Preparation protocol for epifluorescence microscopy when working close to the detection limit

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Impact statement

This study addresses one of the core challenges of cell enumeration routinely encountered in low volume, low biomass samples using epifluorescence microscopy. It outlines a straight-forward and cost-effective preparation that reduces background contamination of non-target cells by almost two orders of magnitude. This enables the reproducible enumeration of cells from environmental samples where cell counts are close to the detection limit.

Abstract

Working with low density, low biomass material can be challenging, especially when working near the detection limit. Although background contamination is a universal consideration in microbiological research, its impact is increased when the cells under assessment approach the same concentration as the background contamination. The aim of this work was to identify and remove laboratory sources of background contamination in the cell mounting process for epifluorescence microscopy to improve the reliability of cell counting for low biomass samples. Microscope slides and coverslips were assessed before and after autoclaving, washing with detergent and rinsing with ethanol solution. The solutions used in sample mounting; 4′,6-diamidino-2-phenylindole, phosphate buffered saline and immersion oil, were tested before and after autoclaving as well as both single and triple filtering with a 0.2 µm membrane filter. Using a combination of detergent and ethanol rinses of glassware and triple filtering of all solutions, we were able to reduce the background contamination by almost two orders of magnitude, down from $1x10^4$(±$4.3x10^3$) cells to 302(±312) cells per filter paper. This method was then validated with low biomass glacial sediment samples from Renardbreen, Svalbard with cell concentrations of $1.8x10^5$(±$2.9x10^4$) cells g^{-1}, close to the reported detection limit of epifluorescence microscopy.

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Introduction

Enumeration of cells using DNA staining fluorophores such as 4',6-diamidino-2-phenylindole (DAPI) is a standard method in environmental microbiology (e.g., Porter and Feig, 1980, Kirchman et al. 1982, Muthukrishnan et al. 2017). However, such methods generally assume cleanliness and the use of sterile materials to limit background contamination. In practice, however, and often in shared laboratories with multiple occupants, this can sometimes be challenging. There are several well-known primary sources of background contamination that can affect sample preparation for microbiological studies in laboratory settings:

1. The sampling environment – Contamination arising in the sampling environment during collection from improper PPE and aseptic technique is a particular concern when working with low biomass samples (e.g., Willerslev et al. 2004, Makinson et al. 2016).
2. The laboratory environment – Contamination can arise easily inside busy and/or drafty laboratory environments including those with active air conditioning systems, where settled contaminants can be resuspended in air (known as bioaerosols) and deposited on samples or other laboratory equipment (e.g., Nikfarjam and Farzaneh 2012, Sanders 2012, Ghosh et al. 2015).
3. Equipment contamination – Ineffective cleaning practices, improper storage, as well as the presence of bioaerosols can contribute to contamination of laboratory equipment (e.g., Eisenhofer et al. 2019, Cando-Dumancela et al. 2021).
4. Reagent contamination – Although typically purchased as sterile, common laboratory reagents have been known to contain contaminants contributing background cells and DNA (Salter et al. 2014, Eisenhofer et al. 2019). It is therefore best practice to filter any reagents even those listed sterile to remove any potential contaminating cells (Kallmeyer, 2011).
5. Cross-contamination between samples – Ineffective cleaning practices of equipment between samples and splashing of reagents when samples are in close contact can result in cross-contamination (e.g., Abatenh et al. 2018, Cando-Dumancela et al. 2021).
6. Researcher-contamination – Poor technique, improper PPE and human error can result in contamination of the sample with a researcher's own microbiome (e.g., Eisenhofer et al. 2019; Hornung et al. 2019).

Each of the above sources of contamination can vary in the same laboratory environment over time, thus frequent and comprehensive background controls during sample preparation are critical for reliable results (Eisenhofer et al. 2019, Weyrich et al. 2019). Due to the number of potential sources of contamination, when compounding components for cell mounting and enumeration it can also be difficult to identify at which specific step contamination may be occurring. For example,

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regardless of the sterility of the fluorescing compound, washing solution and immersion oil, a contaminated glass coverslip will result in anomalously high cell counts. In addition, a completely sterile glass coverslip will be of little benefit if all the solutions at the preparation stage contain some degree of contamination.

The detection limits of cell counting by fluorescence microscopy have been cited at ~10^3-10^6 cells ml^{-1} evenly distributed on a filter membrane (Hobson et al. 1996, Broadaway et al. 2003a, 2003b, Lisle et al. 2004, Madrid & Felice, 2005, Barton et al. 2006, Chae et al. 2008, Pascaud et al. 2009, Singh et al. 2016, Bedrossian et al. 2017a,b, Muthukrishnan et al. 2017, Hoyles et al. 2018, Tyagi et al. 2022). High biomass environmental samples such as soils, have estimated prokaryote cell counts of >10^9 cells g^{-1} which is often enough for the enumeration of cells even with relatively high detection limits (Torsvik et al. 1990, Dunbar et al. 2002, Smith et al. 2006, Liebner et al. 2008, Tecon and Or 2017). However, in low biomass environments (see Table 1), time consuming and complex cell separation techniques are required, and often large volumes of sample are needed to exceed the detection limit. This is not always possible for samples taken from remote and extreme environments where sample volume is limited. Thus, lower limits of detection must be improved and background contamination reduced to achieve reliable and reproducible results.

In published works, the degree of background contamination present in an epifluorescence microscopy protocol, and indeed the detection limit of a particular study are often not reported. This leaves a significant knowledge gap making it difficult to achieve reproducible results using published protocols. To address this issue, we focussed on reducing the amount of background contamination present in the laboratory and materials used for cell mounting. The overall aim being to develop a simple and cost-effective protocol which reduced background contaminant cells to a value below the detection limit of the instrument/protocol that can be employed by others in a reproducible way.

Materials and methods

The following protocol applied sequential additions of each cell mounting component using different cleaning and sterilisation methods to determine the most effective protocol for reducing background contamination. Aseptic techniques, the use of laboratory gloves when handling consumables and samples and the frequent changing of gloves to reduce cross-contamination and sterile single-use plastic materials were used throughout. To reduce the impact of background contamination from the laboratory environment, it is also recommended that filtering and sample preparation is performed in a class II microbiological safety cabinet where possible.

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For the purposes of this investigation, a standard microscopy slide preparation consisted of; a (i) glass microscope slide, (ii) glass coverslip, (iii) a Whatman Cyclopore Track-Etched Membrane (0.2 µm pore size) and iv) immersion oil (Sigma-Aldrich). An initial analysis of all preparatory materials for microscopy was conducted prior to any cleaning procedures. A vacuum manifold with a diameter of 18.5 mm was used to stain, rinse and dry filter papers using a gentle vacuum (≤0.20 millibar pressure). All components of the vacuum manifold were cleaned with detergent (Detergent 8, Alconox), rinsed well with sterile water, and allowed to dry between uses to prevent cross-contamination between samples. This provided a baseline of background contamination present in the current laboratory setting.

Cleaning protocol

Prior to the cleaning protocol, a ten-fold dilution series using a stock solution of *E. coli* culture was performed to determine detection limits. The solution was prepared in 5 ml Luria-Bertani broth (LB) and incubated for 24 hours. A 10-fold dilution series was prepared for microscopy analysis using the slide and enumeration procedure below, counting cells per fields of view (FOV) until a stable mean had been established. Sample 1 being the most concentrated stock solution and each step thereafter prepared with 100 µl of the previous sample and 900 µl of sterile phosphate buffered saline (PBS). This protocol was also repeated after the cleaning protocol to verify the background levels of contamination.

The first step in the cleaning process was to remove any contaminating cells or auto-fluorescing dust and particles from glassware (Fig. 1). Both microscope slides and coverslips were washed with detergent then thoroughly rinsed in a stream of 95% ethanol whilst held with sterile tweezers and allowed to dry.

The solutions used in the preparation of the samples; (i) Gibco pH 7.4 PBS, (ii) immersion oil (Sigma-Aldrich), and (iii) 5 mg ml^{-1} solution of DAPI (Thermo Fisher Scientific) could also be sterilised and the following sterilisation techniques were tested (Fig. 1):

1. Single filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)
2. Triple filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)
3. Autoclaving immersion oil and PBS followed by triple filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)

Preparation of slides and enumeration of cells

To stain the samples, a 5 mg ml^{-1} solution of DAPI was prepared using PBS and stored at -20°C for up to one month. 10 µL of DAPI solution was added to a Whatman Cyclopore Track-Etched

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Membrane (0.2 µm pore size) and incubated in the dark for 5 minutes. A glass coverslip without immersion oil was then placed on top of the filter membrane and the slide was analysed. To test the sterility of PBS, 1 ml of sterile certified on purchase PBS was added to the filter membrane in a vacuum manifold and a gentle vacuum (≤0.20 millibar pressure) was applied. Potential contaminating cells present in the PBS captured on the filter membrane were then stained with 1 µL of 5 mg ml^{-1} DAPI and incubated for 5 minutes. The filter membrane was then rinsed twice with 1 ml of PBS to remove excess DAPI with a gentle vacuum applied between rinses. A glass coverslip without immersion oil was then placed on the filter membrane glass slide and analysed immediately. The same preparation procedure was repeated to test the sterility of the immersion oil but before adding the glass coverslip a drop of immersion oil was placed in the centre of the filter membrane using a sterile pipette tip and a glass coverslip placed on top. The sample was then analysed immediately. Samples were analysed at 40x magnification across a full vertical and horizontal transect of the filter paper accounting for potential cell clumping at the edges of the filter paper where the vacuum effect is strongest. Cell counts were obtained using a running mean to achieve a stable mean value. This equated to an average of 35 FOV on a fluorescence microscope (Olympus BX51) using UV light. Each method was also analysed using three independent replicates by three different researchers to remove any potential counting bias related to human error. An estimate of total cells on the membrane filters was calculated using the following equation (Eq. 1):

$$Cells\ per\ filter\ paper = Average\ cells\ per\ FOV * \frac{Area\ (\pi R2)\ A}{Area\ (\pi R2) B} \quad \textbf{(1)}$$

Where *Area A* is the area of the filter membrane exposed to solution (268.8 mm^2) and Area B is the area of the FOV (0.22 mm^2) providing a conversion factor (CF) to extrapolate the total cells on the membrane (CF – 1221).

Environmental sample

To validate the protocol, the triple filtered consumables, and detergent cleaned glassware was used to enumerate cells from a glacial low volume, low biomass environment. Sediment samples were collected from the surface of Renardbreen, Svalbard originating from a thrust structure within the ice. Samples were collected using sterile gloves into Fischerbrand 20 ml sterile syringes and stored in sterile Whirlpak bags at -20°C.

Cells were extracted from the sediment in three independent replicates using 0.51-0.55 g of sediment vortexed for 2 minutes in 2 ml Eppendorf tubes with PBS. A negative control was also implemented at this stage consisting of 2 ml of the PBS only. Samples and control were then centrifuged at 6,764 g for 1 minute to separate the suspended sediment from the solution containing the cells. The resultant supernatant was then pipetted onto a Whatman Cyclopore Track-

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Etched Membrane. Excess liquid was removed via gentle vacuum before being stained with 10 µL of DAPI solution and incubated in the dark for 5 minutes. Samples were then washed twice with 0.5 ml of PBS and excess liquid removed via vacuum before mounting on a microscope slide with 1 drop of immersion oil and a cover slip. The three replicate samples were analysed by counting cells in 35 FOV on each sample at 40x magnification on an epifluorescence microscope using UV light. The cell concentration in the sample could then be compared with the negative control which would reflect the level of background contamination.

Results and Discussion

Glassware preparation

Cleaning of glassware with detergent before rinsing with ethanol and drying on a hot plate in a sterile petri dish removed any autofluorescence from small fibres and reduced the number of apparent contaminant cells (Table 2). This alone reduced the apparent background contamination from 8.89 cells per FOV or $1x10^4$(±$4.3x10^3$) cells per filter paper to 3.30 cells per FOV or $4.04x10^3$(±$2.97x10^3$) cells per filter paper. The continued presence of contaminant cells after cleaning of the glassware was likely introduced through the solutions required for the cell mounting process i.e., air, DAPI, PBS and immersion oil. This effect was suggested by the gradual increase in number of background cells observed after the cleaning process with the addition of each new component.

Solution sterilisation

Single filtering of DAPI, PBS and immersion oil produced a reduction in background contamination in all steps of the process, with a statistically significant difference seen in cell counts per FOV and per filter paper between filtered and unfiltered consumables (P = <0.01, t = 1.98, n = 105) (Table 2). The largest improvement in background contamination was seen when all solutions underwent a triple filter technique with a statistically significant difference obtained between the single filtering and triple filtering (P = <0.01, t = 1.98, n = 105) (Fig. 3 and Table 2). The triple filtering technique combined with the clean glassware reduced background contamination by almost two orders of magnitude with the total number of cells present on the filter membrane and cover slip down from 8.89 cells per FOV or $1x10^4$(±$5.2x10^3$) cells per filter paper to 0.25 cells per FOV 302(±312) cells per filter paper using all solutions and cell mounting components.

Autoclaving of PBS and immersion oil before triple filtering was also tested to see if further improvements in the reduction of background contamination could be achieved. However, triple filtering after autoclaving showed similar results to triple filtering alone. In fact, the lowest cell concentration was observed using the triple filtered method with no autoclaving (Table 2). This

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196 demonstrated that the addition of autoclaving the solutions did not sterilise the materials further
197 and therefore the added labour and cost of autoclaving was not beneficial in this instance.

198 **Background contamination**

199 Background contamination before employing the cleaning protocol was measured using an *E. coli*
200 dilution series (Fig. 2) and then again with no sample but all components of the cell mounting
201 process for microscopy (Table 2, Fig. 3). This method showed increasing levels of background
202 contamination highlighting the importance of cleaning/sterilising all materials used in the cell
203 mounting process to ensure low background contamination and a reduction in detection limits.

204 Prior to any cleaning procedure, the background contamination value fell within the
205 previously stated detection limit of ~10^3-10^6 cells ml^{-1} for fluorescence microscopy (e.g., Broadaway
206 et al. 2003a, Chae et al. 2008, Pascaud et al. 2009, Bedrossian et al. 2017a, Bedrossian et al. 2017b,
207 Muthukrishnan et al. 2017, Hoyles et al. 2018). Thus, when working close to this lower limit of
208 detection, reducing the background contamination below this level is paramount to achieving
209 accurate and reproducible counts. This was further confirmed using an *E. coli* dilution series which
210 showed cell numbers of 0.08-2 cells per FOV or $3.9x10^2$-$9.8x10^3$ per filter paper at 3-10 fold dilution
211 after employing a slide washing and triple filtering cleaning protocol compared to the previous 1.7-
212 16.8 cells per FOV or $8.3x10^3$-$8.2x10^4$ per filter paper at 3-10 fold dilution prior to any cleaning
213 protocol (Fig. 2).

214 In previous works background contamination during cell enumeration has been accounted
215 for by subtracting cell numbers of negative controls from sample counts (e.g., Foght et al. 2004, Xia
216 et al. 2013, Davis et al. 2023). However, when working with low biomass and thus low cell number
217 samples, there is potential for background contamination to be equal to or surpass those of the
218 actual sample.

219 **Applications for environmental samples**

220 In remote polar environments ice, snow and glacial sediment samples have a generally low biomass
221 with reported cell abundances of 10^1-10^6 cells ml^{-1} of water or g^{-1} of sediment (see Table 1 and
222 references therein). Similarly, ultra-low biomass environmental aerosol samples have a reported cell
223 abundance of 10^1-10^5 cells m^3 (see Table 1 and references therein).

224 Therefore, to validate this protocol, environmental low biomass samples from a glacial
225 environment were also analysed using the triple filtered and detergent washed consumable
226 protocol. Negative controls were used alongside the samples which comprised all the cell mounting
227 components with no sample. The average cell concentration across the three replicates in these

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228 sediment samples was $1.8x10^5$(±$2.9x10^4$) cells g^{-1}. The standard deviation of this value fell within the
229 same level as the background contamination before any cleaning or sterilisation protocol. Whereas
230 the negative control using the most optimal protocol outlined here remained low with an average
231 cell concentration of 44(±$1.9x10^2$) cells per filter paper. This sample falls close to the detection limit
232 of the epifluorescence microscopy method of ~10^3 cells ml^{-1}. By reducing the background
233 contamination, with a negative control slide two-four orders of magnitude lower than the sample,
234 we were able to confidently enumerate cells in this low biomass environmental sample.

235 **Further considerations for protocol optimisation**

236 Further considerations for optimising cell enumeration using fluorescence microscopy include the
237 use of other cell stains. Fluorescence *in-situ* hybridization (FISH) for example allows for fluorescent
238 probes to bind to RNA of the small ribosomal sub-unit (SSU) of cells (Rivera et al. 2023). The probe
239 EUB338 is one such universal probe that binds to bacterial SSU (Amman et al. 1995, Fuchs et al.
240 1998, Rivera et al. 2023). Such probes and analysis aid in the differentiation of true bacterial cells
241 and fluorescing artifacts. Differentiation of live and dead bacteria can also be achieved using 5-
242 cyano-2,3-ditolyl tetrazolium chloride (CTC) which is absorbed by active cells and reduced to the
243 fluorescent formazan (CTF) product detectable using fluorescence microscopy (Rodriguez et al. 1992;
244 Schaul et al. 1993). Both compounds can be coupled with DAPI as outlined here to investigate cell
245 viability.

246 Background contamination may also be reduced by employing and reporting on aseptic
247 laboratory techniques used to; reduce the impact of researcher contamination through the proper
248 use of PPE (e.g., Willerslev et al. 2004, Makinson et al. 2016) and cross-contamination between
249 samples as well as other contaminated surfaces or equipment (e.g., Abatenh et al. 2018, Eisenhofer
250 et al. 2019, Hornung et al. 2019, Cando-Dumancela et al. 2021). Further description of such
251 techniques in future publications as well as routine reporting of background contamination levels
252 and detection limits will better facilitate reproducible cell enumerations.

253 **Conclusions**

254 The protocol outlined here reduced background contamination from $1x10^4$(±$4.3x10^3$) cells per filter
255 paper to 302(±312) cells per filter paper. When this protocol was applied to an *E. coli* dilution series
256 the cells per FOV were reduced from 1.7-16.8 cells to 0.04-1.7 cells per FOV. This is a reduction by
257 *almost two orders of magnitude*. This makes low biomass samples such as the glacial sediment
258 samples presented here, fall within a detectable range above the background contamination. This
259 protocol is particularly applicable to studies whereby background contamination is accounted for by
260 subtracting background cell values in controls from those measured in the sample.

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Conflicts of Interest Statement

The author(s) declare that there are no conflicts of interest.

Data Availability Statement

All data presented in this paper is available in an open access repository, Open Science Framework, at: 10.17605/OSF.IO/952ZA.

Authors' Contribution Statement:

RM was involved in; writing (lead), laboratory co-ordination (lead), conceptualisation (support), data curation (lead), formal analysis (lead), investigation (equal), methodology (lead), project admin (support), software (lead), supervision (support), validation (lead), visualisation (lead), writing original (lead),

LH was involved in: Validation (support), investigation (support), methodology (support), data curation (support), writing review and edit (support).

JO was involved in: Validation (support), investigation (support), methodology (support), data curation (support), writing review and edit (support).

HZC was involved in: Validation (support), investigation (support), methodology (support), data curation (support), writing review and edit (support).

JL was involved in: Validation (support), writing original (support), writing review and edit (support).

JW was involved in: Funding (equal), supervision (equal), writing original (support), writing review and edit (support).

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DAP was involved in: conceptualisation (lead), data curation (support), funding (equal), methodology (support), project admin (lead), resources (lead), supervision (lead), validation (equal), writing original (support), writing review and edit (equal).

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451 **Table 1** Example cell counts, reported detection limits and reported FOV counts from the literature
452 of low biomass ecosystems.

Sample Type	Cell Count	Method for checking background contamination	Reported number of FOV counts	Reference
Air	1×10^4 cells m^{-3}	Not reported	400 cells or 86 FOV	Bauer et al. (2002)
	3.4×10^4 cells m^{-3}	Control samples reported as one order of magnitude lower than cell counts	10 FOV	Xia et al. (2013)
	$6.5\text{-}9 \times 10^5$ cells/m^{-3}	Not reported	10 FOV	Dong et al. (2016)
	$1.1\text{-}3.2\times10^5$ cells m^{-3}	Negative controls	20 randomly selected	Hu et al. (2020)
Sea ice	1.4×10^{11} cells m^{-3}	Negative controls	200 cells and 10 FOV	Sullivan et al. (1984)
	1.0×10^5 cells mL^{-1}	Not reported	Not reported	Piontek et al. (2021)
Soil and sediments	1.15×10^8 - 2.13×10^9 cells g^{-1}	Not reported	Not reported	Garcia-Pichel et al. (2003)
	$1.2 – 23 \times 10^8$ cells g^{-1}	Not reported	1000 cells and 10 FOV	Kobabe et al. (2004)
	$2 – 6 \times 10^8$ cells g^{-1}	Not reported	10 FOV	Buckeridge et al. (2013)
	$10^{6–8}$ cells g^{-1}	Not reported	Not reported	Thomas et al. (2021)
	$2–7 \times 10^6$ cells g^{-1}	Subtracting the average reagent control from 20 FOV of each blank control	10 -20 FOV	Foght et al. (2004)
	1.6×10^7 cells mg^{-1} of organic matter	Not reported	Not reported	Kaštovská et al. (2007)
	10^7 cells g^{-1}	Not reported	Not reported	Lanoil et al. (2009)
	$1.22\text{-}4.42 \times 10^7$ cells g^{-1}	Negative controls	2500 cells and 50 FOV	Pearce et al. (2013)
	1.3×10^5 - 2.6×10^6	Negative controls	25-60 FOV	Davis et al. (2023)
Glacier and snow	$10^1 – 10^5$ cells ml^{-1}	Not reported	Not reported	Harding et al. (2011)
	39 – 363 cells mL^{-1}	Not reported	300 cells	Dayal et al. (2023)
	$10^2 – 10^4$ cells g^{-1}	Negative controls	60 FOV	Montross et al. (2014)
	2×10^3 - 2×10^6 cells ml^{-1}	Blanks with no cells counted in parallel	300 cells	Stibal et al. (2015)
	6.6×10^4 - 3.7×10^5 cells ml^{-1}	Field blank of sterile Nanopure water in parallel	Not reported	Skidmore et al. (2005)

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454 **Table 2** Average cells per FOV in each step of the cleaning protocol identifying the most effective
455 way to reduce background contamination in microbial microscopy preparation. Cells per filter paper
456 were calculated using Equation 1.

Cleaning protocol	Components	Average cells per FOV (±SD)	Average cells per filter paper
Control (no cleaning or sterilisation)	DAPI + PBS + Immersion oil	8.89(±3.52)	$1.08x10^4$(±$4.30x10^3$)
Cleaning of glassware with detergent and ethanol	DAPI + PBS + Immersion oil	6.86(±2.4)	$4.04x10^3$(±$2.97x10^3$)
Single filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)	DAPI	1.89(±1.09)	$2.30x10^3$(±$1.33x10^3$)
	DAPI + PBS	3.03(±2.22)	$3.70x10^3$(±$2.71x10^3$)
	DAPI + PBS + Immersion oil	1.61(±1.27)	$1.97x10^3$(±$1.55x10^3$)
Triple filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)	DAPI	0.2(±0.2)	$2.1x10^2$(±$2.3x10^3$)
	DAPI + PBS	0.61(±0.36)	744(±436)
	DAPI + PBS + Immersion oil	0.25(±0.26)	302(±312)
Autoclaving immersion oil and PBS followed by triple filtering with a sterile syringe fitted with a Filtropur S attachment (0.2 µm)	DAPI	0.02(±0.01)	23.60(±16.69)
	DAPI + PBS	0.20(±0.11)	246(±130)
	DAPI + PBS + Immersion oil	0.36(±0.29)	438(±352)

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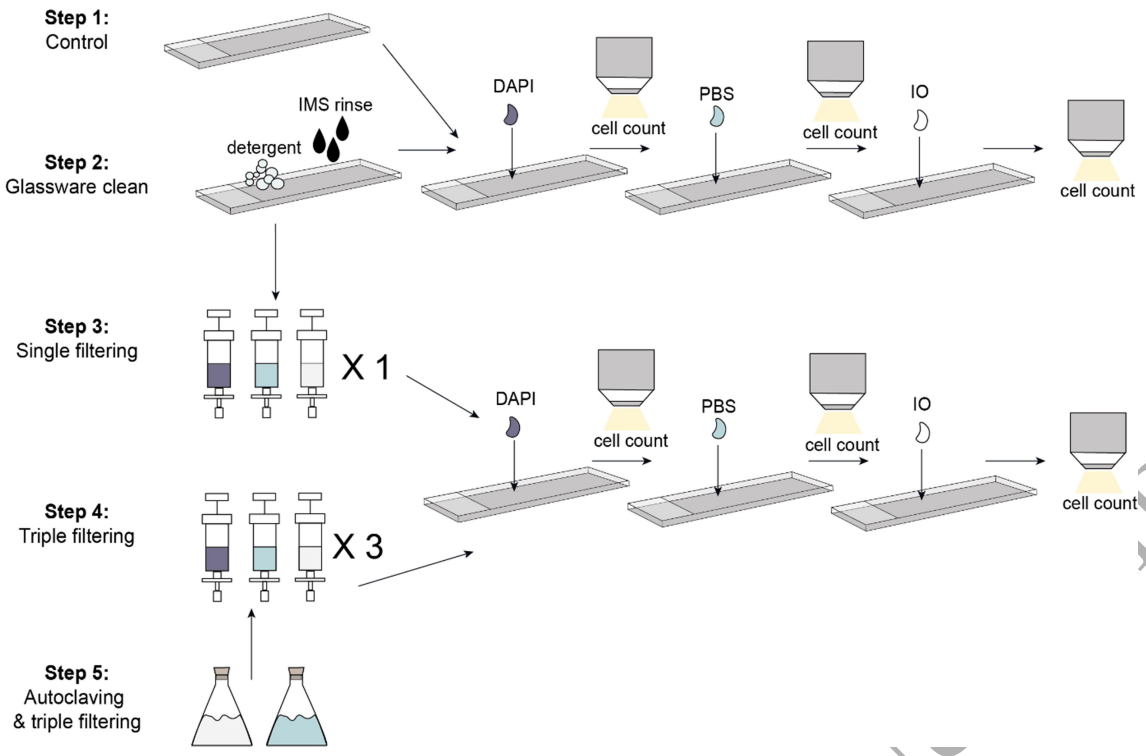

Figure 1 Schematic workflow of the methodological approach of this study detailing the sequential steps and analyses employed to test the sterility at different points of the cell mounting process. DAPI - 4',6-diamidino-2-phenylindole, PBS - Gibco pH 7.4 phosphate buffered saline , IO - immersion oil, IMS – industrial methylated spirit 95% ethanol.

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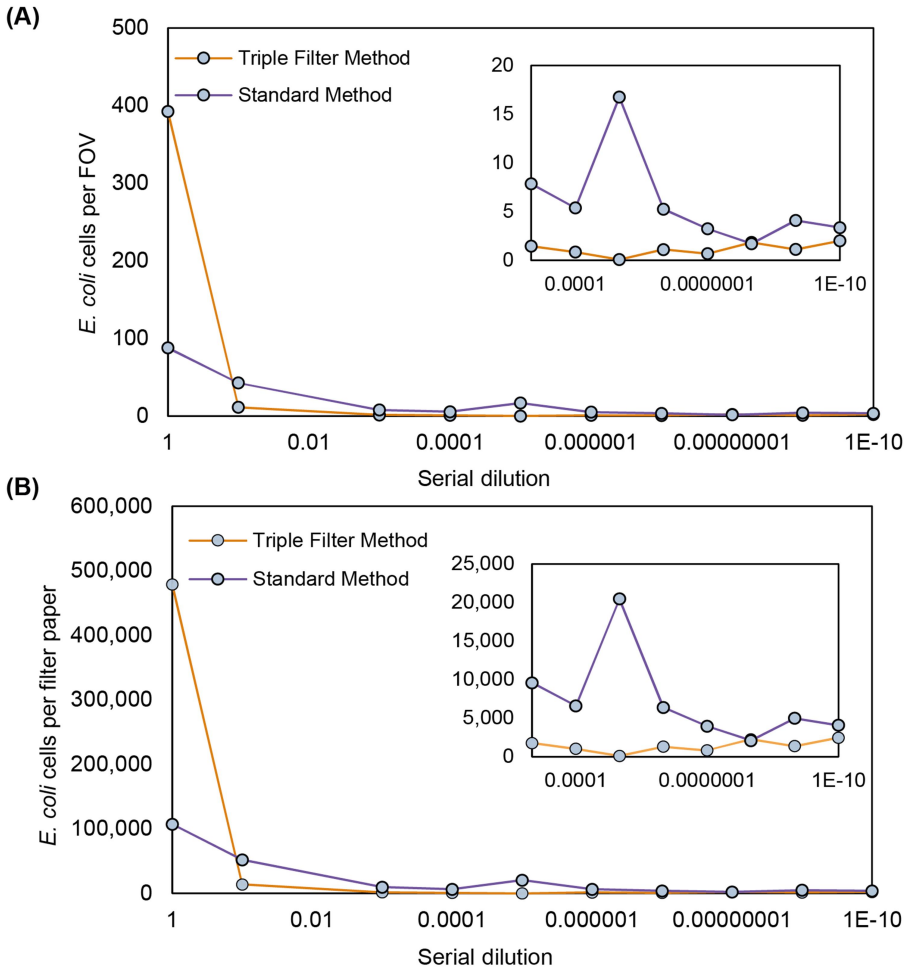

Figure 2 Calibration graphs of serial dilutions of *E. coli* to test background contamination levels. (A) Average cells per FOV in a ten-fold dilution series using the standard method and the triple method. (B) Average cells per filter paper in a ten-fold dilution series using the standard method and triple filter method.

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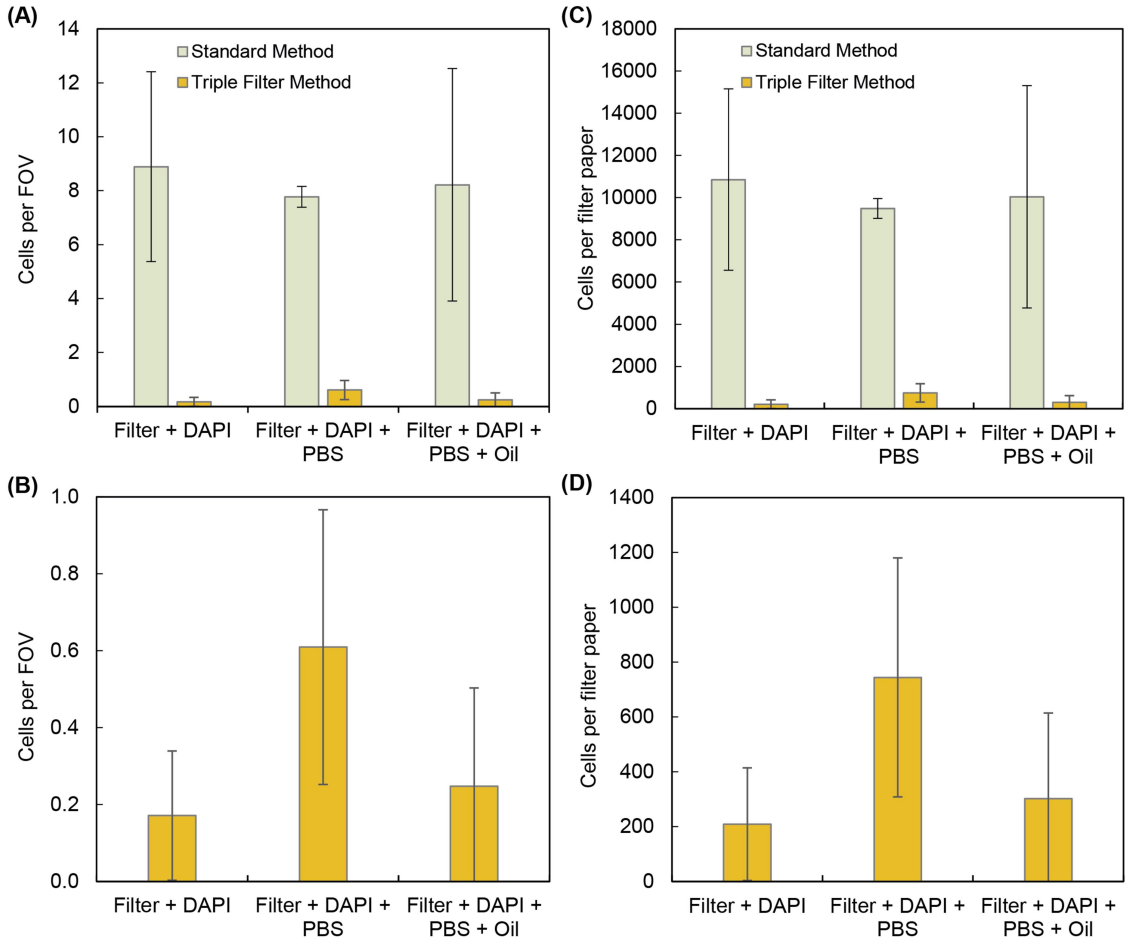

Figure 3 (A) Average cells per FOV of cell mounting components at each stage of the cell mounting process. Error bars represent the standard deviation. (B) Cells per FOV of the triple filtered consumables only. (C) Average cells per filter paper of cell mounting components at each stage of the cell mounting process. Error bars represent the standard deviation. (D) Cells per filter paper of the triple filtered consumables only.

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