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	PullThru Comparison Study	A	00124	DH	14/10/2021

PullThru Comparison Study

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1. Aim/Purpose of the Study

To compare and evaluate the cleaning efficacy of the PullThru wipes against alternative cleaning devices (single-headed brush and double-headed brush) through using VeriTest Blue protein detection reagent and ProReveal protein quantification technology.

This study aimed to determine the most effective cleaning apparatus for the cleaning of internal channels of endoscopic instruments.

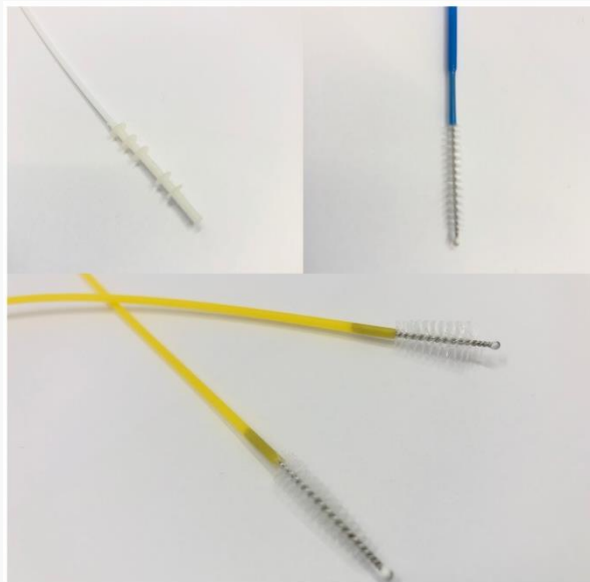


Figure 1. Top Left - PullThru, Top Right - Single Headed Brush, Bottom - Double Headed Brush

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2. Methods & Equipment

2.1 Equipment List

- 10 PTFE tubes (50cm length 3mm internal diameter)
- PullThru Wipes
- ProReveal Channel Safe (600mm length, 3.5mm diameter) + Cone Accessory
- VeriTest Blue (Batch 9:1)
- ProReveal Machine
- Aseptium Ultrasonic Washer
- Single Headed Brush (3mm)
- Double Headed Brush (3-5mm / 4-6mm)
- ProReveal Reagent Spray (Batch 11)
- Hydrogen Peroxide (10%)
- Sterile Water
- Neutral Enzymatic Detergent

2.2 Preparation and inoculation of PTFE test devices

To emulate the internal channels of flexible endoscopes, PTFE tubing (3mm internal wall diameter) was cut into 10 equal sections, each 50cm in length.

The tubes were labelled to ensure they were not re-used more times than other tube sections to minimise the possibility of residue build up after repeated soiling.

The PTFE tubes were cleaned following disinfection with H₂O₂ (10%) and then flushed with sterile water before drying.

Aseptium's VeriTest soil was prepared and diluted to an approximate concentration of 5mg/mL of protein.

The PTFE tubes were then inoculated with 1mL of the 5mg/mL solution over a waste container. It should be noted that some of the 1mL soil dilution was eluted out of the end of the tubing, however the purpose of inoculation at a concentration as high as this was not to have tubes inoculated with 5mg of protein, but to ensure that there was simply and excess of protein within the channel in order to represent a heavily contaminated endoscope channel.

Inoculated tubes were left to dry for a minimum of 1 hour at room temperature before testing.



Figure 2. 1mL Syringe of diluted test soil and inoculated PTFE tubing

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2. Methods (continued)

2.3 Cleaning methods

Aseptium's Ultrasonic washer was used as a basin in which to submerge the inoculated PTFE tubing during the cleaning process.

The chamber was filled to the high level with cold water which was tested in the ProReveal to ensure the background contamination of the water would not impact the results of the study.

Once full, a single inoculate PTFE tube was submerged in the chamber, and the cleaning device of interest was used according to their IFU (adapted for PTFE test apparatus).

Once the cleaning action was performed, the tube was removed from the water and placed on a clean surface prior to analysis via either VeriTest Blue or ProReveal protein quantification.

The washer chamber was then drained to remove contaminated water. The chamber was then subjected to two individual 1 minute rinse cycles to ensure no cross-contamination took place.

This process was repeated for each test.

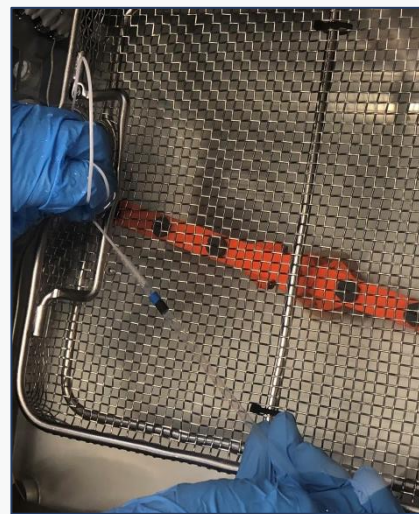


Figure 3. PullThru device being used while submerged in washer chamber

2.4 Testing Methods

2.4.1 VeriTest Blue

Aseptium's VeriTest Blue is a specially designed reagent that changes colour from pale green to dark blue depending on the concentration of protein in the sample.

After removing the PTFE tubing from the chamber, ProReveal Channel Safe was used to pull any residual protein solution out of the tubing. Any protein collected by the Channel Safe would be present on the "head" of the device.

Upon exit of the tube, the head was cut using scissors into an open VeriTest Blue vial which was then closed and vortexed to allow the reaction to take place.

The colour intensity was assessed using the accompanying colour chart and photographs were taken to record the result.

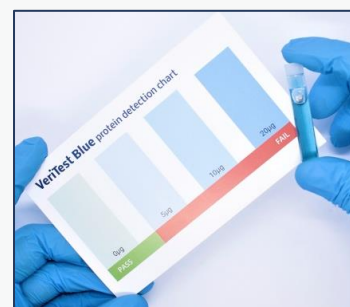


Figure 4. VeriTest Blue Colour Chart.

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2. Methods (continued)

2.4 Testing Methods

2.4.2 ProReveal Protein Quantification

ProReveal technology uses a special reagent that binds to proteins, causing them to fluoresce under UV excitation. This method allows for accurate quantification of protein residue on surfaces.

Using the ProReveal Channel safe in combination with the Cone Accessory, cleaned tubes were wiped with the leading end passing through the cone. Any liquid leaving the tube was collected on the inner surface of the cone, and as the head exited the tube, the Channel Safe was cut.

The cone, containing the head portion of the Channel Safe and any liquid that exited the tubing, was then unfolded and placed within the ProReveal drawer.

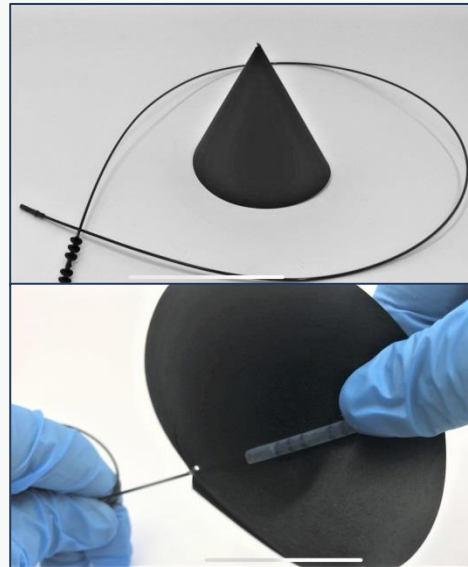


Figure 5. ProReveal Channel Safe & Cone Accessory.


The sample was then sprayed with the ProReveal reagent and tested within the machine. Results were recorded in micrograms.

2.5 Reprocessing of PTFE tubes

PTFE tubes were cleaned prior to re-use, with their number of uses tracked to ensure each tube were used an equal number of times.

After analysis of protein residue, tubes were flushed with 20% concentration Neutral Enzymatic detergent followed by rinsing with H₂O₂ and were subjected to a final rinse using sterile water.

Tubes were then re-inoculated according to methods described in 2.2 for further testing.

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3. VeriTest Blue Results

PullThru



Single-Head Brush



Double-Head Brush



Dirty Control



Clean Control



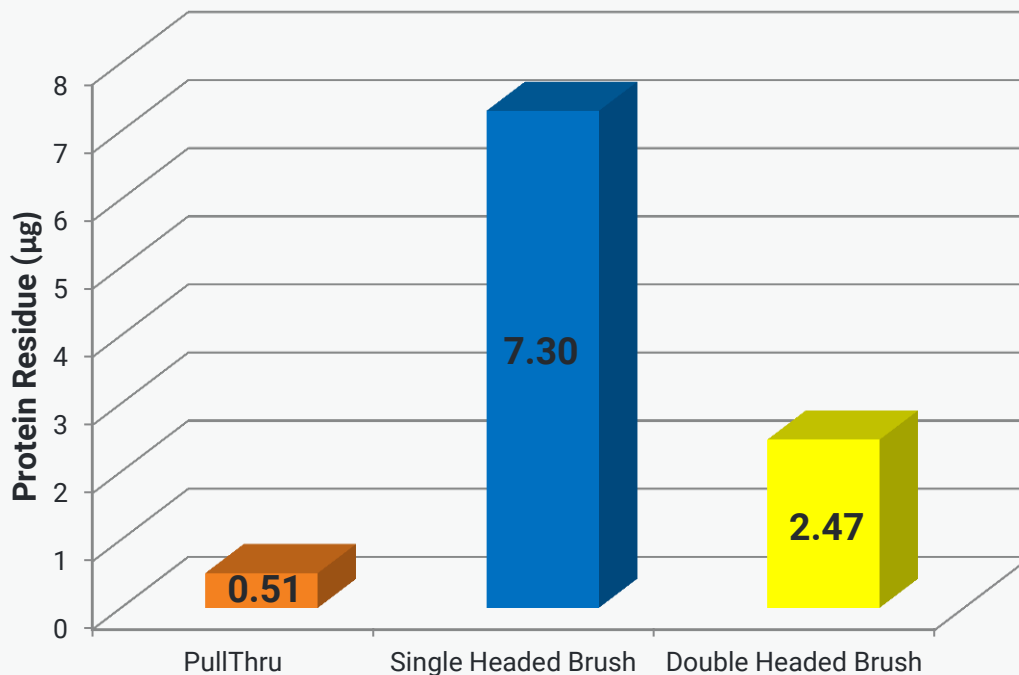
Replicate	PullThru	Single Headed Brush	Double Headed Brush	Control (Clean)	Control (Dirty)
1	0	5-10	5-10	0	>20
2	0	5-10	5-10	0	>20
3	0	10-20	5-10	0	>20
4	0	10-20	5-10	0	>20
5	0	10-20	5-10	0	>20

4. ProReveal Quantification (μg)

The results below were gathered from the ProReveal Protein Quantification. 5 replicates were performed for each condition to improve accuracy and to reduce errors.

Replicate	PullThru	Single Headed Brush	Double Headed Brush	Control (Clean)	Control (Dirty)
1	0.458	4.857	1.834	0.029	8.883
2	0	0.16	1.346	0	78.342
3	2.096	3.578	5.794	0	7.555
4	0	17.474	0.225	0	99.009
5	0	10.423	3.151	0.669	207.982
Average	0.51	7.3	2.47	0.14	80.35
S.D	0.81	6.07	1.91	0.26	73.55

ProReveal Analysis



The PullThru device is seen here in the above graph as the most effective at removing contamination from internal channels.

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4. ProReveal Quantification Continued

The images below are examples of the results gained via ProReveal analysis. The image on the left shows the recorded result after cleaning with a single headed brush. The image on the right shows the recorded protein quantity after using a PullThru device. The full set of ProReveal results can be found in the attached appendix.

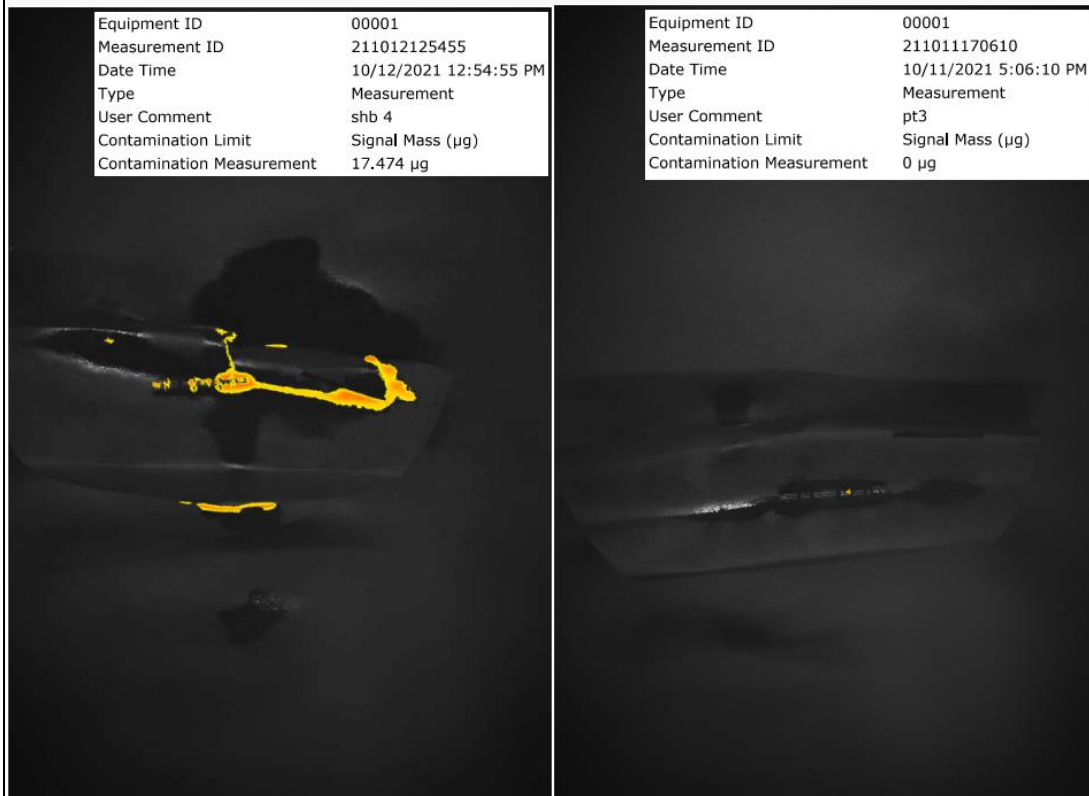


Figure 6. Single Headed Brush ProReveal Result #4.

Figure 7. PullThru ProReveal Result #3.

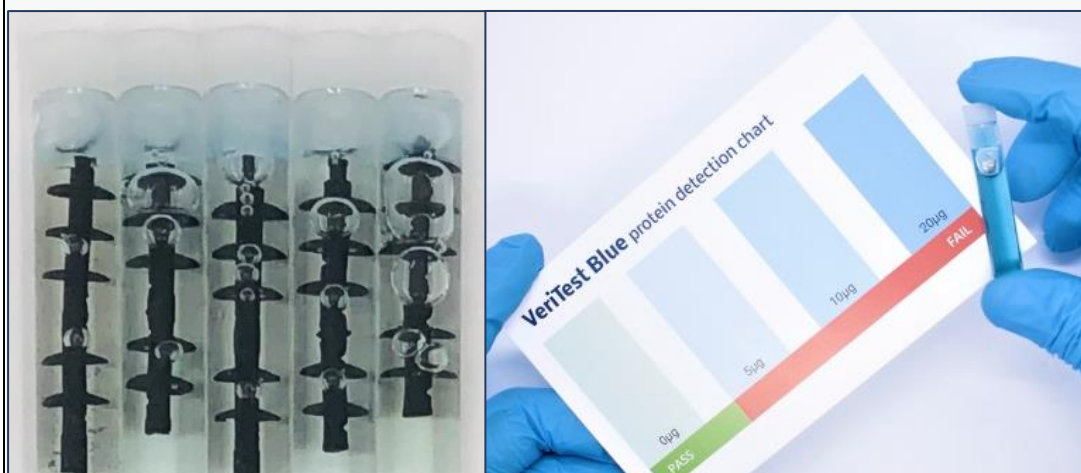
(shb = Single Headed Brush, pt = PullThru, dhb = Double Headed Brush, cc = Clean Control, dc = Dirty Control).

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5. Discussion

5.1 VeriTest Blue Results Discussion

Results gathered from VeriTest Blue analysis demonstrate visually how each cleaning device differs from one another. It is clear from these tests that the PullThru is the most effective of the chosen devices at removing protein residue from internal channels. The image below is again the VeriTest Blue with the Channel Safe tip after cleaning with PullThru devices.



VeriTest Blue protein detection chart

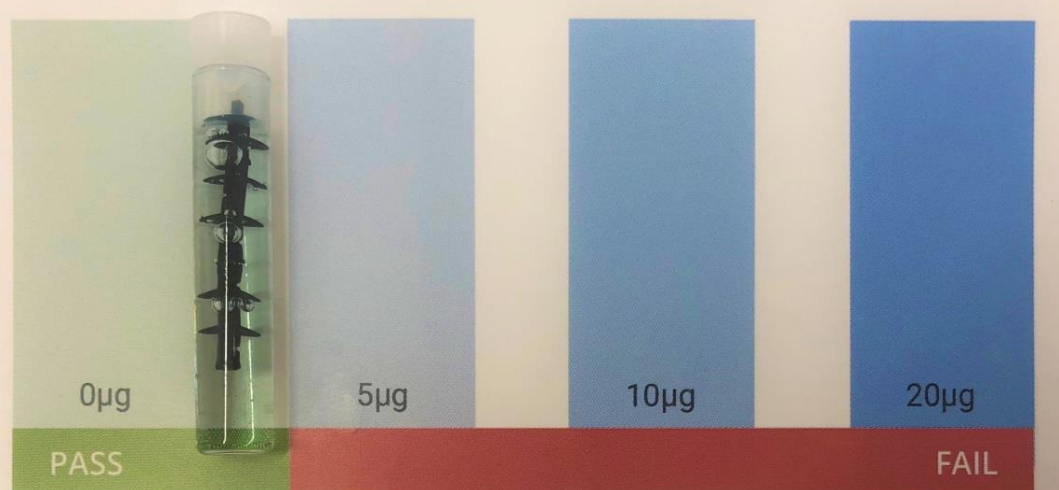


Figure 8. PullThru Analysis with VeriTest Blue. Vial against colour chart.

VeriTest Blue is a fast and effective way at visually demonstrating cleaning effectiveness. However, it does not provide precise quantitative data. For this reason the ProReveal was used as an additional method of measurement.

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5. Discussion (continued)

5.2 ProReveal Results Discussion

The ProReveal and its accessory, the ProReveal Channel Safe, allowed for an accurate measurement of protein residue from the internal channels of the tubing. Results gathered using the ProReveal gave precise values of residual protein at the microgram level.

The cone accessory ensured that any liquid that was present within the tubes was eluted and collected on the surface of the cone, which was then subjected to analysis along with the “head” of the Channel Safe device.

Through a pairwise comparison, the absolute differences of the means were calculated and are represented in the table below. Excluding the comparisons that include control groups, the greatest difference found was between the PullThru and the Single Headed Brush (6.79µg difference).

Group 1	Group 2	Absolute Difference between means (µg)
PullThru	Single Headed Brush	6.7876
PullThru	Double Headed Brush	1.9592
PullThru	Clean Control	0.3712
PullThru	Dirty Control	79.8414
Single Headed Brush	Double Headed Brush	4.8284
Clean Control	Dirty Control	80.2126
Single Headed Brush	Clean Control	7.1588
Single Headed Brush	Dirty Control	73.0538
Double Headed Brush	Clean Control	2.3304
Double Headed Brush	Dirty Control	77.8822

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5. Discussion (continued)

The figure below presents the percentage difference between the means of the three cleaning devices. The PullThru was found to be the most effective method of cleaning, removing 93% more protein than the Single Headed Brush and 79% more than the Double Headed Brush. The Single Headed Brush was determined as the least effective method of protein removal from internal channels.

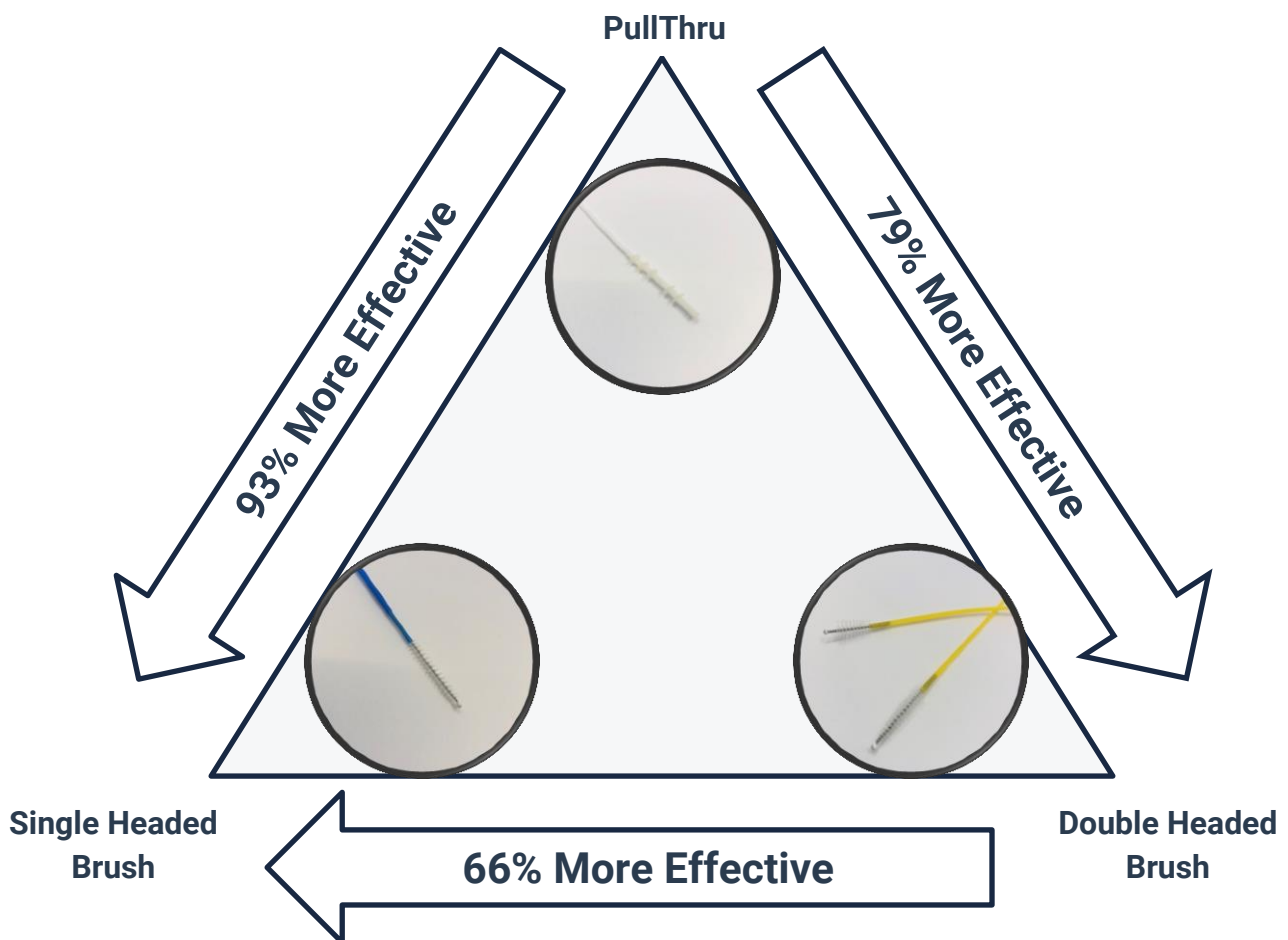



Figure 9. Comparison of Cleaning Efficacy between groups.

6. Summary

This study aimed to determine the most effective cleaning device among three different types of brush. The results indicate that the PullThru device is the most effective of the three at removing contamination from the internal channels of endoscopes, demonstrating a 93% greater efficacy than the Single Headed Brush and a 79% greater efficacy than the Double Headed Brush.

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