

**Quantitative monitoring  
of residual protein in  
cannulated medical  
devices:** Findings from a  
multicenter study

## 1. Introduction

The reprocessing of reusable medical instruments is a critical component of infection prevention in healthcare settings (1,2). Among these instruments, cannulated devices—those with narrow internal lumens such as orthopedic reamers, phacoemulsification handpieces, and endoscope channels—present unique challenges due to their complex geometry, which hinders effective cleaning and visual inspection (3,4). Despite adherence to manufacturers' instructions for use (IFU) and compliance with standard cleaning protocols, studies have consistently reported residual contamination—particularly of proteins and other organic materials—within these devices following both manual and automated reprocessing (5–8).

In recent years, there has been a paradigm shift toward the application of quality management systems (QMS) in medical device reprocessing, emphasizing validated cleaning instructions and routine monitoring to ensure consistency and effectiveness (1,9). The importance of adequate cleaning has been underscored by numerous outbreaks of device-associated infections linked to retained bioburden and biofilm, especially in cannulated and flexible instruments (6,7,10). Research has shown that the presence of residual proteins and biofilm inside lumens can impair the efficacy of disinfection and sterilization processes, including high-level disinfection (HLD) and steam sterilization, ultimately increasing the risk of patient cross-contamination (5,11).

While automated washer-disinfectors (WDs) have improved the reproducibility of cleaning processes for many devices, they may not be suitable or sufficient for complex or delicate instruments (3,12). Moreover, there is limited standardization in cleaning efficacy indicators for WDs, because cleaning performance is influenced by multiple interacting variables, including device design, lumen diameter and length, soil composition, water quality, detergent chemistry, mechanical action, and cycle parameters (3,12). While standards such as ISO 15883 define performance requirements and test soils for validation of washerdisinfectors (12), they do not fully replicate the wide variability of real clinical soils or the complexity of modern cannulated and flexible instruments.

In addition, many cannulated devices present geometric and material challenges that limit the effectiveness of automated washing alone (3,4). Narrow internal channels, dead spaces, valves, distal tips, and complex interfaces can impair fluid dynamics and reduce shear forces necessary for soil removal (3). Biofilm formation and proteinaceous

residues may persist if mechanical brushing or directed flushing is not performed prior to automated processing (6,11).

For these reasons, manual cleaning remains a necessary and critical step for many cannulated instruments (2,13). Manual pre-cleaning ensures direct mechanical action within internal channels, improves detergent contact with residual bioburden, and enhances overall cleaning efficacy before subsequent washer-disinfection or sterilization steps (2,13).

Current detection strategies include rapid biochemical assays (e.g., ninhydrin-based tests), total organic carbon (TOC) analysis, adenosine triphosphate (ATP) bioluminescence, and fluorescence-based imaging techniques (14–16). However, many of these methods lack sensitivity for detecting low-level residues or are not feasible for internal lumen inspection due to physical access limitations (14,16). Additionally, ATP tests cannot confirm sterility and may yield false reassurance if viable microorganisms persist despite low ATP readings (15).

Advancements in Automated Endoscope Reprocessors (AERs) have led to FDA-cleared cleaning cycles that replace manual cleaning steps, improving compliance and reducing variability due to human error. However, concerns remain regarding their effectiveness in cleaning complex areas such as the lever and lever recess of duodenoscopes, especially in light of recent outbreaks of multidrug-resistant organisms (17,18). As a result, the FDA requires revalidation of AER cleaning protocols and continues to recommend strict adherence to manual cleaning for certain devices (17).

A study by Alfa et al. demonstrated that biofilm may persist inside endoscope channels despite full reprocessing according to IFU under worst-case conditions, and that detergent type significantly influenced biofilm removal efficiency (6). Inadequate cleaning impaired the success of subsequent peracetic acid sterilization, reinforcing the critical role of proper cleaning in preventing biofilm persistence.

Guidelines such as AAMI ST91 and CDC recommendations support routine (preferably daily) monitoring of manual cleaning and emphasize ongoing staff training, audits, and gap analysis (2,13). Failure to monitor and correct deficiencies in reprocessing can result in serious consequences—including infection outbreaks and regulatory action (17,18).

### Objective

This work focuses on the evaluation of a protein

detection system specifically designed for application within cannulated medical devices. The goal is to address the technical advantages of monitoring with a sensitive, non-destructive solution capable of detecting clinically relevant levels of protein in lumens. By improving the ability to verify the effectiveness of cleaning protocols, this system aims to reduce the risk of device-related infections and support the implementation of robust QMS in medical reprocessing departments.

## 2. Materials and methods

Three leading healthcare institutions in the province of Buenos Aires, Argentina, participated in the study:

- A. Hospital Italiano.
- B. Fundación Favaloro (University Hospital).
- C. Hospital El Cruce Dr. Néstor Kirchner.

For the evaluation and quantification of residual protein inside cannulated instruments, the following tools were used (see Annex 1):

- Chemdye® Pro1 Endo
- Chemdye® SWE high-absorption swabs, 2.5 m in length and with diameters of 1.7 mm, 2.0 mm, 2.7 mm, and 3.0 mm
- Bionova® MiniPro reader

Annexes 3 – 5 present the list of materials used for the evaluation, along with their main characteristics (diameter and length).

### 2.1 Operating principle of the protein detection system Chemdye® Pro1 Endo

The Chemdye® Pro1 Endo Hygiene Monitoring System is designed to verify the cleanliness of cannulated instruments by detecting protein residues that may remain after the cleaning process. The system is used in conjunction with Chemdye® SWE high-absorption swabs, specially designed for cannulated instruments. These swabs must be inserted through the internal channel of the cannulae to sample for contaminants that may remain after the cleaning process.

The Chemdye® Pro1 Endo system provides quantitative results in the range of 1 to 50 µg of protein with a limit of detection (LOD) of 0.5 µg, when incubated in the Bionova® MiniPro reader.

Chemdye® Pro1 Endo operates based on the well-known BCA (Bicinchoninic Acid) assay. The Bicinchoninic Acid (BCA) assay is a colorimetric method used to determine the total protein concentration in a sample. It is based on the biochemical reduction of copper ions (Cu<sup>2+</sup> to

Cu<sup>+</sup>) by proteins in an alkaline medium, followed by the formation of a purple-colored complex between BCA and Cu<sup>+</sup> ions (see Annex 2).

How the BCA Reaction Works in Chemdye® Pro1 Endo

**1. Sample Collection:** A swab is used to collect any residual protein from the internal surface of a medical device, such as a cannulated instrument.

**2. Reagent Activation:** The swab is immersed in the Chemdye® Pro1 Endo reactive solution, which contains:

- Bicinchoninic acid (BCA).
- Copper(II) sulfate (CuSO<sub>4</sub>).
- An alkaline buffer.

**3. Protein-Copper Reduction Reaction:** Proteins in the sample (particularly peptide bonds) reduce Cu<sup>2+</sup> to Cu<sup>+</sup> under alkaline conditions. This reaction is similar to the Biuret reaction, but the BCA method is more sensitive.

**4. Color Development:** The Cu<sup>+</sup> ions form a purple-colored complex with BCA. The intensity of this purple color is directly proportional to the amount of protein present in the sample.

**5. Incubation Conditions:** Incubation is done at 60°C for 4 minutes.

**6. Quantification and Readout:** The absorbance of the resulting solution is measured spectrophotometrically at 562 nm. The intensity of absorbance correlates to a specific amount of protein based on a calibration curve developed using known concentrations of bovine serum albumin (BSA), a standard protein used in biochemical assays.

### 2.2 Reprocessing procedures and post-cleaning verification testing

The study was conducted under real processing conditions, without artificial load standardization, in order to evaluate the system's performance under representative use scenarios. The methodology applied is described below.

#### 2.2.1 Manual cleaning

##### Preparation

1. The appropriate personal protective equipment (PPE) was donned according to institutional policy.
2. The device manufacturer's instructions for use (IFU) were reviewed to confirm compatibility with selected detergents and cleaning parameters.
3. A fresh enzymatic detergent solution was prepared according to the manufacturer's specified dilution, temperature, and contact time.

## Cleaning

4. The cannulated medical device was fully immersed in the prepared detergent solution to prevent aerosolization and ensure complete surface contact.
5. The internal lumen(s) were filled with the detergent solution using an appropriately sized syringe or flushing adapter to ensure full internal contact.
6. The device was allowed to soak for the contact time specified by the detergent manufacturer.
7. External surfaces were cleaned using a soft, non-abrasive brush.
8. Each lumen was brushed with a correctly sized, single-use or properly reprocessed lumen brush, ensuring contact with the entire internal surface.
9. The lumen was flushed repeatedly with detergent solution until no visible soil was observed.
10. Particular attention was given to joints, distal tips, connectors, and difficult-to-access areas.

## Rinsing

11. The device was removed from the detergent solution and immediately rinsed with abundant treated water.
12. External surfaces were thoroughly rinsed to remove all detergent residues.
13. Each lumen was flushed with clean water using a syringe or irrigation device until no visible foam or detergent traces remained.
14. According to the institutional protocol, a final rinse with critical or treated water was performed.

## Drying

15. Excess external water was removed using a lint-free cloth.
16. Each lumen was purged with medical-grade compressed air until no visible moisture was expelled.
17. The device, including internal lumens, was visually inspected using a lighted magnification system and, when applicable, a borescope inspection device to confirm the absence of visible residual moisture.
18. The device was allowed to air dry in a clean environment if necessary, in accordance with the IFU.

### 2.2.2 Automated ultrasonic cleaning

#### Preparation and set-up

1. Appropriate PPE was donned according to institutional policy.
2. The device IFU and the ultrasonic washer manufacturer's instructions were reviewed to confirm that ultrasonic cleaning was permitted for

the specific device and to verify required parameters.

3. The ultrasonic tank was inspected to ensure it was clean, filled to the correct level, and operated at the specified temperature range.
4. A compatible cleaning solution (enzymatic detergent) was prepared and added at the manufacturer-recommended concentration.
5. The ultrasonic unit was degassed for the time recommended by the manufacturer to optimize cavitation performance.

#### Pre-cleaning (before ultrasonics)

6. Gross soil was removed under the waterline to reduce aerosolization.
7. The device was disassembled (if applicable) per IFU, and removable components were separated.
8. Each lumen was pre-flushed with the prepared detergent solution to wet internal surfaces and remove loose debris.
9. If required by the IFU, each lumen was brushed with an appropriately sized lumen brush prior to ultrasonic cleaning.

#### Loading the ultrasonic washer

10. Instruments were placed in an ultrasonic basket or tray to prevent contact with the tank bottom.
11. Cannulated devices were positioned to avoid overlap and to allow solution contact with all surfaces.
12. Lumens were oriented to allow filling and venting (to minimize trapped air).
13. When required by the IFU or local practice, lumen adapters/tubing were connected so that cleaning solution circulated through internal channels during the ultrasonic cycle.
14. Hinged instruments were kept in the open position to expose hidden surfaces.

#### Ultrasonic cleaning cycle

15. The basket was lowered into the tank, ensuring full immersion of all components.
16. The ultrasonic cycle was run for the time, temperature, and detergent concentration specified by the device IFU and/or ultrasonic washer manufacturer.
17. During the cycle, the solution was allowed to cavitate without interruption, and instruments were not stacked or repositioned mid-cycle.
18. After completion, the basket was raised and allowed to drain back into the tank.

#### Post-ultrasonic rinsing

19. Instruments were removed and immediately rinsed with abundant water to remove loosened soil and detergent residues.

**20.** Each lumen was flushed thoroughly with clean water using a syringe or irrigation device until no foam or residue was observed.

**21.** A final rinse with treated/critical water was performed.

### Drying

**22.** External surfaces were dried using a lint-free cloth.

**23.** Each lumen was purged with medical-grade compressed air until no visible moisture was expelled.

**24.** The device was allowed to air dry in a clean area if needed, in accordance with the IFU.

### 2.3 Hygiene monitoring with Chemdye® PRO1 ENDO and Chemdye® SWE Swabs

For instruments cleaned manually or automatically, the following procedure was performed after completion of the cleaning process:

**1.** The device was placed on a clean surface and the swab corresponding to the instrument's internal channel diameter was selected according to the suggested table in product's IFUs.

**2.** The Chemdye® PRO1 ENDO device was removed from refrigeration at least 30 minutes prior to use or allowed to equilibrate to a temperature of 20–25 °C.

**3.** The Chemdye® SWE swab was removed, ensuring that the absorbent tip was not touched.

**4.** The swab was moistened by immersing it for 5 seconds in the moisturizer

The swab was not pressed against the walls of the tube nor shaken.

**5.** The swab was inserted and passed through the internal channel of the endoscope or cannulated instrument in a single way (one-way direction).

**6.** The swab was cut approximately 20 cm from the absorbent tip using scissors, avoiding contact with the absorbent portion.

**7.** The Chemdye® PRO1 ENDO device was activated by pressing the plunger until it reached the stop position.

Once activated, the following steps were performed immediately.

**8.** The device was vigorously shaken downward until the solution turned green and reached the reference volume indicated in the Chemdye® PRO1 ENDO IFU.

If the device was not shaken adequately to achieve complete emptying of the internal compartment or if large liquid droplets were observed, the results were considered invalid.

**9.** The device handle was removed.

**10.** The swab was inserted into the device until it was fully immersed in the solution contained in

the reading tube.

**11.** The swab was gently maintained in the solution for 10 seconds.

**12.** The swab was slid upward and removed from the device.

**13.** The handle was reinserted.

The device was incubated immediately for final reading.

Solutions that turned a dark purple color after agitation were not incubated, as this could indicate excessive protein levels and invalidate quantification.

**14.** The Chemdye® PRO1 ENDO device was incubated at  $60 \pm 2$  °C for 4 minutes in the Bionova® MiniPro incubator

The device was positioned correctly in the incubator and allowed to rest at the bottom without movement.

Incubation was performed with the swab removed from the solution (reading cone) and immediately after activation.

**15.** The distal end of the swab channel was pulled to remove the remaining swab segment.

**16.** The medical device was reprocessed according to institutional protocol.

## 3. Results analysis

### 3.1 Overview of tested instruments and institutions

A total of 19 instrument types across three participating institutions — Hospital Italiano, Hospital El Cruce, and Hospital Favaloro — were evaluated using the Chemdye® Pro1 Endo protein detection system. The instruments represented a diverse range of cannulated surgical and endoscopic devices, including ureteroscopes, cystoscopes, Bettocchi hysteroscopes, various suction tubes (De Bakey, Frazier, Poole), aspiration cannulas (coronary, hepatic, neuro), laparoscopic accessories, cholangiography forceps, resectoscopes, and pump cannulas. Their internal channel diameters ranged from 1 mm (Poole thoracic cardio cannula) to approximately 10 mm (cystoscope), and their channel lengths ranged from as short as 12 cm (neuro aspiration cannula) to over 120 cm in flexible endoscopic instruments. Both manual and automated (ultrasonic) cleaning processes were represented, enabling a comparative assessment of cleaning efficacy across different methodologies and instrument categories.

### 3.2 Protein residue detection across instrument categories

Across all instruments with available quantitative

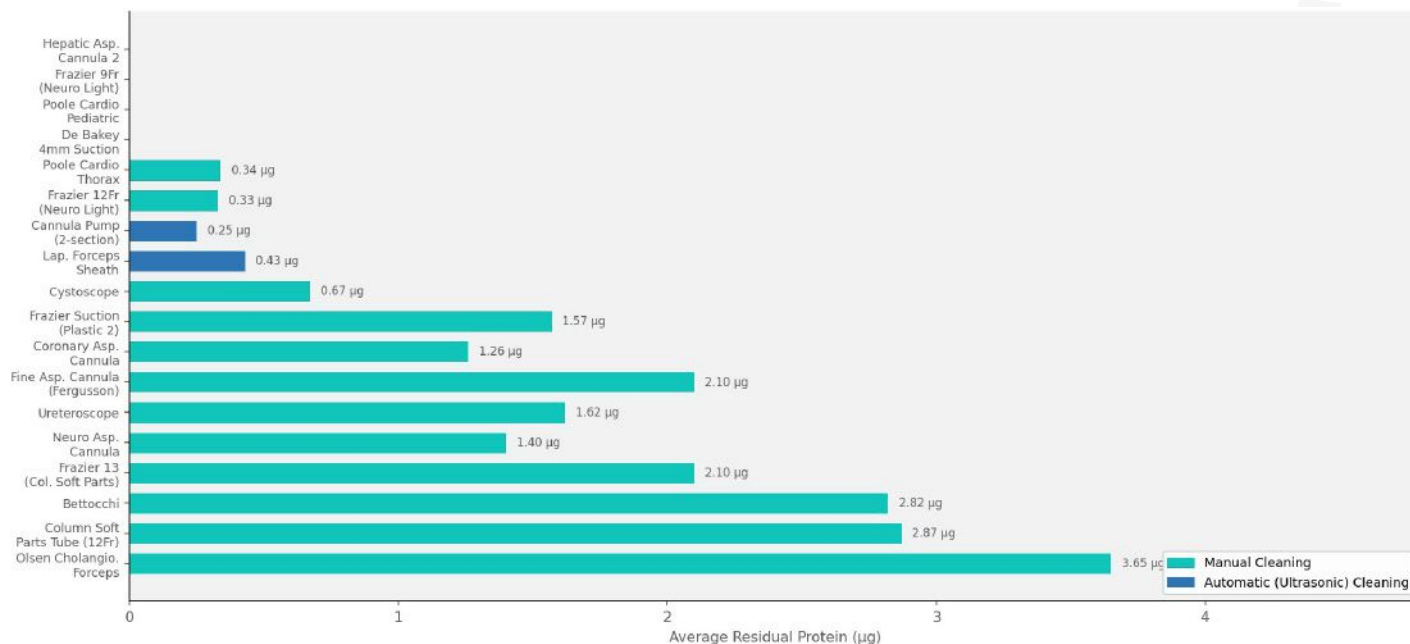
results, the Chemdye® Pro1 Endo system successfully detected protein residues in a significant proportion of samples despite the instruments having undergone standard institutional cleaning protocols. Results ranged from 0 µg (undetectable) to 6.3 µg of protein, with several instruments demonstrating values well above the system’s limit of detection (LOD) of 0.5 µg. Annexes 3 – 5 present the average µg detected per instrument.

Among the most clinically relevant findings were those observed in instruments with narrow or complex geometries. The Olsen Cholangiography Forceps (2 mm internal diameter, 32 cm channel length), tested at Hospital Favaloro using manual cleaning, exhibited the highest average protein residue of approximately 3.65 µg across replicate measurements, with a maximum reading of 6.3 µg. This result is particularly noteworthy given the forceps’ narrow lumen, its length, and its complex articulated design, all of which limit the mechanical effectiveness of manual brushing and flushing. Similarly, the Frazier 13 Suction Tube (4.3

mm diameter, 18 cm length) from Hospital Cruce showed 6.3 µg in one replicate; upon re-washing and retesting, it still registered 4.9 µg, and only after a second rewashing with ultrasonic assistance did it reach 0 µg. This sequence demonstrates not only the persistence of proteinaceous contamination after conventional cleaning, but also the critical value of the Pro1 Endo system as a real-time feedback tool that guides corrective action.

The Neuro Aspiration Cannula (2 mm diameter, 12 cm length), tested at Hospital Favaloro using manual cleaning, consistently yielded protein levels between 1.0 and 1.7 µg across all three replicates, indicating a reproducible residual contamination pattern for this instrument type. Likewise, the Fine Aspiration Cannula (Fergusson/Aesculap GF373R, 3 mm diameter, 18–25 cm length) at Hospital Cruce showed variable results (0.0, 2.9, and 3.4 µg) across replicates, suggesting inter-procedural variability in the extent of organic soil deposited and in the thoroughness of manual cleaning between uses.

Figure 1. Average residual protein by instrument type after standard cleaning



### 3.3 Manual vs. automated (ultrasonic) cleaning performance

A comparative analysis between manual and automated ultrasonic cleaning reveals a meaningful difference in average protein residues. Instruments processed by manual cleaning

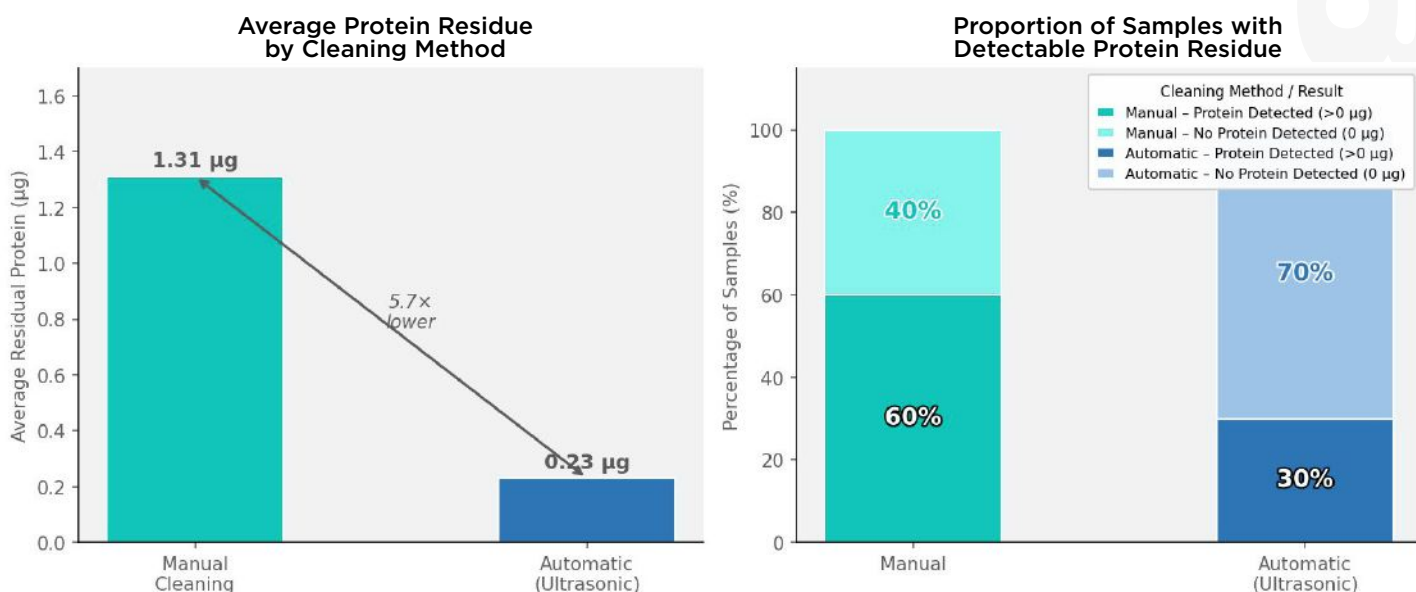
showed an average residual protein level of approximately 1.31 µg, whereas instruments processed via automated ultrasonic cleaning showed a substantially lower average of approximately 0.23 µg. This difference is consistent with the established principle that automated cleaning with directed mechanical

energy (cavitation) achieves more reproducible and thorough decontamination of internal channel surfaces compared to manual techniques, which are inherently subject to operator variability.

However, it is important to note that automated cleaning did not guarantee zero contamination. The Cannula Pump (two-section, Hospital Favaloro) processed by ultrasonic cleaning showed 1  $\mu\text{g}$  in one of four replicates, and the

Laparoscopic Forceps Sheath at Hospital Favaloro also showed 1.3  $\mu\text{g}$  in one replicate. These findings reinforce the importance of post-cleaning verification regardless of the cleaning modality employed. Instruments with particularly complex geometries — such as multi-section or multi-lumen designs — may present areas of incomplete cavitation access, making protein detection testing an essential complementary step even after automated processing.

Figure 2. Manual vs. automatic (ultrasonic) cleaning performance



### 3.4 Influence of instrument geometry on residual protein levels

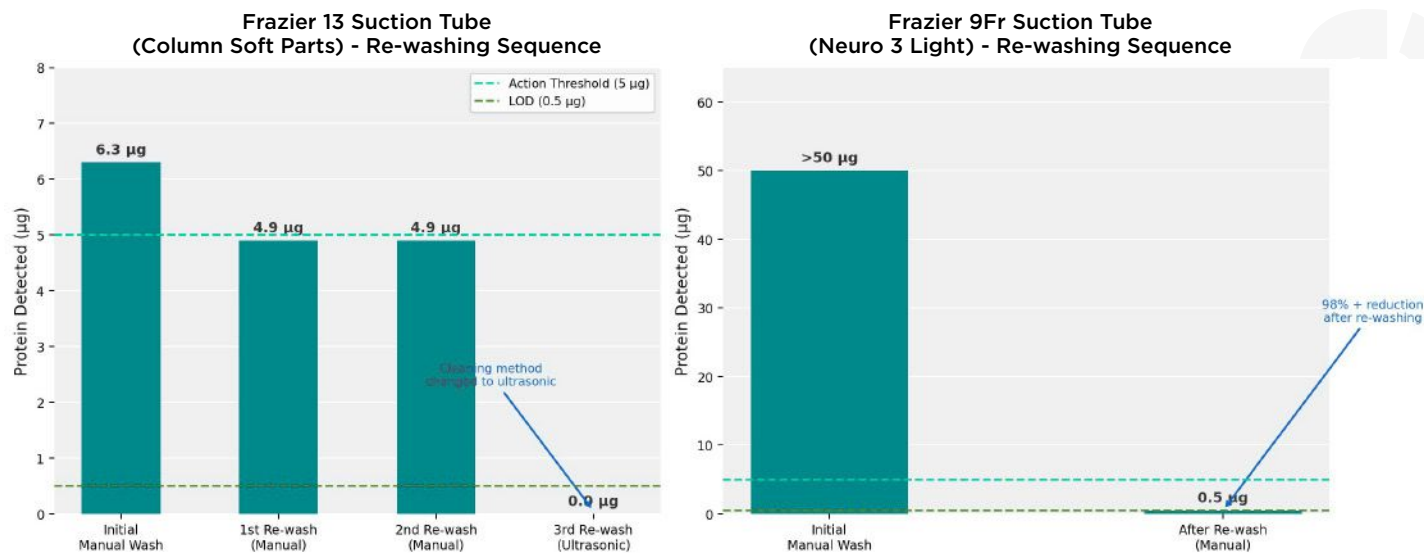
When results are normalized to internal surface area (expressed as  $\mu\text{g}$  of protein per  $\text{cm}^2$ ), instruments with very narrow channels display a disproportionately high protein load per unit area. The Olsen Cholangiography Forceps (2 mm diameter, 32 cm length) showed an average surface density of approximately  $0.48 \mu\text{g}/\text{cm}^2$ , compared to  $0.028 \mu\text{g}/\text{cm}^2$  for the Ureteroscope and  $0.010 \mu\text{g}/\text{cm}^2$  for the Cystoscope. This pattern indicates that narrow, long-channel instruments accumulate proportionally more protein per unit of internal surface, likely due to reduced fluid shear forces, limited mechanical access, and the tendency for soil to adhere to fine lumen walls under turbulent-to-laminar flow transitions. These instruments therefore represent the highest risk category in the context of inadequate cleaning.

### 3.5 Actionable detection and real-time protocol adjustment

A particularly noteworthy aspect of the study was the documented use of Pro1 Endo results as an immediate corrective feedback mechanism. In at least two cases — the Frazier 9 Fr Suction Tube (Neuro 3 Light) and the Frazier 13 Suction Tube (Column Soft Parts) at Hospital Cruce — instruments with initial results above  $5 \mu\text{g}$  were returned to the cleaning process and retested. In the case of the Frazier 9 Fr tube, the second wash reduced the result to below  $1 \mu\text{g}$ . For the Frazier 13 tube, even after two standard re-washings the result remained at  $4.9 \mu\text{g}$ , and only after switching to ultrasonic cleaning did the result drop to  $0 \mu\text{g}$ . This real-world application demonstrates the critical importance of routine monitoring with a quantitative protein detection system: without post-cleaning verification, these instruments

could have proceeded to sterilization carrying residual protein loads that could compromise disinfection efficacy and patient safety.

Figure 3. Pro1 Endo as a real-time corrective tool: re-washing case studies at Hospital El Cruce



### 3.6 Instruments with consistently negative results

Several instrument types yielded consistently zero or near-zero protein results across all replicates. These included the De Bakey Suction Tube (4 mm diameter, 20 cm length) and the Poole Aspiration Cannula (Cardio Pediatric, 2 mm diameter, 22 mm length) at Hospital Cruce, both processed by manual cleaning. The Hepatic Aspiration Cannula 2 (4 mm diameter, 25 cm length) at Hospital Favalaro, processed by ultrasonic cleaning, also showed 0 µg across all three replicates. These results suggest that, for certain instrument geometries — particularly those with relatively larger diameters, shorter channels, and simpler internal architecture — standard cleaning protocols are sufficient to remove protein to below the system’s detection threshold. Nonetheless, the high variability observed even within the same instrument type across replicates (as seen in the ureteroscope and Frazier tube series) underscores that favorable geometry alone does not guarantee consistently adequate cleaning.

## 4. Conclusions

This multi-institutional case study demonstrates that residual protein contamination in reusable

cannulated medical instruments is a real and measurable risk under routine clinical reprocessing conditions. The following conclusions can be drawn from the data collected across Hospital Italiano, Hospital El Cruce, and Hospital Favalaro:

- 1. Residual protein is present in a significant proportion of instruments after standard cleaning.** Multiple instrument types, across all three hospitals and under both manual and automated cleaning protocols, yielded detectable levels of protein residue. This finding confirms that current standard cleaning procedures, while necessary, are not universally sufficient to eliminate all organic contamination from the internal channels of cannulated instruments. The persistence of protein residues — even at levels well below visual detection thresholds — constitutes a latent patient safety risk, as these residues can shield microorganisms from disinfectants and sterilants.
- 2. Automated ultrasonic cleaning outperforms manual cleaning in reducing internal protein load.** Instruments processed via automated ultrasonic cleaning showed a notably lower average protein residue (approximately 0.23 µg) compared to those cleaned manually (approximately 1.31 µg). This approximately 5.7-fold difference highlights the advantage of

directed cavitation energy in accessing difficult internal channel surfaces. Nevertheless, ultrasonic cleaning did not guarantee zero residual protein in all cases, and its effectiveness remained dependent on instrument geometry and proper cycle parameters. These findings support the adoption of automated cleaning as a preferred method wherever instrument compatibility permits, while reinforcing the need for ongoing monitoring to verify cleaning outcomes.

### **3. Narrow-lumen, long-channel instruments present the greatest cleaning challenge.**

Surface-normalized analysis confirms that instruments with internal diameters of 2 mm or less and longer channel lengths accumulate disproportionately higher protein loads per unit of internal surface area. The Olsen Cholangiography Forceps, the ureteroscope, and the neuro aspiration cannula all represent high-risk categories for residual contamination. These instrument types should be considered priority candidates for enhanced cleaning protocols, dedicated monitoring programs, and — where feasible — transition to single-use alternatives.

### **4. The Chemdye® Pro1 Endo system provides actionable, quantitative results that directly improve reprocessing outcomes.**

The most compelling finding of this study is the direct impact of Pro1 Endo testing on clinical decision-making. In documented cases, instruments that failed the protein test were returned to cleaning, retested, and in at least one instance required a change in cleaning method (from manual to ultrasonic) before achieving a passing result. This demonstrates that Pro1 Endo functions not merely as a passive quality indicator, but as an active component of the cleaning verification loop — one that prevents contaminated instruments from proceeding to sterilization and subsequent patient use. This capability is not achievable with conventional visual inspection, ATP bioluminescence, or other currently used methods.

### **5. Pro1 Endo is the only system capable of quantitatively sampling protein from deep within internal instrument channels.**

The Chemdye® SWE swabs, available in four diameters (1.7 mm, 2.0 mm, 2.7 mm, and 3.0 mm) and at 2.5 m in length, enable direct sampling of the entire internal channel length in a single pass. This design advantage is unique among commercially available systems and addresses a fundamental limitation of prior methods: the inability to access and quantify contamination within long, narrow, or complex lumens. The compatibility of the swab range with the full spectrum of cannulated instruments evaluated in this study — from sub-2

mm channels in ophthalmic instruments to wider channels in urological and endoscopic devices — confirms the system's broad clinical applicability.

### **6. Integration of Pro1 Endo into routine reprocessing workflows supports quality management system (QMS) compliance.**

The quantitative, documented results provided by the Bionova® MiniPro reader create an objective, traceable record of cleaning verification for each instrument tested. This data trail supports compliance with ISO 13485, AAMI ST79, and other regulatory and accreditation frameworks that require evidence-based monitoring of reprocessing quality. In an environment of increasing regulatory scrutiny — particularly regarding complex devices such as duodenoscopes and ureteroscopes — the ability to demonstrate systematic, instrument-level cleaning verification represents a significant institutional advantage in terms of patient safety, liability management, and accreditation readiness.

In summary, this case study provides clear, real-world evidence that residual protein contamination in cannulated medical instruments is not a theoretical concern but a confirmed, measurable phenomenon occurring under standard institutional conditions. The Chemdye® Pro1 Endo system demonstrated superior sensitivity, broad instrument compatibility, and direct clinical utility as a real-time corrective tool. Its adoption as a routine component of the reprocessing verification workflow is strongly supported by the data gathered across these three institutions, and its use is expected to meaningfully reduce the risk of device-associated infections in patients undergoing procedures with cannulated instruments.

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## Annex 1- Terragene's protein detection system



Chemdye® Pro1 Endo



Bionova® MiniPro auto-reader

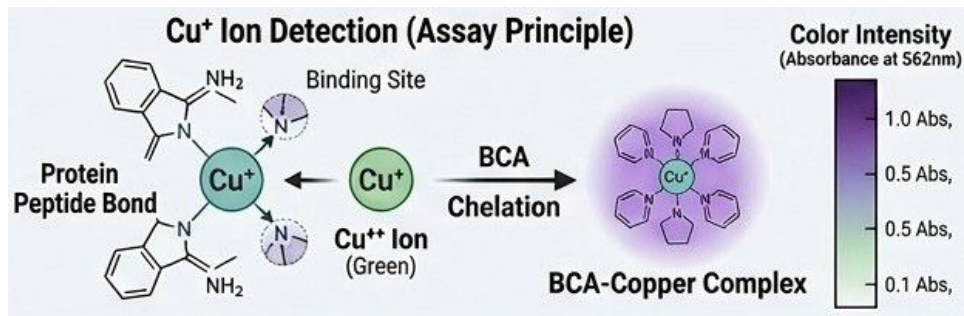


Chemdye® SWE: protein test swab



Chemdye® Moisturizer

## Annex 2- Chemdye® Pro1 Endo chemical reaction (BCA Assay)



## Annex 3- Instruments with internal diameter between 2 - <3 mm: characteristics and results

Instrument	Internal Channel (mm) Diameter	Channel Length (cm)	Cleaning Process	SWE Swab Used	Result (µg protein)
Ureteroscope	2 - <3	40 - 45	Manual	1.7 mm	1.6
Ureteroscope	2 - <3	40 - 45	Ultrasonic	1.7 mm	3.4
Neuro Aspiration Cannula	2 - <3	12	Manual	1.7 mm	1.5
Neuro Aspiration Cannula	2 - <3	12	Manual	1.7 mm	1.0
NeuroAspiration Cannula	2 - <3	12	Manual	1.7 mm	1.7
Ozen Cholangiography Forceps	2 - <3	32	Manual	1.7 mm	6.3
Ozen Cholangiography Forceps	2 - <3	33	Manual	1.7 mm	1.0

## Annex 4- Instruments with internal diameter between 3 - <4 mm: characteristics and results

Instrument	Internal Channel (mm) Diameter	Channel Length (cm)	Cleaning Process	SWE Swab Used	Result (µg protein)
Fine Aspiration Cannula (Fergusson) Aesculap Gf373r (Hepatic Implant 1)	3 - <4	25	Manual	3.0 mm	0.0
Fine Aspiration Cannula (Fergusson) Aesculap Gf373r (Hepatic Implant 1)	3 - <4	18	Manual	3.0 mm	2.9
Fine Aspiration Cannula (Fergusson) Aesculap Gf373r (Hepatic Implant 1)	3 - <4	18	Manual	3.0 mm	3.4
Suction Tube Frazier 9 Fr (Neuro Liviano 1)	3 - <4	18	Manual	2.0 mm	0.0

## Annex 5- Instruments with internal diameter between 4 - <5 mm: characteristics and results

Instrument	Internal Channel (mm) Diameter	Channel Length (cm)	Cleaning Process	SWE Swab Used	Result (µg protein)
Bettocchi	4 - <5	30	Manual	3.0 mm	1.5
Bettocchi	4 - <5	32	Manual	3.0 mm	1.4
Bettocchi	4 - <5	35	Manual	3.0 mm	6.2
Bettocchi	4 - <5	39	Manual	3.0 mm	3.0
Frazier Suction Tube (Plastic 2)	4 - <5	19	Manual	3.0 mm	1.1
Frazier Suction Tube (Plastic 2)	4 - <5	19	Manual	3.0 mm	1.7

Instrument	Internal Channel (mm) Diameter	Channel Length (cm)	Cleaning Process	SWE Swab Used	Result (µg protein)
Frazier Suction Tube (Plastic 2)	4 - <5	19	Manual	3.0 mm	1.9
Frazier 12 Fr Suction Tube (Neuro Liviano 1)	4 - <5	18	Manual	3.0 mm	0.0
Frazier 12 Fr Suction Tube (Neuro Liviano 1)	4 - <5	18	Manual	3.0 mm	1.0
4 mm Suction Tube (12 Fr) (Column 2 Soft Parts)	4 - <5	16	Manual	3.0 mm	3.0
4 mm Suction Tube (12 Fr) (Column 2 Soft Parts)	4 - <5	16	Manual	3.0 mm	3.4
4 mm Suction Tube (12 Fr) (Column 2 Soft Parts)	4 - <5	16	Manual	3.0 mm	2.2
Frazier 13 Suction Tube (Column Soft Parts)	4 - <5	18	Manual	3.0 mm	0.0
Frazier 13 Suction Tube (Column Soft Parts)	4 - <5	18	Manual	3.0 mm	6.3
Coronary Aspiration Cannula	4 - <5	30	Manual	3.0 mm	1.2
Coronary Aspiration Cannula	4 - <5	30	Manual	3.0 mm	0.0
Coronary Aspiration Cannula	4 - <5	30	Manual	3.0 mm	2.6
Laparoscopic Forceps Sheath	4 - <5	32	Ultrasonic	3.0 mm	0.0
Laparoscopic Forceps Sheath	4 - <5	32	Ultrasonic	3.0 mm	1.3