




## Article

# An Alternative Approach to Validate the Cleaning Efficiency of a Skin Cleansing Wipe

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**Abstract:** A key functionality for cleansing wipes is their efficiency in removing dirt and microbial contamination from the skin to safe or non-detectable levels, traditionally determined using the gravimetric method, which has been reported to be prone to experimental errors. This study evaluates the efficiency of a water-based cleansing wipe, WaterWipes<sup>®</sup> (WaterWipes, UC, Drogheda, Ireland), for removing synthetic faecal matter (Feclone<sup>™</sup>, SiliClone Creations LLC, Havertown, PA, United States) and *Escherichia coli* (NCTC 10538) from volunteers' skin, the former using a dermal analytical device called the Antera 3D<sup>™</sup> camera (Miravex Ltd., Dublin, Ireland), and the latter using standard microbiological methods. Feclone<sup>™</sup> was applied to participants' forearms and the Antera 3D<sup>™</sup> camera captured detailed images of the skin surface before and after wiping. The Antera 3D<sup>™</sup> camera approach was found to be effective in measuring cleaning efficiency, with the wipe removing all detectable traces of the Feclone<sup>™</sup> applied. The total pore area (mm<sup>2</sup>), pore count, and total pore volume (mm<sup>3</sup>) in test participants post-wiping were observed to be reduced on average by 39.05%, 34.39%, and 39.98%, respectively. The wipe removed 99.99% of *E. coli* (NCTC 10538) applied, as measured using the microbial plate count method. In conclusion, the Antera 3D<sup>™</sup> camera method was observed to be effective in evaluating removal of topically applied Feclone<sup>™</sup>.

**Keywords:** cleansing wipes; Antera 3D<sup>™</sup> camera; Feclone<sup>™</sup>; WaterWipes<sup>®</sup>; bacterial removal



**Citation:** Marisa, A.; Shadrach, W.; Holohan, K.; Abu Alhajja, A.A.; Gilligan, E.; Sommerville, J.; Burke, N.; Yeomans, T. An Alternative Approach to Validate the Cleaning Efficiency of a Skin Cleansing Wipe. *Cosmetics* **2024**, *11*, 172. <https://doi.org/10.3390/cosmetics11050172>

Academic Editor: Maria Manconi

Received: 31 July 2024

Revised: 13 September 2024

Accepted: 26 September 2024

Published: 1 October 2024



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## 1. Introduction

Wipes can be described as cleaning agents and are generally divided into dry and wet wipes, with the use of wet wipes increasing in recent years, especially in relation to baby and infant hygiene [1]. Studies show that the use of wet wipes improves baby diaper cleaning outcomes, offering efficient cleaning and reduced skin erythema, suitable for both unimpaired and impaired skin surfaces when compared to the use of water and cloth [2–5]. Baby wet wipes typically consist of base sheet materials formulated with a mild cleaning surfactant, preservatives, and pH-buffering agents. Water is generally used as a base component in baby wipes for the purpose of cleaning babies' sensitive skin and faecal residues [4,6]. It has been recommended by a European roundtable meeting that wet wipes designed primarily for baby use should not have the potential to cause skin sensitivity reactions and should be formulated with ingredients that are safe for long-term usage [7]. Wet wipes designed for cleaning and disinfection applications undergo standard testing, such as microbiological tests, wiping efficiency, wipe wet pick-up, and lotion formulation [1], with wiping efficiency traditionally determined using wipe-clean gravimetric methods (such as the melted chocolate recovery technique) which have been reported to be prone to experimental errors [1,8].

Hence, this study seeks to design and evaluate a new non-gravimetric method with potential future use for assessing the cleaning efficiency of new and existing commercial wipe products. The designed method involves the use of an Antera 3D™ camera known for its real-time dermal imaging, with advantages such as the delivery of accurate, quick, and objective data in different areas of skin studies and cosmetic product evaluation [9–12]. The Antera 3D™ camera (Miravex Ltd., Dublin, Ireland), under European Patent No. 2400890, operates on the principle of skin illumination from multiple angles, with skin surface data reproduced in a 3D manner using computer-enabled software to interpret the reflected light from the skin surface. Relevant skin data that can be generated with this instrument include skin pore counts, wrinkles, roughness, and pigmentation analysis [13]. Skin pores play a role in the release of sebum, sweat, and cell debris from the skin. Treatment of enlarged facial pores (with therapies such as ultrasound, broadband light, radiofrequency, and fractional non-ablative lasers) has been reported to be a leading cosmetic demand [14].

The designed method was evaluated using WaterWipes® (WaterWipes UC, Drogheda, Ireland) a commercially available brand of surfactant-free wet wipe made with minimal ingredients. Its basic composition comprises plastic-free fibrous materials (moistened sheets of non-woven spun lace fabric 100% viscose). The sheets are moistened with ultra-purified water (99.9%) and citrus grandis (grapefruit) seed extract 0.1% [15]. Its cleaning efficiency was tested against topical applications of Feclone™ and *E. coli* in two separate human volunteer trials. Feclone™ (SiliClone Creations LLC, Havertown, PA, United States) is a proprietary brand of artificial simulated faecal material with reported applications in the testing of food products as well as incontinence and baby wipes [16,17]. The adapted Antera 3D™ camera method was used to evaluate the cleaning efficacy of a specific commercially available wipe product in the Feclone™ trial and the plate counting method for the *E. coli* trial.

## 2. Materials and Methods

### 2.1. Evaluation of Wipe Product Cleaning Efficiency in Removing Feclone™

This study was conducted based on ethical approval from Munster Technological University's Human Research Ethics Committee (HREC-FER-24-004) and operated in line with BS EN 1500:2013 [1]. Inclusion criteria for the study were males and females over the age of 18 with healthy unbroken and non-sensitive adult human skin, while exclusion criteria were vulnerable adults, skin complaints, skin sensitivity (self-reported), or broken skin on forearm. The resulting panel consisted of 25 human volunteers (18 female and 7 male) volunteers; samples and images taken were anonymized to participants. The age range of participants in this study was 18–50. The investigation was conducted in a controlled setting at the Shannon Applied Biotechnology Centre Laboratory (MTU, Kerry, Ireland) with ambient temperature ( $25 \pm 2$  °C) and relative humidity ( $50 \pm 5\%$ ).

#### 2.1.1. Feclone™ Preparation

In total, 120 mL of distilled water was heated to 99 °C in a covered vessel; simultaneously, 40 g of Feclone™ was pre-warmed for 5 min using a hot plate. Upon reaching the desired temperature, the heated water was carefully added to the Feclone™ (SiliClone Creations LLC, Havertown, PA, USA), followed by thorough stirring for approximately 20 s using a spatula. The mixture was then covered with aluminium foil and incubated at 99 °C for a minimum of 30 min, with the mixture gently stirred after the first 10 min. At the end of the incubation period, a final brief stirring was performed before aliquoting the hot solution into 50 mL tubes. The tubes were placed at 4 °C until further use.

#### 2.1.2. Feclone™ Application on Human Forearm

Volunteers sat with one arm rested at a table and with their forearm exposed and allowed to acclimatise to the room conditions for 3 min. The purpose of this study was

to determine the cleaning efficiency of the wipe, and so skin temperature was deemed to be a lower priority. Within an  $8 \times 8$  cm section, a  $4 \times 4$  cm area was secured by placing a template onto the volunteer's forearm. An amount of 2 g Feclone™ was applied to the  $4 \times 4$  cm area and evenly spread using a spatula. The cleaning procedure involved wiping the contaminated area vertically and horizontally as follows: left to right with one wipe surface, followed by right to left with another wipe surface, then top to bottom with a fresh wipe surface, and finally bottom to top using a new wipe surface, all from the same sheet.

An image of the marked area was captured using an Antera 3D™ camera before and after cleaning, as well as directly after application of the Feclone™, and analysed using the Antera 3D™ software (version 3.1.8) to compare pre-wipe and post-wipe conditions. The percentage removal was calculated using the software volume parameter, which measures the depression and elevation above a normalised reference surface. Skin volume ( $\text{mm}^3$ ) post-wiping was subtracted from skin volume pre-application of Feclone™; the obtained value was subtracted from the skin volume ( $\text{mm}^3$ ) with Feclone™ pre-wiping and divided by the skin volume ( $\text{mm}^3$ ) with Feclone™ pre-wiping. This value was then multiplied by 100 to calculate the percentage removal (formula in Section 3.1) with result obtained found in Section 3.1 (Table 1).

**Table 1.** A table showing the Feclone™ cleaning efficiency of the wipe product on skin using the Antera 3D™ camera. A 100% cleaning efficiency was obtained post-wipe, and the experiment was carried out using freshly prepared 2 g Feclone™ on 25 healthy volunteers.

Volunteer Number	% Feclone™ Removed	Volunteer Number	% Feclone™ Removed
1	99.96	14	100.06
2	100.33	15	100.02
3	100.10	16	99.63
4	101.35	17	99.50
5	100.26	18	99.93
6	108.90	19	102.75
7	98.52	20	100.06
8	100.02	21	99.91
9	99.89	22	100.25
10	99.79	23	103.57
11	100.06	24	100.05
12	99.90	25	108.93
13	99.97		
%Total average	100.92		

## 2.2. Evaluation of Wipe Product Impact on Human Skin Pores

This study was carried out on the bare forearm of each volunteer (25 in total). The test area was marked out on the arm as described previously so that before and after pictures were of the exact same area. The area was then photographed, followed by an application of 2 g of Feclone™. The subsequent cleaning procedure was carried out as outlined in Section 2.1.2 and results obtained can be found in Section 3.2 (Table 2). The formula used for the different pore parameters from the Antera 3D™ software (version 3.1.8) include:

- Total pore volume ( $\text{mm}^3$ )  $V = \text{sum of depth for every pixel} * \text{pixel area}$ .
- Total pores area ( $\text{mm}^2$ ) = number of depressed pixels \* pixel area.
- Pore count = isolated depression islands inside the selected region of interest.

**Table 2.** A table showing the impact of the test wipe on skin pores. Data were obtained and analysed using the Antera 3D™ camera method. A significant reduction in mean pore count, volume, and area was observed post-wipe as compared to the pre-wipe imaging of the same skin surface.

Pore Parameters	Pre-Wipe (Average)	Post-Wipe (Average)	% Reduction (Average)
Pore total area (mm <sup>2</sup> )	42.07	25.64	39.04
Pore count	341.4	224	34.38
Pore total volume (mm <sup>3</sup> )	0.59	0.35	39.98

### 2.3. Evaluation of Wipe Product Cleaning Efficiency against *E. Coli* (NCTC 10538)

This study was conducted using KWIK STIK *E. coli* (NCTC 10538), bacterial culture media, and 25 human volunteers. Preparation of culture media and bacterial starter cultures were prepared prior to testing on volunteers.

#### 2.3.1. Bacterial Culture

In total, 30 g of Tryptone soya broth (TSB) powder was added to 100 mL of distilled water. The mixture was thoroughly stirred until the powder was completely dissolved. The volume was then adjusted with distilled water to achieve a final volume of 1 L. Subsequently, the media was sterilised by autoclaving. Tryptone soya agar (TSA) media was prepared in a similar manner, but using 40 g/L of TSA powder.

*Escherichia coli* (*E. coli*) cells (NCTC 10538) were initially inoculated onto TSA plates and then placed in an incubator set at 37 °C overnight. The following day, a single colony was selected and transferred to another TSA plate, which was then incubated under the same conditions. On the subsequent day, a single colony was picked and introduced into 10 mL of TSB media. This culture was incubated overnight at room temperature with shaking. The next day, the optical density (OD) of the culture was measured using a spectrophotometer, and the cells were diluted with TSB media until an OD of 0.15 was reached.

#### 2.3.2. Application of Bacteria to the Forearm of Volunteers

This method was based on the guidelines outlined in BS EN 1500:2013 [1]. *E. coli* broth cultures with an optical density (OD) of 0.15 were consistently used throughout the experiment; the average number of *E. coli* used was  $1.55 \times 10^8$  CFU/mL. An aliquot of this culture was used for enumeration using the plate count method. The volunteers cleaned their hands and arms with a non-antibacterial soap, followed by swabbing the cleansed area, which was placed in 1 mL of phosphate-buffered saline (PBS) media for enumeration via the plate count method (serving as the control). A 4 × 4 cm area was marked out on the volunteer's skin for testing. Subsequently, 100 µL of overnight culture at OD of 0.15 was pipetted onto the test area, gently spread using an L-shaped spreader, and allowed to air dry for 3 min.

This was followed by the cleaning procedure using the wipe product as described in Section 2.1.2. The used wipe was then submerged in 10 mL of Dey-Engley neutralising media (D3435, Merck, Darmstadt, Germany), vortexed for 60 s, and serially diluted twice with PBS media. Subsequently, 0.1 mL of the bacteria was applied to an agar plate for overnight culture and subsequent quantification. Following the wiping process, the surface of the test area of the forearm was swabbed with a moistened swab. The swab was then placed into 1 mL of PBS media, and 0.1 mL was applied to an agar plate for quantification. Serial dilutions of neat,  $10^{-1}$ , and  $10^{-2}$  were applied to an agar plate for quantification in triplicate. The original stock of bacteria was serially diluted at  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  and plated on agar plates for colony enumeration. For each area, CFU/mL was calculated using the following formula:

$$\text{CFU/mL} = (\text{N} * \text{DF}) / \text{VC}$$

where CFU/mL = Colony forming unit per mL:

N = Number of colonies (total number of colonies counted on the plates within the optimal range).

DF = Dilution Factor (the reciprocal of the dilution used for plating).

VC = Volume of Culture Plated (the volume of the diluted culture plated onto the agar plate). The table showing the percentage bacteria removed can be found in Section 3.3 (Table 3).

**Table 3.** A table showing the cleaning efficiency of test wipe product on skin inoculated with *E. coli*. An average cleaning efficiency of 99.99% was reported post-wipe.

Volunteer Number	%Bacteria Removed	Volunteer Number	%Bacteria Removed
1	99.99	14	99.99
2	99.99	15	99.99
3	99.99	16	99.99
4	99.99	17	99.99
5	99.99	18	99.99
6	99.99	19	99.99
7	99.99	20	99.99
8	99.99	21	99.99
9	99.99	22	99.99
10	99.99	23	99.99
11	99.99	24	99.99
12	99.99	25	99.99
13	99.99		
%Total average	99.99		

### 3. Results

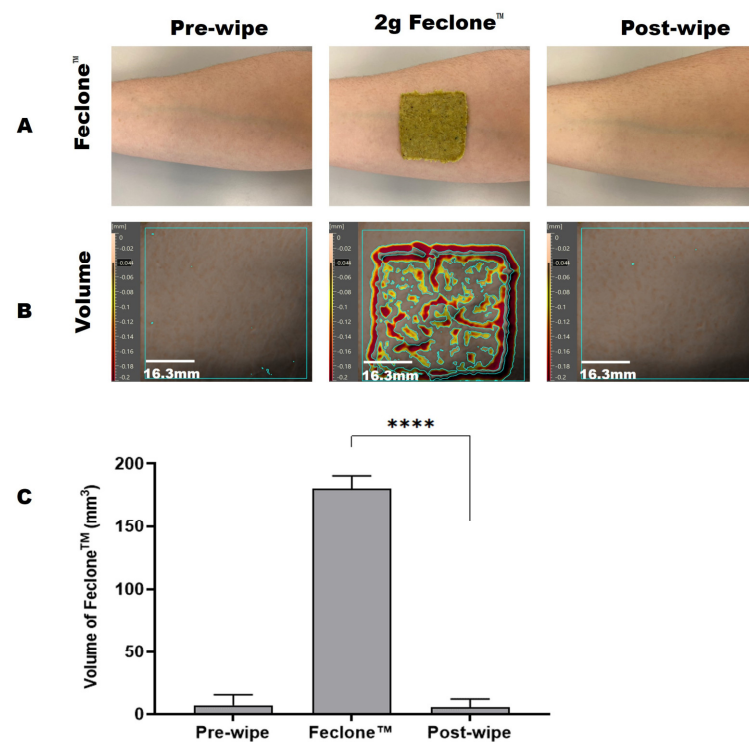
#### 3.1. Evaluation of Wipe Product Cleaning Efficiency in Removing Simulated Faecal Material (Feclone™)

The adapted Antera 3D™ method was used to evaluate the cleaning efficiency of test wipes regarding the removal of Feclone™ from volunteer's forearms. The results were analysed using the volume parameter of the Antera 3D™ software. Volume refers to the overall thickness or bulkiness of any residue or contaminants on the skin surface, and the reduction in volume post-cleaning indicates successful removal (see Figure 1). The percentage removal was calculated using the following formula:

$$sv_0 - sv_1 / sv_0 \times 100$$

where SV0 = Skin volume (mm<sup>3</sup>) with Feclone™ pre-wiping.

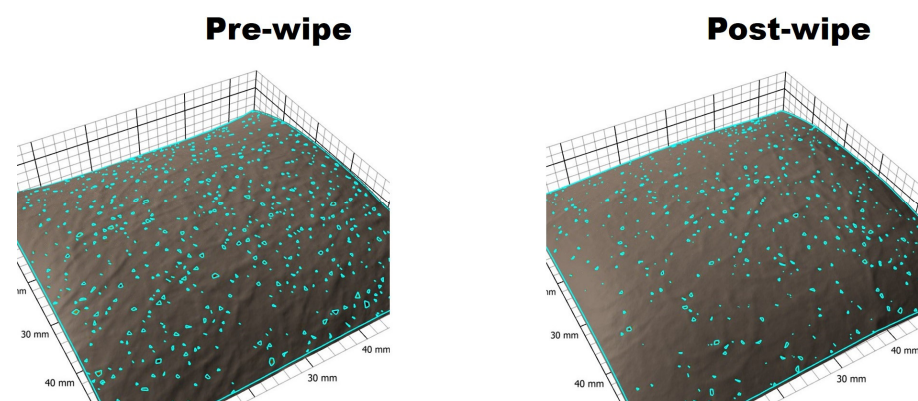
SV1 = (Skin volume (mm<sup>3</sup>) post-wiping—skin volume (mm<sup>3</sup>) before application of Feclone™).



**Figure 1.** (A) Representative images showing the human skin forearm before and after Feclone™ was applied and removed using the test wipe product; imaging was performed using a phone camera. (B) Volume of Feclone™ detected on the forearm skin surface using the Antera 3D™ method and cleaning with the wipe product. Area of application for analysis was 40 mm × 40 mm. The vertical scale bar represents the skin volume and topography, whereas the horizontal scale bar refers to the 2D measurement of the defined skin region. (C) Shows the pre-wipe, Feclone™, and post-wipe skin volumes observed with the Antera 3D™ method. The wipe product was noted to reduce the Feclone™-induced increase in skin volume down to preapplication levels. A paired *t*-test analysis of Feclone™ administered, and post-wipe residual skin volume showed a highly significantly difference (\*\*\*\*  $p < 0.0001$ ). Graph was plotted as mean with SD (standard deviation) using GraphPad Prism 10 (GraphPad Software, 225 Franklin Street, Fl. 26 Boston, MA 02110, USA). This represents an average cleaning efficiency of 100.92%.

### 3.2. Evaluation of Wipe Product on Human Skin Pores

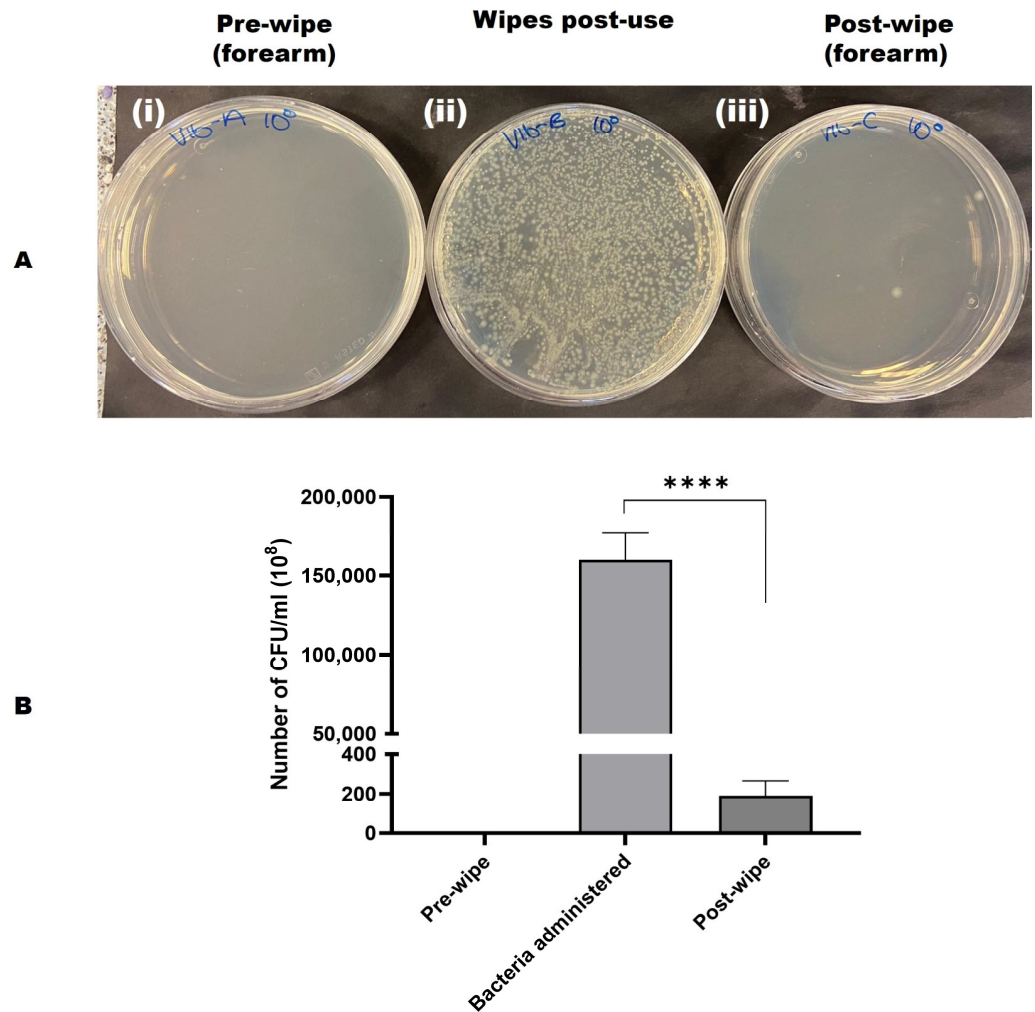
A reduction in pore parameters such as total pore count, pore volume, and area was observed after wiping the volunteer's forearm skin with the wipe product, (Figure 2).



**Figure 2.** A representative image from the Antera 3D™ software showing the observed reduction in detectable skin pores (count, total area, total volume) after cleaning with wipe product.

### 3.3. Evaluation of Wipe Product Cleaning Efficiency in Removing *E. Coli*

The efficacy of the wipe product in removing bacterial contamination was also assessed. The number of CFU/mL was calculated for each area, and the averages were determined. These findings indicate that the wipe product effectively removed 99.99% of bacterial cells and efficiently cleaned the contaminated skin areas. This was validated by harvesting the used wipes for bacterial quantification after cleaning, as well as swabbing the clean area for culturing on TSA plates (see Figure 3A,B).



**Figure 3.** (A) A representative image showing the bacterial removal efficiency of test wipes from contaminated human skin. (i) Swab pre-wiping plated (ii) Used wipe plated (iii) Swab post-wiping plated. V = volunteer, A = Pre-wipe sample, B = Wipe post-use, C = Post-wipe sample, 10<sup>0</sup> = neat/undiluted sample. (B) Graph of bacterial removal efficiency of wipe product using *E. coli* and microbiological counting. Test wipes were observed to have 99.99% bacteria removal of what was administered. Paired *t*-test analysis of bacteria administered, and post-wipe count showed a highly significant difference (\*\*\*\*  $p < 0.0001$ ). Graph was plotted as mean with SD (standard deviation) using GraphPad Prism 10. V = volunteer, A = Pre-wipe sample, B = Wipe post-use, C = Post-wipe sample, 10<sup>0</sup> = neat/undiluted sample.

## 4. Discussion

This study aimed to design and evaluate an alternative method for carrying out cleaning efficiency studies of wipe products using an Antera 3D™ camera, a dermal imaging and analytical device. Commercially available baby wipes (WaterWipes®) were used in this study and were tested for their cleaning efficiency with synthetic faecal matter

(Feclone™) and contaminating *E. coli* from the skin surface of volunteer's forearms. The Antera 3D™ camera, a validated cosmetic product assessment tool [11], has been employed in many cosmetic studies such as those involving the quantification of wrinkles, acne, and pigmentation [11,18,19], and so an adaptation of the European standard BS EN 1500:2013 [1] incorporating the Antera 3D™ camera for quantitative and qualitative evaluation of the wipes' cleaning efficiency was developed and tested using the wipe product. The objective analysis of the Feclone™ test used the software volume parameter to provide an in-depth evaluation of the wipes' cleaning efficiency.

Results from visual assessment and Antera 3D™ camera analysis demonstrated that this method was appropriate in evaluating the removal of Feclone™ from human skin using wipes, which showed a 100.92% Feclone™ cleaning efficiency. We hypothesise that the removal value being >100% could be due to the sensitivity of the Antera 3D™ camera method in detecting wipe removal of both applied Feclone™ and any pre-existing skin debris. A similar imaging approach for determining wipe cleaning efficiency was reported by Lee et al. [8] and consists of a computer with a scanner and image analysis software; however, this study was conducted using a contaminant spread on glass plate and not human skin. Imaging techniques therefore provide an alternative to the traditional gravimetric method used for assessing the cleaning efficiency of wipes, which has been reported to be prone to experimental errors.

The gravimetric method typically involves recording the wipe weight before and after wiping to determine the cleaning efficiency. This technique focuses on analysing the wipe and not the surface being cleaned. The method has been reported to be prone to errors which could arise from the process, such as from the handling of wipes during the weighing process which could lead to moisture transfer from the wipe to the tester's gloves and unavoidable moisture evaporation from the test wipes, all of which could affect the obtained results and should be accounted for as experimental variables. However, with the Antera 3D™ camera method, which is an optical based approach, there is less concern for wipe moisture evaporation and handling, as cleaning efficiency will be deduced from the skin surface directly and not via the difference in wipe weights, making this method potentially helpful in tackling errors common to gravimetric analysis. Other advantages associated with the proposed Antera 3D™ camera methods include its 3D skin imaging ability, ease of use, precision, reproducibility, and its multi-readout for skin parameters such as pore size, blemishes, wrinkles, and roughness. However, there are some limitations observed with the proposed models, which include uncertainty about its applicability in other areas of wet wipe efficiency characterisation such as wipe drying rate analysis (rate of moisture evaporation from wipe when exposed to the atmosphere over time), wipe pick-up analysis (ability of the wet to pick up lotion), and lotion transfer studies (release of lotion from wipe to skin) [1,8,20]. Since the proposed method uses a specific device, the Antera 3D™ camera and associated software are required to perform or reproduce the method, a potential limitation of widespread application. The model is an optical technique with variables such as fixed distance, degree of resolution (0.1 mm), and spectral band (seven) obtainable with the Antera 3D™ camera, making the reconstructed skin topography an estimation [21]. The sensitivity of the proposed technique allows it to detect what is present on the skin (hair, pores, sebum, scars, freckles, etc.); however, this is not expected to influence or cause artefacts in the data generated, as the protocol states that skin baseline readings be taken first.

Interestingly, it was observed that cleaning the skin with the wipe product in question had an impact on reducing the total mean pore count, volume, and area, and this is of noteworthy importance, as various studies have shown a relationship between a lower pore count and healthier skin [14,22,23]. This is likely attributed to the combination of the formulation and the wipe composition, whereby a low surface tension formulation spreads and wets the skin pores sufficiently to allow the wipe to effectively cleanse the pores of sebum, sweat, and other cell debris. The reduction in mean pore count and size



may also be aided by the cooler temperature of the wipes themselves. The temperature of the wipes appears cooler than skin temperature; therefore, this could be the reason for the pores temporarily closing. Some studies have reported that cooling the skin leads to a reduction in pore size and count via cutaneous vasoconstriction and skin tightening [24–26]. Additionally, grapefruit seed extract (GSE), a skin conditioning ingredient and an ingredient in the wipe product used, is reported to have anti-microbial properties [27–30]. It is also reported to possess antioxidative activity and help to relieve skin congestion by purifying, cleansing, and clearing clogged skin pores for smooth and brightened skin radiance, as well as tightening skin pores due to its mattifying capacity [31,32]. It is proposed that these wipes, WaterWipes® (WaterWipes UC, Drogheda, Ireland), could find use as a less invasive way of cleaning pores and minimising their appearance, given that, when taken as a whole, the change in the pore area, volume, and count post-wipe could be a combination of all of the above reasons; however, this is a separate topic, and more research needs to be carried out to validate these findings and determine the exact mechanism of action, since the temperatures of the wipes and volunteers' skin were not taken into account in this study. Nonetheless, the Antera 3D™ camera (Miravex Ltd., Dublin, Ireland) pore visualisation and measurement capabilities provided additional understanding of the effect that the wipe product has on the skin.

## 5. Conclusions

In conclusion, the findings from this study demonstrate that the Antera 3D™ camera method can be an effective alternative technique in evaluating the efficiency of cleansing wipes. Its benefits include its simplicity of use, data reproducibility, controlled light in the internal image area, and the fact that it is a portable unit with a high resolution and multi-parameter read out. However, since no comparison was carried out with other methods of cleaning efficiency evaluation (such as the gravimetric technique), this proposed technique is intended to serve as a potential alternative to existing methods. The wipe product used in this study was found to have good cleaning efficiency regarding the substantial removal of both Feclone™ and *E. coli* from human skin. Further research could explore the wipes' performance on different skin types and body sites and under varying environmental conditions to broaden their applicability and understanding of efficiency using the developed method. Overall, this study contributes valuable insights into the effectiveness of the Antera 3D™ camera method for measuring the cleaning efficiency of cleansing wipes.

**Author Contributions:** Conceptualization, T.Y., N.B., J.S. and E.G.; methodology, A.A.A.A., A.M.; K.H. and N.B.; Investigation and data analysis, A.M., K.H. and W.S.; writing—original draft preparation, W.S.; writing—review and editing, W.S., N.B., J.S., E.G. and T.Y.; resources, E.G., J.S. and T.Y.; supervision, N.B. and T.Y.; project administration and funding, T.Y., E.G. and J.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received funding from WaterWipes UC.

**Institutional Review Board Statement:** This human volunteer trial was conducted in accordance with the Declaration of Helsinki and approved by the Munster Technological University Human Research Ethics Committee (reference MTU-HREC-FER-24-004). The governance of the Munster Technological University Human Research Ethics Committee is as outlined in the University Human Research Ethics Policy [33], and aligns with the National Policy Statement on Ensuring Research Integrity in Ireland (Research Integrity National Forum (2019) [34]. All research activity that involves humans as research participants is required to undergo formal ethical review, prior to commencement.

**Informed Consent Statement:** All volunteers provided written informed consent before participating in this study.

**Data Availability Statement:** All data can be found in this article.

**Acknowledgments:** All volunteers in both studies are acknowledged for their role in data collection.

**Conflicts of Interest:** Authors Jill Sommerville and Emer Gilligan were employed by the company WaterWipes UC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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