

ANNEX: Methodology

Overview of Washing Methods

To assess microfibre release under controlled and reproducible conditions, two complementary and internationally recognised testing systems were used: the **GyroWash** (ISO 105-C06) and the **Wascator** (ISO 6330). Each method captures a different dimension of shedding, allowing both fibre counts and total fibre mass to be measured.

GyroWash Method (ISO 105-C06)

The GyroWash is a laboratory-scale laundering device consisting of sealed stainless-steel vessels partially filled with filtered water and ten stainless-steel balls, which provide controlled mechanical abrasion comparable to light domestic washing. In this study, each vessel contained 150 mL of water and washed a standardised **4 × 10 cm** fabric sample.

Because the washed surface area is fixed and clearly defined, the GyroWash enables precise and comparable fibre counting between materials. This method is well suited for quantifying the number and size of fibres released under controlled conditions and avoids bias associated with variable surface areas.

Wascator Method (ISO 6330)

To replicate full-garment domestic laundering, the same temperature, duration and level of mechanical action used in the GyroWash were reproduced using a **Wascator-type washing machine**, a standard front-loading system widely used in textile laboratories. The stainless-steel drum (≈520 mm diameter; 61 L volume) operates at programmable speeds of 20–59 rpm, with extraction speeds of 200–1100 rpm, maintaining water temperature within ± 1 °C.

The Wascator enables washing of whole garments, where the volume of shed fibres is too high to count individually. For this reason, mass-based fibre release is determined by weighing the filters before and after washing, allowing the total amount of shed material to be quantified accurately.

Rationale for Using Both Methods

Using both systems was essential to ensure methodological integrity. The GyroWash provides controlled, repeatable fibre counts from small, standardised samples, while the Wascator provides mass-based shedding data under realistic domestic washing conditions. Combining the two allows comparison between fibre number and fibre mass, strengthens scientific credibility, and makes the results more robust against methodological criticism.

Test Standards

The experiments were carried out in accordance with TS EN ISO 105-C06:2010 (*Textiles — Tests for Colour Fastness – Part C06: Colour Fastness to Domestic and Commercial Laundering*) and TS EN ISO 6330:2012 (*Textiles — Domestic Washing and Drying Procedures for Textile Testing*).

The A1S washing procedure defined in ISO 105-C06 was adopted, representing mild domestic laundering conditions at $40\text{ °C} \pm 2\text{ °C}$ for 30 minutes, using 150 mL of filtered water per test vessel and 10 stainless-steel balls ($\approx 6\text{ mm}$ diameter) to provide mechanical agitation and abrasion.

The same washing temperature, duration, and mechanical motion were reproduced in the Wascator system in accordance with ISO 6330 to ensure comparability.

No detergent or auxiliary chemicals were used, ensuring that fiber release originated solely from mechanical and thermal stresses.

Washing Devices

Two standardised washing systems were used:

GyroWash-type laboratory laundering unit — a sealed multi-vessel device conforming to ISO 105-C06, comprising two independent test baths, each containing eight stainless-steel test vessels (16 in total). Each test vessel (diameter $\approx 75 \pm 5\text{ mm}$; height $\approx 125 \pm 10\text{ mm}$; capacity $\approx 550\text{ mL}$) rotated at $40 \pm 2\text{ rpm}$ in a thermostatically controlled bath maintained at $40\text{ °C} \pm 2\text{ °C}$.

Wascator-type domestic laundering simulator — a front-loading, horizontal-drum washer conforming to ISO 6330. The stainless-steel drum (diameter $\approx 520\text{ mm}$, depth $\approx 315\text{ mm}$, volume $\approx 61\text{ L}$) operated at programmable washing speeds of 20–59 rpm and extraction speeds of 200–1100 rpm, maintaining temperatures between 4 °C and 97 °C ($\pm 1\text{ °C}$).

This system reproduced the A1S washing conditions on a domestic scale.

Sample Preparation

A total of 51 garments made from cotton, polyester, recycled polyester, polyamide, and recycled polyamide were examined. For GyroWash testing, specimens measuring approximately $100 \times 40\text{ mm}$ were prepared in accordance with ISO 105-C06 (A1S).

Each Wascator garment served as the source material for three GyroWash specimens ($4 \times 10\text{ cm}$ each), which were cut from representative regions of the same garment. After these subsamples were removed, the cut edges on both the remaining garment and the GyroWash specimens were sealed using a fast-curing adhesive to prevent fiber release from the open edges. This ensured that edge shedding was consistently controlled for both testing systems. The remaining garment portion (after subsampling) was rejoined along the cut lines and washed as a whole item in the Wascator, under equivalent temperature and duration parameters.

Thus, each garment provided both GyroWash and Wascator results, enabling direct comparison between laboratory-scale and domestic-scale laundering behavior.

Prior to washing, all specimens were conditioned at 20 ± 2 °C and 65 ± 5 % RH and weighed on an analytical balance with a precision of ± 0.001 g.

Among the 51 total samples, 11 garments (S13, S16, S34, S35, S36, S37, S38, S40-1, S40-2, S45, and S46) exhibited distinct printed areas or local structural variations.

Because a 4×10 cm cut would not represent their material heterogeneity, these garments were washed only in the Wascator system as whole pieces.

The remaining 40 garments underwent both tests:

GyroWash: three replicates per garment ($n = 3$), under A1S conditions.

Wascator: remaining garment body after GyroWash specimen removal.

This dual-scale experimental design allowed direct correlation of microfiber release between small-scale and full-scale laundering.

2.4 Washing and Rinsing Procedure

In the GyroWash system, the instrument consisted of two independent test baths, each containing eight stainless-steel test vessels. Each vessel was filled with 150 mL of filtered water, 10 stainless-steel balls, and one fabric specimen, and rotated for 30 min at $40 \text{ °C} \pm 2$ °C as specified in the A1S procedure.

To ensure contamination control, in each test bath seven vessels contained specimens, while one vessel (blank control) contained only water and steel balls. After washing, the liquor from this blank control vessel was filtered and microscopically examined for possible background fibers.

Following each run, the liquor from every test vessel was poured into the filtration system. The inner walls of the test vessels were rinsed with filtered water using a handheld pressure sprayer, and the rinsate was also filtered to ensure total fiber recovery.

For the Wascator tests, the same temperature and duration were applied.

Ballast fabrics were enclosed in triple-layer leak-proof polyethylene bags to prevent external fiber transfer.

After each washing cycle, the Wascator performed an automatic 120-second rinse program to flush any remaining fibers from the drum. The rinse water was collected and combined with the wash effluent of the corresponding sample for analysis.

2.5 Effluent Collection and Filtration

All wash and rinse effluents were filtered using a vacuum manifold filtration apparatus (ISOLAB). Before filtration, the Wascator effluents passed through a plankton-net prefilter to retain larger debris.

The filtration assembly employed Milipore™ mixed cellulose ester (MCE) filter membranes (filters) with a pore size of 0.45 μm and 47 mm diameter, mounted on stainless-steel filtration bases under -0.7 bar vacuum pressure.

The wash liquor and subsequent the post-wash rinse water obtained from the GyroWash tests were both transferred to the same filtration setup. Similarly, the combined wash and 120-second rinse effluents from the Wascator were filtered through the same apparatus.

After each filtration, the filters were carefully removed using stainless-steel forceps, placed in sterile Petri dishes, and air-dried at room temperature.

Fiber Quantification and Analysis

Each filter contained approximately 130 grid squares. 13 randomly selected fields were examined under an optical microscope, and the mean fiber count was extrapolated to the total filter area.

The number of released fibers was expressed as fibers per gram of fabric, while fiber length (μm –mm) was measured using a calibrated microscope scale.

For the Wascator tests, filters were air-dried at room temperature and reweighed to calculate mass-based fiber release (mg of fibers per g of fabric). All values were expressed as the mean $\pm$ standard deviation from three replicates.

Data processing and calculations

This section details the equations used for quantifying fiber release in both GyroWash and Wascator tests. Equations are formatted for publication and include full unit definitions.

Notation (units)

n_i : counted fibers in grid field i

$k = 13$: number of counted fields

$G = 130$: total number of grid fields on the filter

l_j : length of fiber j (mm); m : number of measured fibers

w : tested sample mass for GyroWash (g)

W : Wascator sample mass (g)

M : Wascator shed mass (g)

B : blank control fibers (count)

b : blank mass (g)

GyroWash (fiber number & length)

The total number of released fibers (N_{filter}) was estimated from counts in 13 out of 130 grid fields and extrapolated to the whole filter area. The normalized fiber release (R_{count}) was calculated by dividing N_{filter} by the mass of the tested specimen (w , in g).

A1) Extrapolated total fibers on the filter

$$N_{\text{filter}} = (G / k) \times \Sigma(n_i) = (130 / 13) \times \Sigma(n_i) = 10 \times \Sigma(n_i)$$

$$\text{Blank correction: } N_{\text{filter,corr}} = F_{\text{filter}} - B$$

A2) Normalized fiber release (fibers per gram)

$$R_{\text{count}} = N_{\text{filter,corr}} / w \quad (\text{fibers} \cdot \text{g}^{-1})$$

A3) Mean fiber length

$$\text{Mean length: } \bar{\ell} = (1/m) \Sigma \ell_i$$

$$\text{Standard deviation: } s_{\ell} = \sqrt{[(1/(m-1)) \Sigma (\ell_i - \bar{\ell})^2]}$$

Wascator (mass-based)

$$R_{\text{mass}} = ((M - b) / W) \times 1000 \quad (\text{mg} \cdot \text{g}^{-1})$$

Replicate statistics (n = 3)

$$\text{Mean: } \bar{x} = (1/3) \Sigma x_r$$

$$\text{Standard deviation: } s_x = \sqrt{[(1/2) \Sigma (x_r - \bar{x})^2]}$$

Report as: $\bar{x} \pm s_x$

Cleaning and Cross-Contamination Control

GyroWash control: After each washing cycle, all test vessels were rinsed with filtered water. Each eight-vessel bath included **one blank control vessel** containing only water and steel balls.

The filtered liquor from these control vessels was analyzed microscopically.

Wascator control: After each washing run, the machine automatically performed a 120-second rinse program, and the rinse water was filtered and analyzed together with the corresponding sample effluent. This ensured that any fibers retained inside the drum were included in the results and that subsequent tests began with a clean system.

Both cleaning controls confirmed that all fibers collected originated exclusively from the tested textile specimens, guaranteeing the reliability and reproducibility of the experimental data.