Tokyo, Japan) and Kyowakiden Industry Co., Ltd (Nagasaki, Japan). Nano washer does not use detergent compared to Manual washing because the washing process is done with electrolyzed water. The Nano washer named the initials of 1: Normal temperature and pressure: applicable to precision mechanical equipment, 2: Automatic ultrasonic washing: efficiently removing high risk microbial pathogens, 3: No detergent: highly safe to human bodies and environment, 4: Only use salt and water: eco-friendly. The procedure takes about 30-50 min per endoscope, and was performed as follows (Fig. 2). The objects to be cleaned were placed in the tank of the Nano-washer and fully automatic washing and disinfection was performed according to a set programme that includes pre-wash, alkaline electrolytic wash, acid electrolytic water wash, and rinse components, as follows. After a pre-wash with tap water for 1 min, ultrasonic washing is performed with alkaline electrolytic water for 20 min to remove remaining grease and proteins, followed by ultrasonic washing with tap water for 1 min to remove suspended dirt and residual alkaline water. The components are then washed with acidic electrolytic water for 20 min

and contaminants are removed by sterilisation and oxidative decomposition, and then washed with tap water for 1 min, followed by re-sterilisation, and removal of decomposition residues and residual acidic water. Any remaining water droplets are then air-dried.

Protein quantification

Samples for analysis of residual protein were collected from the lens of the endoscope before and after Nano-washing and manual washing. The tip of the lens was swabbed and the sample was then dissolved in phosphate-buffered saline solution. Proteins were extracted using DC Protein Assay Reagent (Bio-Rad Laboratories, California, USA). The extract was applied to a F16 MaxiSorp loose NUNC-Immuno Module (Thermo Fisher Scientific, MA, USA) and absorbance was then measured using a MultiScan JX Spectrum instrument (Thermo Fisher Scientific).

Methodology of 16S rRNA PCR

Samples for analysis of residual bacterial count were collected before and after Nano-washing and manual washing. Samples were collected by circumferential rubbing of the sides of the endoscope within 5 cm of the tip. Assessment of residual bacteria was performed by 16S rRNA PCR. DNA was extracted using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, USA). PCR primer sequences for mRNA were 16s341 forward, 5'-CCTACGGGGAGGCAGCAG-3', and 16s518 reverse, 5'-ATTACCGCGGCTGCTGGG -3'; and PCR reactions were performed with GeneAce SYBR® qPCR (Roche Molecular Biochemicals, IN, USA). Quantitative RT-PCR was performed on a Roche LightCycler 480 system (Roche Molecular Biochemicals).

Quantification data were analysed using Light Cycler analysis software (Roche Molecular Biochemicals), with each sample analysed in triplicate. Results were corrected by dividing the constant copy number (/uL) by the rubbing area (cm^2). One patient in the Nano-washer group was excluded from the analysis because of incomplete data.

Statistical Analysis

Data are reported as the mean ± standard deviation. Comparisons between groups were performed using the Mann–Whitney U test and t-test. Values of $p<0.05$ were considered statistically significant. All statistical analyses were performed using JMP PRO (version 15; SAS Institute Inc, Cary, NC).

Results

Table 1 lists patients' demographic and clinical backgrounds according to washing group. In lobectomy patients, the presence of comorbidities was significantly higher in the Nano-washer group (85% vs 40%, p=0.031). There was no statistically significant difference between Nano washer group and Manual washing group in terms of age sex, body mass index, performance status, operative technique, operative time, blood loss, post-operative complications, or length of hospital stay.

Figure 3 shows residual protein values before and after washing on endoscopes used in lobectomy and colorectal resection. In both cases, residual protein levels were significantly lower after Nano-washing (Fig. 3a). No significant difference was observed after manual washing (Fig. 3b).

Figure 4 compares residual bacteria by 16S rRNA PCR, before and after washing, for endoscopes used in lobectomy and colorectal resection. Endoscopes used in lobectomy

had significantly lower residual bacterial levels after Nano-washing (9,437 copies/cm^2 vs 4,612 copies/cm^2, p=0.024) (Fig. 4a). No significant difference was observed after manual washing (Fig. 4b).

Discussion

In the present study, the usefulness and safety of fully automated washing with the Nano-washer were compared with those of manual washing. The Nano-washer was as effective or more effective than manual washing in terms of protein and bacterial removal. Furthermore, the Nano washer uses no detergents compared to manual washing.

The increasing variety of surgical techniques and medical equipment has led to an increase in the number and type of surgical instruments that require washing prior to re-use. Inadequate washing of medical equipment causes the formation of various inorganic and organic residues that provide a breeding ground for micro-organisms and also increase the risk of fever and infection in individual patients (22). It has been reported that medical equipment can act as a vector for the transmission of infectious agents to susceptible hosts, leading to healthcare-associated and nosocomial infections (3-6).

Among the transmission risks, it has been reported that some bacteria can form biofilms that cannot be removed by normal decontamination methods and cause high rates of iatrogenic infection (3, 23). Among infectious proteins, prion proteins are known to cause Creutzfeldt–Jakob disease, which is lethal and incurable (10). To date, patient-to-patient transmission due to prion contamination of medical instruments such as endoscopic instruments has not been proven (24). Although the infectivity of prion proteins is reported to be basically restricted to the central nervous system, there have been recent reports of prion protein detection also in peripheral tissues. Contaminated surgical

instruments have been reported as the cause of iatrogenic infection (25, 26).

To prevent this and other risks of infection transmission, manufacturers' written instructions for use and AORN guidelines recommend appropriate washing methods (22). Recently, the importance of chemical washing for sufficient antimicrobial efficacy has been reported (16). However, some sensitive medical devices such as endoscopes and high-resolution cameras cannot withstand high temperatures or strong chemicals (3, 4). In addition, adverse effects on the global environment due to the accumulation of wastewater discharged after washing are also a problem. The development of effective and atraumatic washing and eco-friendly for sensitive medical devices is therefore of great importance.

Electrolysed water has recently been used in the process of washing medical devices such as endoscopes (17). Ionising a sodium chloride solution produces acidic water with excellent antibacterial and antiviral properties (27-29) and does not irritate the eyes, skin or respiratory organs, or leave residue (10). Alkaline ionised water has been reported to contribute to protein removal and inactivation of infectious proteins, in addition to having bactericidal effects (10). In this study, we developed a washing method using electrolysed water and ultrasonication as a fully-automated surgical instrument cleaner, and investigated its efficacy in comparison with manual washing. We considered it an ideal environmentally friendly method, as it also minimizes wastewater emissions.

We used the 16S rRNA PCR method to measure residual bacteria before and after washing. 16S rRNA gene polymerases are present in all bacteria as conserved and variable regions (30, 31). In previous reports, 16S rRNA has been shown to identify up to 90% of genera and 66%–90% of species of bacteria (32-34). 16S rRNA PCR is reported to be useful for the detection and identification of bacterial pathogens in clinical samples

in which infection is suspected but bacterial culture results are negative (32-34). It can also detect non-soluble bacterial DNA in sterile patients after antibiotics have been administered (35). Recent guidelines recommend the administration of antibiotics immediately before and every three hours during surgery to prevent surgical site infection in scheduled surgery, and it is difficult to detect residual bacteria by bacterial culture (36). All of the present patients were treated with preoperative antibiotics, but residual bacterial levels were measurable by 16S rRNA PCR, and 16S rRNA levels in endoscopes used for lobectomy were significantly reduced after Nano-washing.

Residual protein levels have been reported as an indicator of the effectiveness of washing methods (15, 37, 38). Indeed, it has been reported that when contaminated instruments were properly washed, protein reductions of 99%–100% were achieved (15, 38). In the present study, before and after washing, a statistically significant reduction in residual protein was observed in the Nano-washer group, and a trend towards protein reduction was also observed in the Manual washing group. However, the present protein reduction rates tended to be slightly lower than in previous reports, 97.7%–98.1% in the Nano-washer group and 75.6%–98.3% in the Manual washing group. One possible explanation is that no complicated cases with massive intra-operative bleeding or prolonged surgery were included in this study, and that there was less endoscopic contamination prior to washing. Fushimi et al. studied the amount of residual protein before and after soft gastrointestinal endoscope washing (37). The mean residual protein decreased from 36 mg/sample before washing to 20 mg/sample after washing, but the difference did not reach statistical significance. They considered that this was due to the low protein concentration even before washing, as there were few cases of invasive surgery with endoscopic treatment, which supports the present results. Another possible

explanation is that in actual surgery, operations are always performed in a clean field and the endoscope is cleaned with sterile water and sterile gauze whenever it becomes contaminated, even intraoperatively. This may also have been a factor in the low protein levels before washing. In the future, it is desirable to establish an indicator of residual protein suitable for assessing the effectiveness of washing.

Both manual and automatic washing are currently in use for washing medical instruments. In general, manual washing has the advantage that it enables detailed washing without a machine. Previous reports have demonstrated that manual washing sufficiently reduces microorganisms on surgical instruments (39). However, manual washing has disadvantages such as high subjectivity of the cleaner, risk of human error, risk of infection of the cleaner, and labour costs. Currently, the ANSI/AAMI ST7922 guideline supports the effectiveness of automatic washing and disinfection of equipment for reprocessing of surgical instruments, and aims to ensure process reproducibility and efficiency (40). However, there is no consensus that automatic washing is more effective than manual washing, and little subsequent quality monitoring has been established (39, 41, 42).

The Nano-washer evaluated in this study is an automatic washer. We consider that this device has notable advantages. Used equipment placed in the tank is cleaned and disinfected fully automatically according to a set program, which is efficient because it reduces the need for manual intervention and has a relatively short washing time. Our preclinical and clinical examination of safety, in terms of physical damage caused by automatic washing, found no visible damage to endoscopes. In addition, the Nano-washer does not use the surfactants employed in manual washing, and thus produces no detergent residue that requires rinsing, which is advantageous for reducing environmental

emissions.

There were several limitations of this study. First, this is a retrospective, single-centre study, and the sample size was small. Second, due to a lack of data, we were unable to compare cost between manual washing and Nano-washers. Third, residual bacteria before washing differed between the Nano-washer and manual washing groups. This may be because the evaluations were performed at different times and the backgrounds were not aligned. Future studies need to be conducted prospectively in randomized clinical trials.

Despite the limitations of the study, the automatic Nano-washer is a promising device for washing endoscopic equipment that is as effective or more effective than manual washing.

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Authors' Contributions

Akiko Fukuda, Keitaro Matsumoto, and Takeshi Nagayasu designed the study. Tetsuro Tominaga, Takamune Matsumoto, Takashi Nonaka, and Keitaro Matsumoto performed surgery. Kosuke Kosai and Katsunori Yanagihara performed analysis of residual bacterial and protein count. Takumi Inoue and Hiromi Irie performed washing. Yoshiaki Miyoshi, Tomomi Sugio, Takatoshi Sakai, Eiji Sakae, and Masahisa Hamada maintained the Nano washer. Takeshi Nagayasu supervised this study. All co-authors contributed substantially to this study and fulfilled the requirements for authorship as per the guidelines of the International Committee of Medical Journal Editors. All authors have read and approved

the final version of the manuscript.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Data Availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

This study was performed in line with the principles of the 1964 Declaration of Helsinki and its later amendments. Approval was granted by the Clinical Research Review Boards of all participating hospitals.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Figure legends

Figure 1: Experimental processes

Figure 2: Nano-washing process

Figure 3: Residual protein before and after Nano-washing (a) and manual washing (b) on endoscopes used in lobectomy and colectomy.

Figure 4: Residual bacteria before and after Nano-washing (a) and manual washing (b) on endoscopes used in lobectomy and colectomy.

Figure 1: Experimental processes

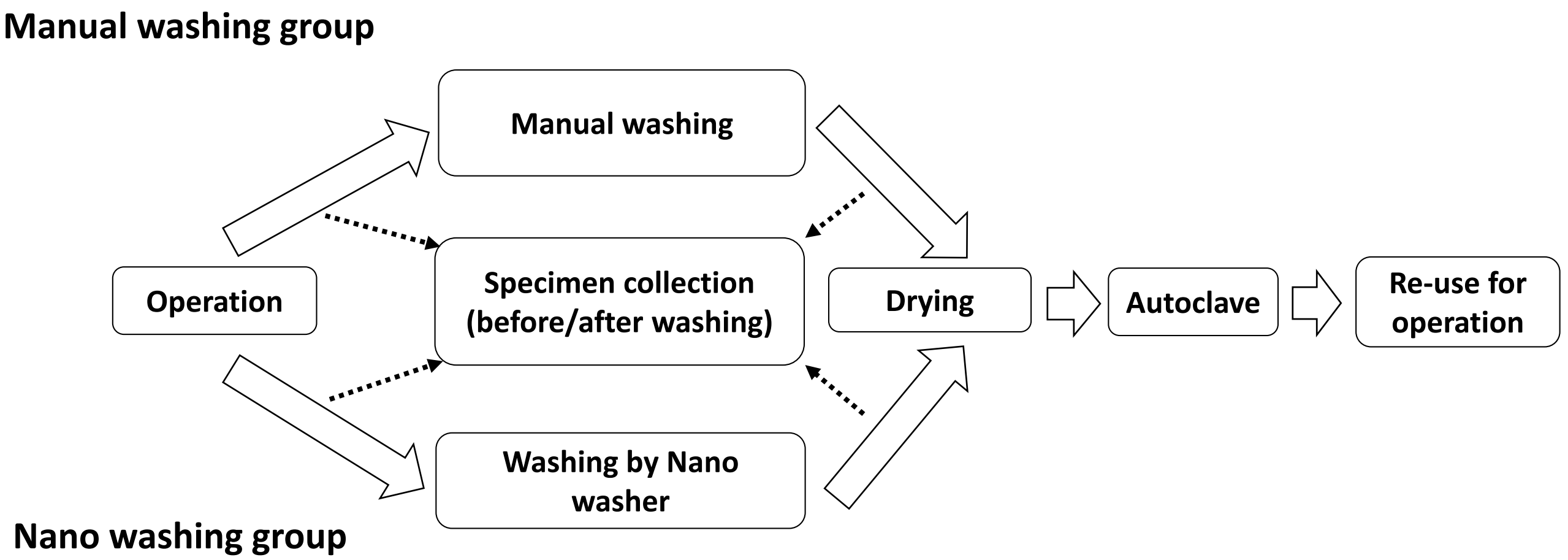


Figure 2: Nano washing process

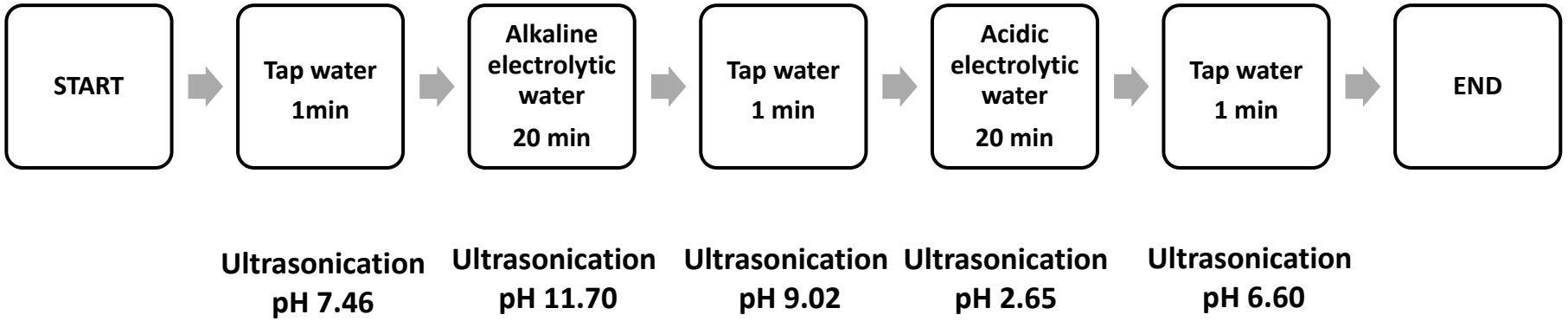
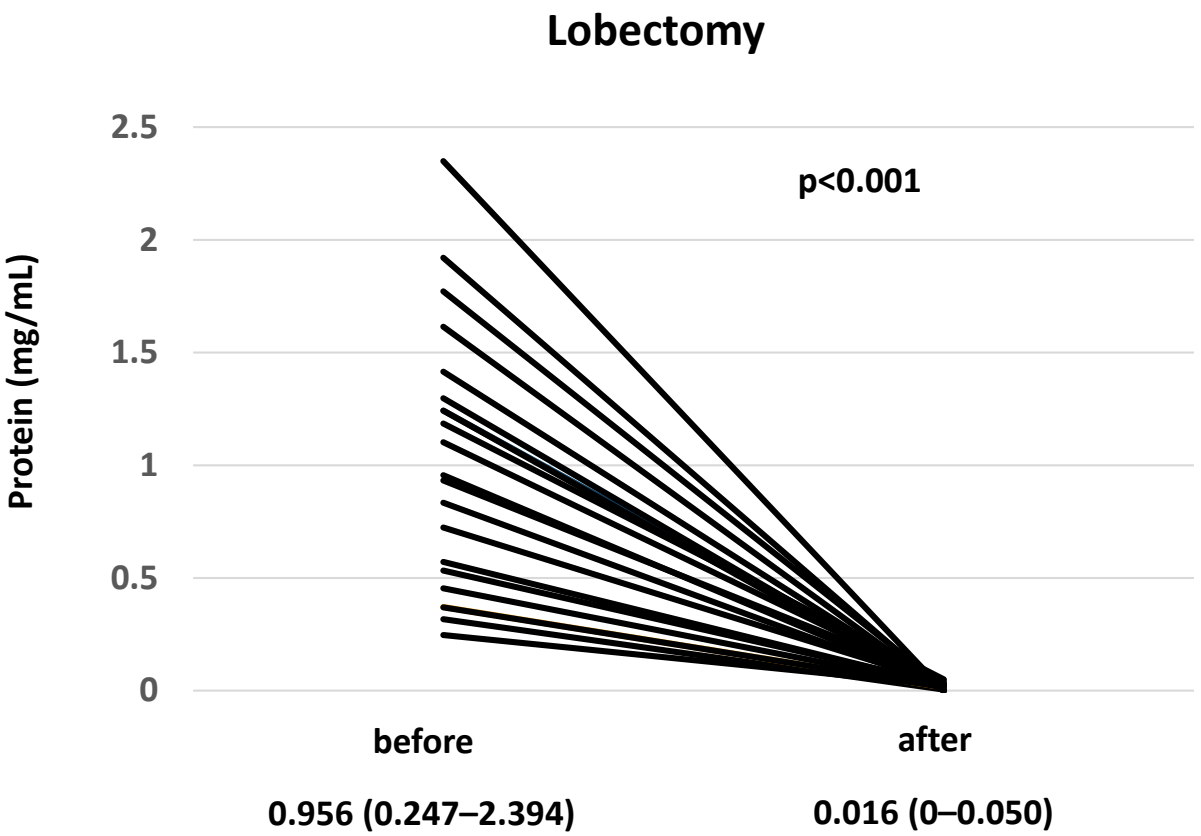


Figure 3a: Residual protein before and after Nano washing



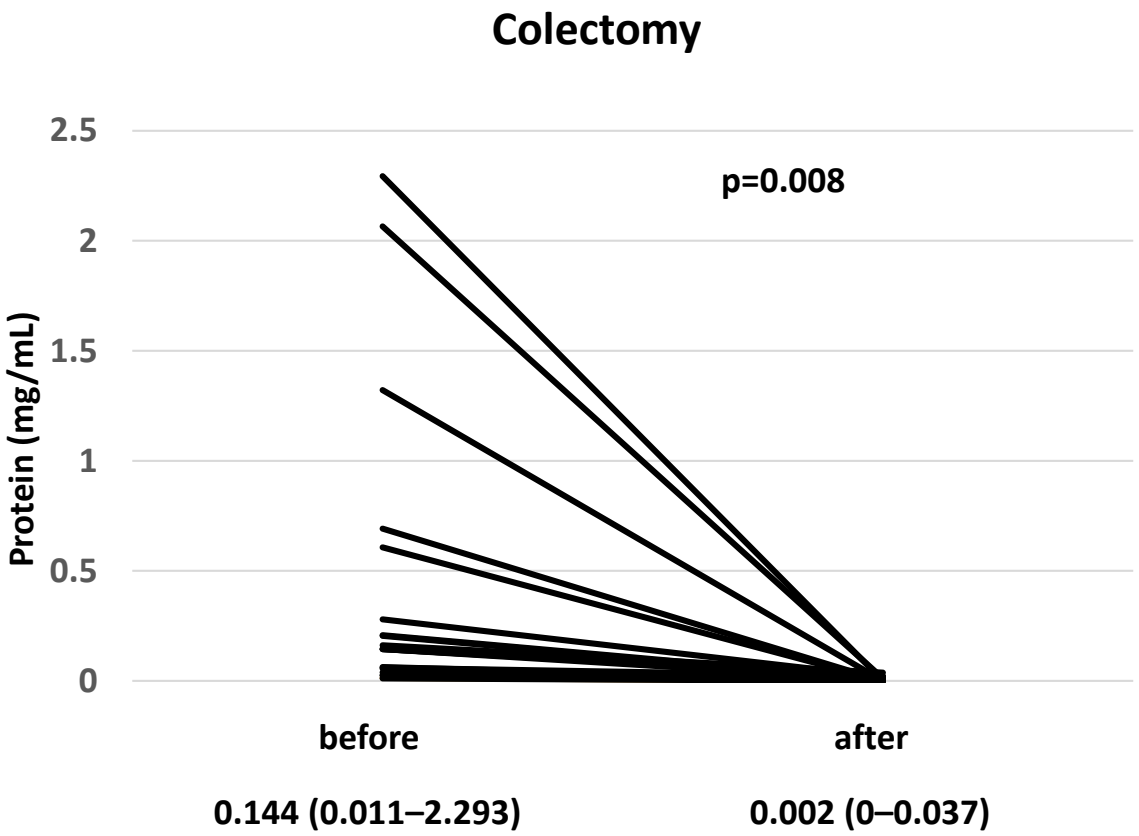
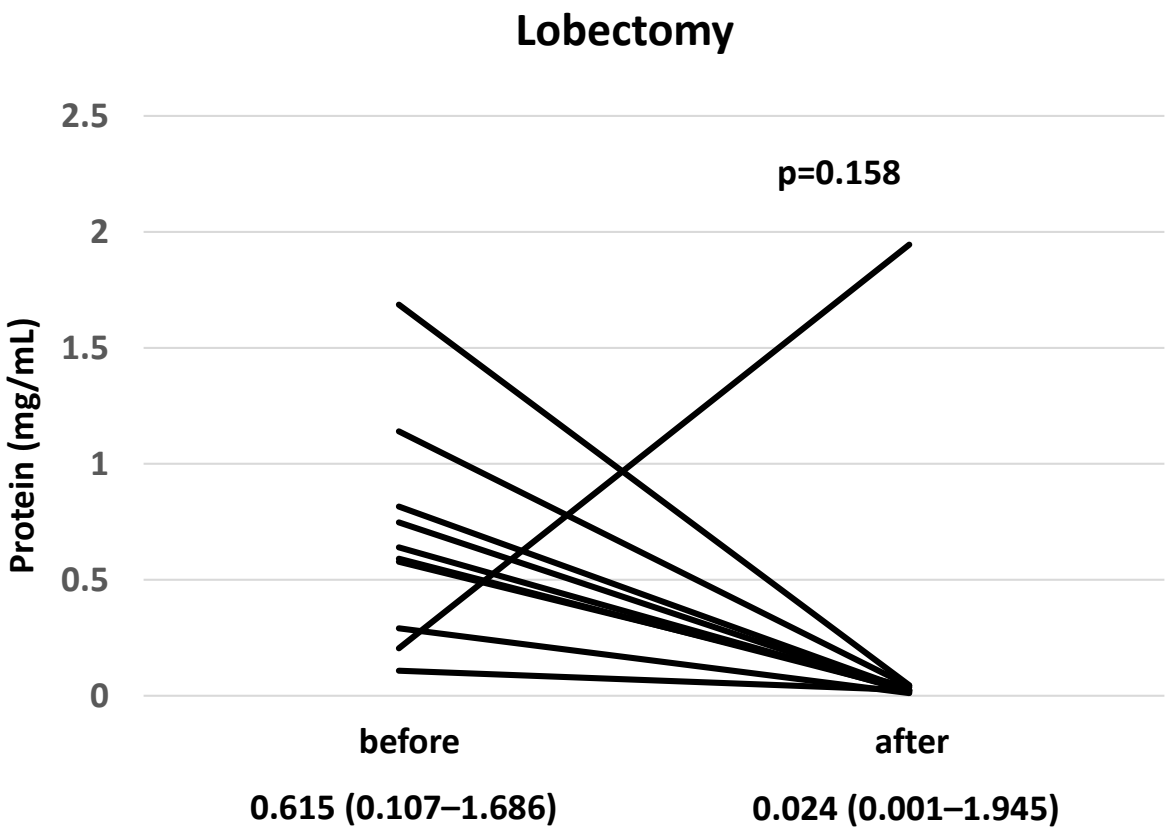


Figure 3b: Residual protein before and after manual washing



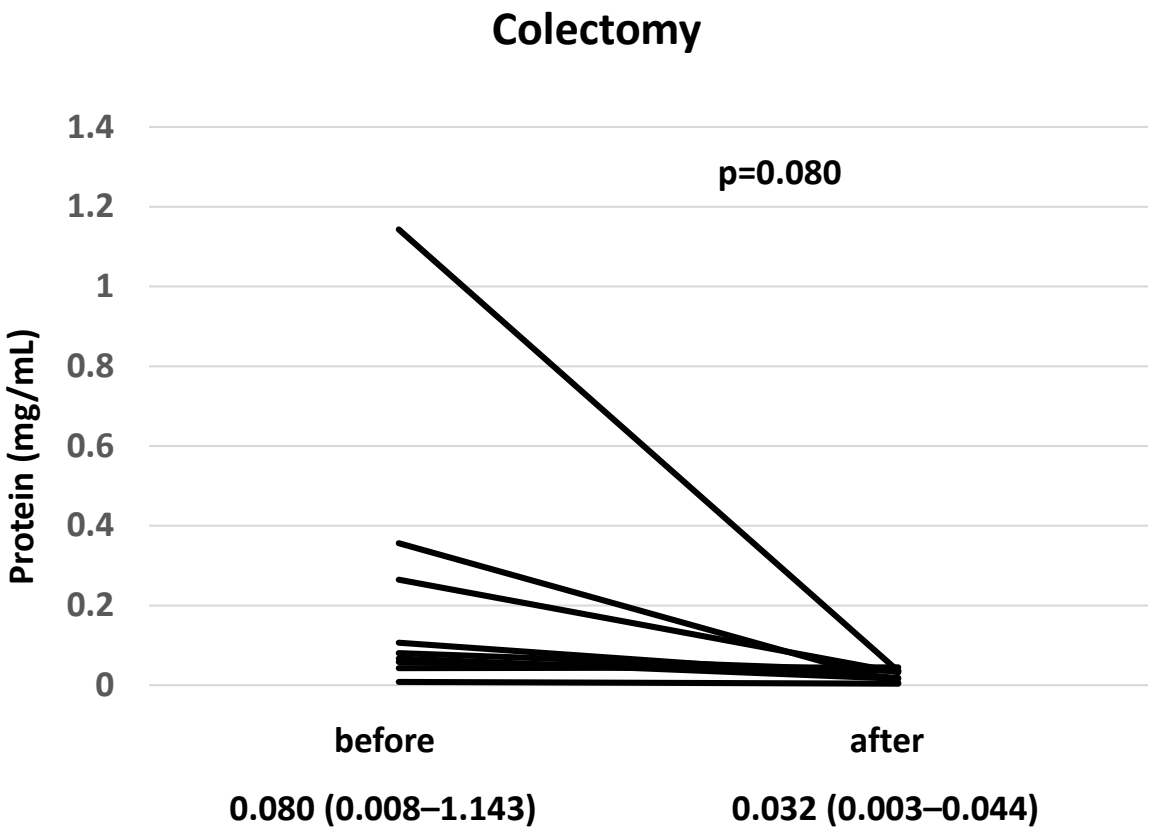
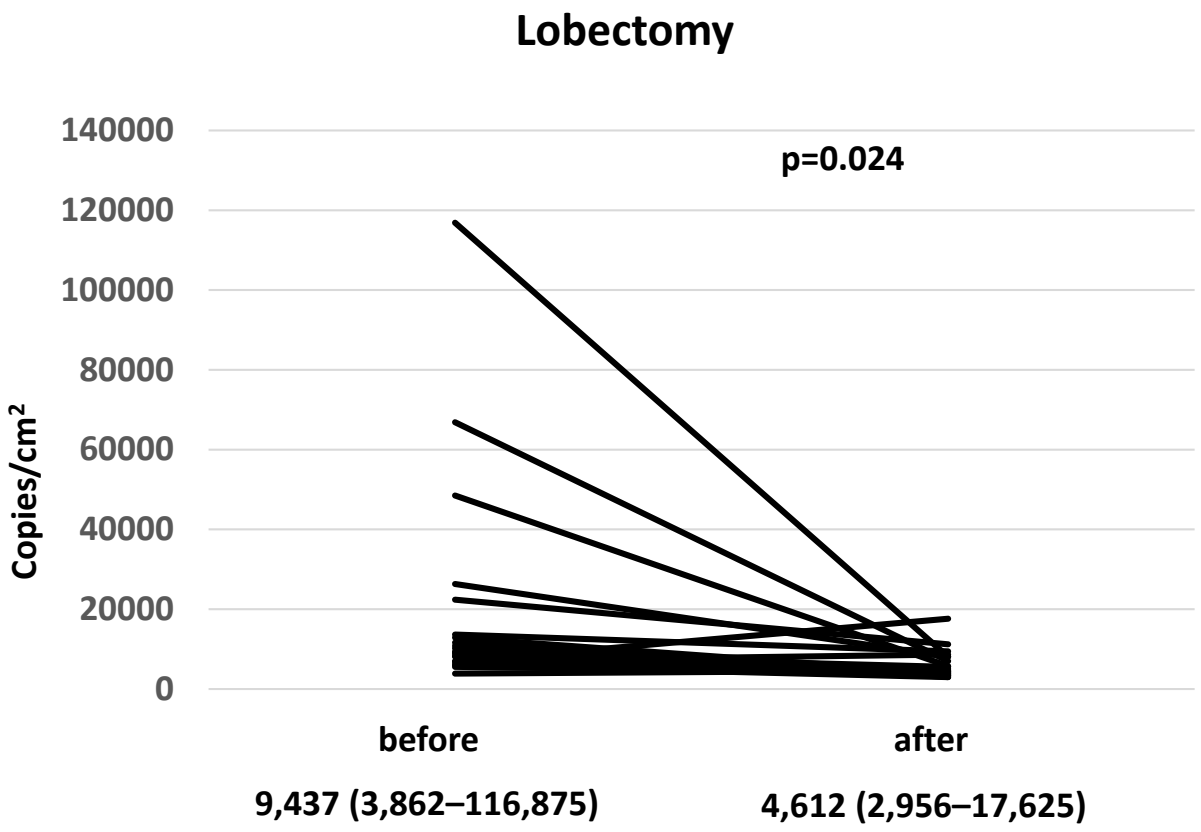


Figure 4a: Residual bacteria before and after Nano washing



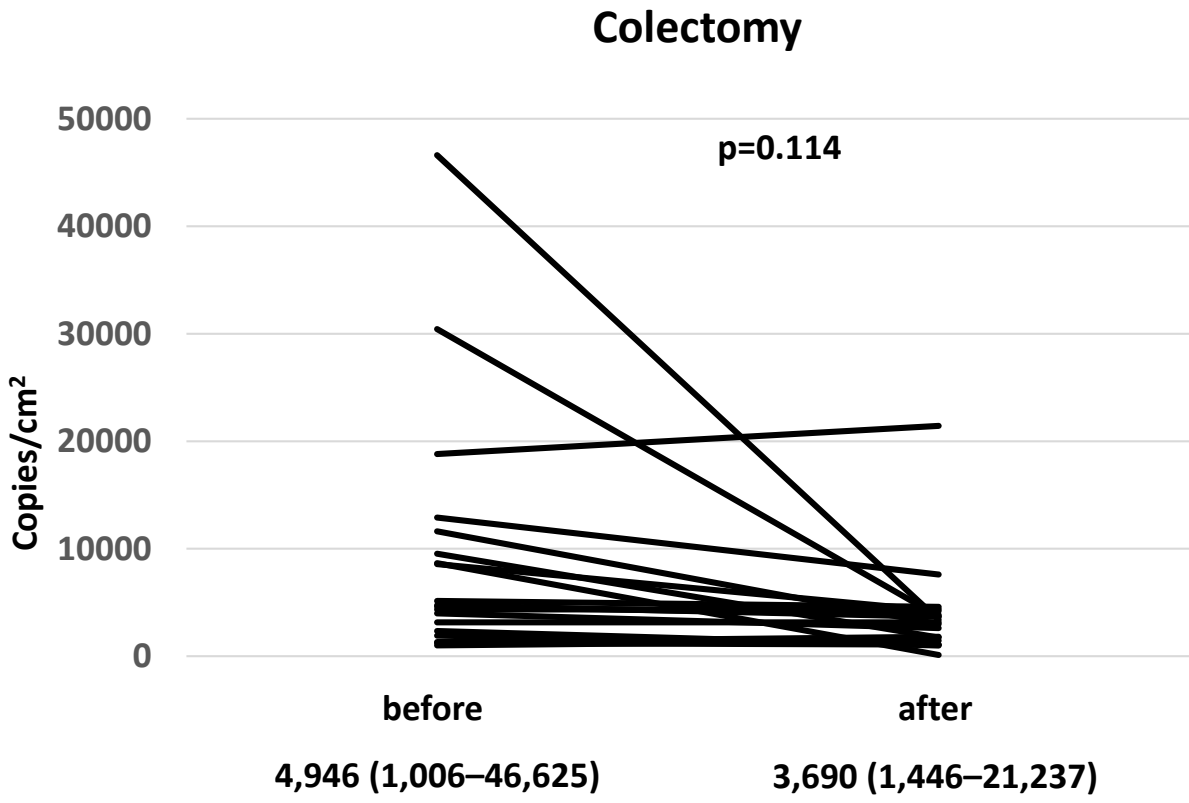


Figure 4b: Residual bacteria before and after manual washing