Terragene®

Chemdye® PRO1 MICRO
Hygiene monitoring system

Cleaning verification implementation protocol

Absolute protein monitoring
A process improvement approach for Central Sterile Services Departments

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1. Introduction

The transition in Central Sterile Supply Departments (CSSDs) from a paradigm of assuming cleanliness to one of documenting evidence marks a critical evolution in patient safety, as sterilization failure is increasingly recognized to originate not in the sterilizer, but in the washing phase. While often used interchangeably in clinical practice, washing and disinfection are sequential but biologically distinct processes; washing is a physical action designed to remove organic and inorganic matter, whereas disinfection is a biochemical action intended to inactivate microorganisms. The success of the latter is entirely dependent on the former, as retained organic soil provides protective niches for bacteria, supplies nutrients for microbial growth, and neutralizes the antimicrobial activity of chemical agents. This is particularly critical in the management of biofilms—biological communities encased in an extracellular matrix that are up to 1,000 times more resistant to disinfectants than planktonic cells—which represent one of the most significant threats to effective reprocessing.

The scientific justification for using protein as the primary marker for cleaning verification, rather than Adenosine Triphosphate (ATP), lies in its structural stability and clinical relevance. ATP is a labile, unstable molecule that auto-degrades and only indicates the presence of living cells; consequently, it does not represent a significant challenge to the washing process. In contrast, proteins are the main constituents of all cells and tissues, characterized by high adherence to stainless steel and extreme complexity, making them the most challenging residues to remove. Furthermore, protein testing is the only direct method capable of assessing the risk of prions—misfolded pathogenic proteins that contain no DNA or ATP and exhibit extreme resistance to heat, steam, and standard chemical disinfectants.

As medical device designs become increasingly complex, visual inspection has become a necessary but insufficient tool for safety assurance. These methods cannot detect molecular-level contamination or early-stage biofilms that can lead to outbreaks of multidrug-resistant microbes.

Global regulatory frameworks, including HTM 01-01:2016, ISO 15883:2021, ANSI/AAMI ST98:2022, and ANSI/AAMI ST91:2021, have addressed this quality assurance gap by requiring or recommending objective proof of cleaning through quantitative protein assays, establishing critical action thresholds. By integrating routine quantitative verification into standard unit practice through gap analysis, standardized frequency, and continuous traceability, CSSDs can transform simple cleaning verification into a comprehensive risk management strategy, ensuring that every device is chemically and biologically safe for patient use.

This protocol describes the implementation of absolute protein monitoring using the Terragene® Chemdye® PRO1 MICRO Hygiene Monitoring System. Results are expressed in micrograms (µg) of total residual protein recovered from a complete instrument side. This approach enables CSSDs to establish a measurement baseline, detect process failures, and drive progressive reduction of protein burden over time through targeted corrective actions.

2. Scope and applicability

This protocol applies to any healthcare facility operating a CSSD that processes reusable rigid surgical and medical instruments. It is intended for:

- Hospital sterile processing units (CSSD, TSSU, UPSS)
- Dental clinics with instrument reprocessing workflows
- Distributors and product specialists supporting end-user implementation

NOTE: The PRO1 MICRO system provides both qualitative (color) and quantitative protein results. Quantitative readout requires a Bionova® auto-reader incubator (MiniPro, IC10/20FR, or IC10/20FRLCD). Semi-quantitative visual interpretation is possible using the IC10/20 incubator without an auto-reader.

3. The PRO1 MICRO System

The Terragene® Chemdye® PRO1 MICRO is based on the BCA (Bicinchoninic Acid) microassay, a colorimetric method that detects the reduction of Cu(II) to Cu(I) mediated by protein peptide bonds under alkaline conditions at 60 °C. The resulting purple color is proportional to the protein concentration and is read automatically by Bionova® auto-readers or interpreted visually against a reference color guide. The system is calibrated against Bovine Serum Albumin (BSA), the reference protein recommended by HTM 01-01 and ISO 15883-5.

Parameter	Value	Reference
Limit of Detection (LOD)	0.5 µg BSA	Technical Report Rev. 7
Limit of Quantification (LOQ)	1.0 µg BSA	Technical Report Rev. 7
Quantification range	1.0 - 50.0 µg BSA	IFU Rev. 15
Incubation temperature	60 ± 2 °C	IFU Rev. 15
Reading time (MiniPro)	4 minutes	IFU Rev. 15
Reading time (IC10/20FR / IC10/20FRLCD)	7 minutes	IFU Rev. 15
Swab recovery (fresh protein)	>85% from stainless steel	Technical Report Rev. 7

3.1 Regulatory context

HTM 01-01:2016, published by the UK Department of Health, establishes that the residual protein on any instrument side after cleaning should not exceed 5 µg. Results are measured directly from the instrument surface per side, without normalization to surface area. The guidance further recommends that facilities monitor cleaning performance over time using statistical control charts (IQAS framework: Individual Value Chart, Moving Range Chart, X-bar, and R Charts), enabling detection of process drift and long-term performance trends.

ISO 15883-5:2021 establishes protein measurement per unit surface area (µg/cm^2) for washer-disinfector validation, with an Alert level of ≥3 µg/cm^2 and an Action level of ≥6.4 µg/cm^2. This protocol applies the per-side measurement logic of HTM 01-01 to support process improvement programs in facilities that do not yet apply surface-area normalization.

4. Instrument selection

Instrument selection is the most critical decision in any cleaning verification program. The instruments monitored must represent the greatest challenge to the cleaning process. Selecting instruments that are easy to clean produces results that look acceptable but provide no real assurance for the instruments that matter most.

Instruments should be selected based on the following criteria:
1. Structural complexity: instruments with joints, box locks, ratchets, serrated jaws, or deep crevices are the highest priority. These areas consistently carry the highest residual protein loads after washing.
2. High-risk use: instruments that contact blood or are used in procedures with high protein-load exposure, such as orthopaedic or cardiovascular surgery.
3. Known problematic items: instruments associated with previous reprocessing incidents, visible soil after washing, or complaint events.
4. Category representation: include at least one item from each major rigid instrument group handled in the CSSD.

RATIONALE: Testing simple flat surfaces produces results that look acceptable but do not reflect the real protein burden on the instruments that matter. The goal is to characterize and control the worst case. If the most challenging instruments pass, simpler instruments will too.

5. Instrument side selection and sampling consistency

Each instrument side presents a different geometry and a different level of challenge to the cleaning process. For this monitoring program, one side of each selected instrument is designated as the reference side for all sampling events. This side is selected once, documented before the program begins, and sampled consistently at every subsequent test event.

The reference side must be the one presenting the highest-risk features: the side that exposes the box lock and hinge, the ratchet mechanism, the jaw serrations, or the complex working geometry. Swabbing covers the full accessible surface of that side, including crevices and joints, applying firm pressure throughout. The following table provides guidance on side selection by instrument type.

Instrument type	Reference side to swab
Scissors, forceps, needle holders	The side exposing the box lock and hinge —swab the full inner face, pressing firmly into the joint crevice and rotating the swab to access the hinge mechanism
Hemostatic clamps, ratchet instruments	The side presenting the ratchet teeth and jaw serrations —swab the full length of the jaw and the entire ratchet mechanism
Retractors and broad instruments	The concave or inner face of the blade — swab the full accessible surface of that side
Dental instruments	The working-end side with serrations or complex geometry — swab all accessible surfaces of that face

IMPORTANT: Document which side has been selected for each instrument before the program begins. Photograph it or provide a written description that any operator can reproduce independently. Consistent side selection across all operators and all test events is the foundation of result comparability in this program.

6. Swabbing procedure

6.1 Materials

- Terragene® Chemdye® PRO1 MICRO protein pen (one per instrument)
- Bionova® auto-reader incubator (MiniPro, IC10/20FR, or IC10/20FRLCD) for quantitative results
- Bionova® IC10/20 incubator for semi-quantitative visual interpretation
- Disposable gloves and surgical mask
- Monitoring log or Bionova® Cloud plataform

6.2 Pre-sampling conditions

- Allow the PRO1 MICRO pen to equilibrate to ambient temperature (15–25 °C) before use. Cold pens produce underestimated results.
- Sample instruments immediately after the cleaning/washing cycle, before any drying, packaging, or sterilization step.
- Do not allow instruments to dry before sampling. Long drying times significantly reduce protein recovery by the swab. If a temporary hold is unavoidable, keep instruments moist.
- Wear gloves. Do not touch the swab tip or the interior of the device.

6.3 Step-by-step procedure

1. Remove the PRO1 MICRO pen from its pouch.
2. Extract the swab from the device. Apply 2 drops of the provided moisturizer to the swab tip.
3. Swab the full reference side of the instrument: move in a zigzag pattern in one direction, then repeat perpendicularly. Press firmly and rotate the swab throughout to maximize sample recovery, paying particular attention to crevices, joints, and serrated areas.
4. Replace the swab into the device.
5. Activate by pressing the pen firmly downward.
6. Shake vigorously downward until the solution turns green and reaches the reference volume mark.
7. Shake vigorously downward for 15 seconds with the swab submerged.
8. Slide the swab upward without removing it from the device.
9. Shake vigorously downward again and collect solution to the reference volume mark.
10. Confirm the swab tip is positioned above the readout cone.
11. Incubate immediately in the Bionova® incubator at 60 ± 2 °C.

CRITICAL: Incubate immediately after activation, do not delay. If using visual (semi-quantitative) reading, interpret the result within 5 minutes of removing the pen from the incubator. Results read beyond this window are invalid.

7. Interpreting results

The auto-reader reports a result in µg of BSA-equivalent protein recovered from the instrument side. HTM 01-01 establishes a limit of 5 µg per instrument side as the maximum acceptable residual protein level. The table below provides a complete interpretation framework, from the quantification limit to gross contamination, aligned with that reference.

Auto-reader result	Classification	Guidance
< 1 µg	**Excellent**	Below quantification limit. Protein not detectable at the method's sensitivity threshold. Process performing at its best.
1 –5 µg	**Acceptable — HTM 01-01 limit met**	Within the HTM 01-01 limit of 5µg per side. Continue routine monitoring and watch for upward trends.
5 –10 µg	**Elevated — Investigate**	Exceeds the HTM 01-01 limit. Review swabbing technique, instrument condition, and WD cycle parameters. Reprocess and retest after corrective action.
> 10 µg	**High — Act**	Significant contamination. Do not proceed to sterilization. Full investigation and corrective action required before reprocessing.
> 50 µg (reported as >50)	**Gross contamination**	Instrument failed cleaning. Do not sterilize. Full manual clean required. Investigate WD cycle.

NOTE: HTM 01-01 establishes 5 µg per instrument side as the action limit. The program's value lies in demonstrating a measurable, sustained reduction from baseline toward and below that limit over successive monitoring cycles.

8. Monitoring frequency

HTM 01-01 recommends a minimum of 50 tests per quarter for routine monitoring. The testing frequency should also be adapted to the maturity of the program and the current state of the cleaning process. The following phased schedule is recommended:

Phase	Frequency	Objective
Baseline Weeks 1–2	Daily, all selected instruments	Generate the first objective picture of the cleaning process. Establish mean and range per instrument side. Identify worst-performing instruments.
Improvement Weeks 3–8	2–3 times per week, same instruments	Track the response to corrective actions. Confirm that interventions produce a measurable reduction from baseline.
Routine Week 9+	Weekly minimum (HTM 01-01 recommends 50 tests/quarter)	Track the response to corrective actions. Confirm that interventions produce a measurable reduction from baseline.
After WD change or maintenance	Immediately, full instrument panel	Revalidation following any cycle change, detergent change, WD servicing, or equipment replacement.

9. Implementation roadmap

The following roadmap describes how to build a cleaning verification program from the ground up using this protocol. The phases are sequential but the boundaries are flexible — the progression to the next phase depends on achieving stable, consistent results at the current one, not on calendar time alone.

Phase	Activities	Expected outcome
Baseline Weeks 1–2	Daily sampling of all selected instruments on their reference side. Record all µg values. Calculate mean and range per instrument. Identify which instruments and sides carry the highest protein burden.	First objective characterization of the cleaning process. Identifies worst-case instruments and informs internal alert and action levels.
Analysis Weeks 3–4	Identify the instruments and sides with results above the HTM 01-01 limit (5 µg). Investigate probable causes using the corrective action framework in Section 10.	Root causes identified for elevated results. Corrective actions selected and documented.
Intervention Weeks 5–8	Implement targeted corrective actions. Continue sampling 2–3 times per week on the same instruments. Compare all new results to baseline values.	Measurable, documented reduction in protein levels from baseline. Corrective action effectiveness confirmed.
Routine Week 9+	Weekly sampling at minimum. Review trend data monthly. Use Bionova® Cloud control charts to monitor stability. Trigger investigation on any result above the HTM 01-01 limit.	Sustained process control. Documented trend data. Regulatory-ready evidence of monitoring program.

GOAL: The objective of this monitoring program is a progressive, documented reduction of residual protein per instrument side toward and below the HTM 01-01 limit of 5 µg. Any sustained reduction from baseline is a documented quality improvement, even before the limit is reached. The data generated constitutes evidence that the cleaning process is under active measurement, review, and control.

10. Corrective action guidance

The following framework should be applied whenever a result exceeds 5 µg per instrument side (HTM 01-01 limit), or whenever a consistent upward trend is detected in control chart data, even if individual values remain below the limit.

Possible cause	Investigation	Corrective action
Insufficient mechanical action	Review WD cycle parameters: temperature, time, water pressure, spray arm function and coverage	Adjust cycle. Add manual pre-cleaning step for complex instruments.
Inadequate detergent	Verify dosing pump calibration and detergent compatibility with the soil type and instrument material	Recalibrate dosing. Consider enzymatic pre-treatment for heavily soiled instruments.
Protein dried before cleaning	Review time interval from instrument use to WD loading	Enforce maximum holding time (<30 min). Use moist holding until cleaning begins.
Swabbing technique error	Observe operator performing the procedure. Verify full side coverage and moistener application.	Retrain operator. Retest with standardized technique on same instrument.

Possible cause	Investigation	Corrective action
Instrument surface damage	Inspect surface under magnification: pitting, corrosion, damaged serrations	Remove from service. Repair or replace instrument.
Washer-disinfect or malfunction	Check WD maintenance log, filter status, spray arm integrity, and cycle documentation	Service WD. Full IQ/OQ/PQ requalification before returning to routine use.

11. Documentation and statistical control

All cleaning verification activities must be documented. Records constitute regulatory evidence, support trend analysis, and are required during inspections and audits.

11.1 Minimum record requirements per test event

- Date and time of sampling
- Instrument identification (type, code, set number, or unique ID)
- Reference side sampled (and reference to the zone documentation or photograph)
- Auto-reader result (µg)
- Classification (Acceptable / Elevated / High)
- Corrective action taken, if applicable
- Operator name

11.2 Statistical control charts

HTM 01-01 recommends monitoring cleaning performance over time using the IQAS framework.

The Bionova® Cloud platform generates the following control charts automatically from stored results:

- Individual Value Chart (X Chart): plots each result individually to detect shifts and outliers
- Moving Range Chart (MR Chart): monitors process variability between successive results
- X-bar and R Charts: used when multiple instruments of the same type are sampled per event

Control charts should be reviewed at a minimum monthly, and findings presented in CSSD quality management reviews. A shift above the control limit or a consistent upward trend across consecutive results is a signal to investigate, even if individual values remain below the 5 µg limit.

> **TIP:** Once the program has generated at least 20–25 data points per instrument, define internal alert and action limits from the baseline data (e.g., the 75th and 95th percentile of baseline values). These facility-defined limits allow earlier detection of process drift than the HTM 01-01 limit alone.

12. Getting started — Implementation checklist

Step	Action	Responsible
1	Select 3–5 most challenging instruments from each major category in the CSSD.	CSSD Supervisor
2	Define and photograph the reference side for each instrument. Store documentation in the monitoring log.	CSSD Supervisor
3	Set up Bionova® incubator. Confirm operating temperature at 60 ± 2 °C.	CSSD Technician
4	Train all operators on the swabbing procedure (Section 6). Emphasize full-side coverage and consistent zone selection.	Product Specialist
5	Begin daily baseline sampling — 2 weeks minimum.	CSSD Operators
6	Calculate mean and range per instrument side. Identify instruments exceeding 5 µg.	CSSD Supervisor
7	Define internal alert and action levels from baseline data. Document in SOP.	Quality / Supervisor

Step	Action	Responsible
8	Implement corrective actions for elevated instruments (Section 10).	CSSD Supervisor
9	Continue sampling 2–3×/week. Confirm measurable reduction from baseline.	CSSD Operators
10	Transition to weekly routine monitoring. Generate control charts in Bionova® Cloud.	CSSD Supervisor
11	Review trends and control charts monthly in quality meetings.	Quality Manager

References

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