

HyWa-CHECK BIOMONITORS⁺

Risk class 1 Biomonitorers for the assessment of Process Hygiene in textile washing processes



HyWa-Check Biomonitorers with *Staphylococcus arlettae* 10⁵

Art. Nr. 503

HyWa-Check Biomonitorers with *Enterococcus faecium* 10⁵

Art. Nr. 504

HyWa-Check Biomonitorers with *Pseudomonas fluorescens* 10⁵

Art. Nr. 505

HyWa-Check Biomonitorers with *Saccharomyces cerevisiae* 10⁴

Art. Nr. 506



Introduction:

Due to changing washing habits in household laundry (like the extended use of liquid detergents instead of heavy duty powder detergents, the decreasing washing temperatures and the lowering water consumption) a more critical hygiene situation in household laundry is expected. This is probably not an issue for healthy persons, but for babies, elderly, pregnant and immunocompromised persons, it could serve as health risk. Ill family members (influenza, diarrhea, athlete's foot) can be as well a risk for sufficient laundry hygiene. With the HyWa-Check biomonitorers, we provide a useful tool for the surveillance, the hygiene assessment and the development of textile washing processes. The use of risk class 1 test germs ensures a safe carrying out of the test.

Description:

HyWa-Check Biomonitorers are used for the quantitative hygiene assessment of textile washing processes. The cotton swatches are contaminated with a minimum microbial load of 1x10⁵ germs per swatch (Bacteria) and 1x10⁴ germs per swatch (Yeast). The behaviour of the used risk class 1 test germs have been compared and adjusted to reference risk class 2 test germs of the most commonly used standards and guidelines (RKI / DGHM / NSF P172) and reach comparable reduction rates.

Analysis:

The analysis of the biomonitorers is variable. For a quantitative result, it is recommended to dissolve the biomonitorers in a buffer, perform a dilution series, plate the single dilutions and determine the microbial plate counts.

The biomonitorers can alternatively be shipped to a microbiological lab for analysis and determination of microbial reduction rates.

Advantages:

The use of risk class 1 germs has several advantages. The transnational shipment of the biomonitors is possible. The handling is simplified and safe and the HyWa-Check biomonitors can be applied without having a fully equipped microbiological lab. No potentially dangerous germs will be implemented in washing machines due to the hygiene assessment tests. A variable analysis allows an optimization of accuracy and costs of the tests.

Validation:

The HyWa-Check Biomonitors reach comparable results as the NSF Protocol P172 "Sanitization Performance of Residential and Commercial, Family-Sized clothes washers" (Test report Nr. 201020102 NSF/EMPA). This test series were done in cooperation with NSF International.

Protocol performance of Hygiene control of a laundry cycle and determination of microbial counts of the biomonitors by microbial plating:

1. 8 biomonitors were used per test germ and washing cycle.
2. For one washing process, 5 biomonitors per test germ are transferred to a blue cotton bag. This is done with sterile forceps..

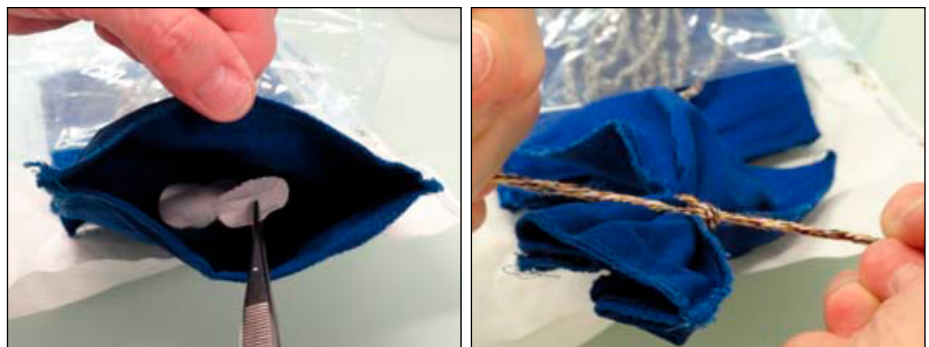


Fig. 1: Per test germ, 5 biomonitors are transferred into a blue cotton bag. The cotton bag is sealed.

3. The blue cotton bag is placed in the middle of the load and the washing process is fully processed.

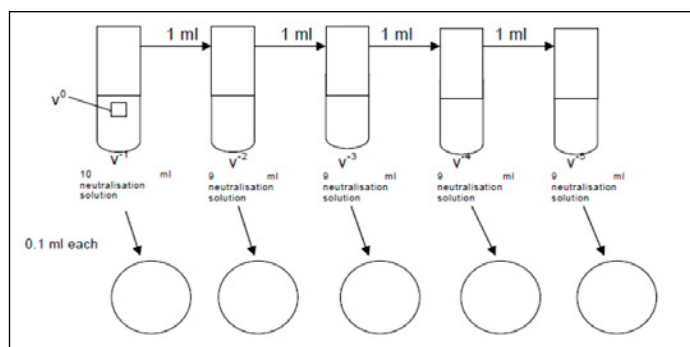


Fig. 2: The cotton bags are placed in the middle of the load without touching each other

4. After the washing process, the blue cotton bag is opened on a clean surface (e.g. a sterilized surface, vessel, foil). The biomonitors are taken out with sterile forceps and every single biomonitor is added to a buffer vial (phosphate buffer with 1 % Tween and 0.2 % Sodium thiosulfate pentahydrate). The vial is marked with the name of the test germ and if the biomonitor represents the initial counts or went through the washing process.
5. Three biomonitors are the initial counts and do not go through the washing process. Each of the three biomonitors are added to a buffer vial and the vial is marked with

the name of the test germ and if the biomonitor represents the initial counts or went through the washing process.

6. Every biomonitor is shaken on a Vortex shaker for 1 minute. After that, it is left for 60 minutes at room temperature, and then it is again 1 min shaken on a Vortex shaker.
7. With the biomonitor solution, a dilution series is done. For that, 1 ml was taken out of the solution with a sterile pipette and is transferred into a vial containing 9 ml Phosphate buffer solution. This vial is shortly shaken on the Vortex. Then again 1 ml is transferred to another 9 ml Phosphate buffer vial up to dilution v^5 .



8. 0.1 ml of every dilution is transferred to an agar plate, and is plated out evenly with a Drigalski spatula. For the bacterial strains tryptic soy agar (TSA) and for the *Saccharomyces cerevisiae* Sabouraud dextrose Agar (SDA) is used. For every plate a sterile Drigalski spatula shall be used.
9. The agar plates are incubated at 30 °C (*Saccharomyces cerevisiae*) and 36 °C (*Pseudomonas fluorescens*, *Staphylococcus arlettae*, *Enterococcus faecium*) for 1 to 5 days.
10. Agar plates with 15 to 300 colonies are counted and used for calculation of the reduction rate.
11. Calculation of microbial load of N_0 and N . The microbial load of the single biomonitors is calculated as follows:

Microbial counts plate 1 = (microbial counts plate 1 / $10^{\text{dilution step}}$) * 10 If two plates can be used for the calculation of the microbial load:
 Microbial counts = (microbial counts plate 1 + microbial counts Plate 2) / 2
 Calculation of the arithmetic mean of N_0 (3 biomonitors before laundry process) and N (5 biomonitors after laundry process)
 Calculation of the standard deviation of N_0 (3 biomonitors before laundry process) and N (5 biomonitors after laundry process)

12. Calculation of the microbial reduction = $\log(N_0) - \log(N)$

Protocol performance of Hygiene control of a laundry cycle and microbial analysis in a microbial lab:

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2. For one washing process, 5 biomonitors per test germ are transferred to a blue cotton bag. This is done with sterile forceps.



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- 6. The biomonitors are shipped to Swissatest Testmaterials AG (Lerchenfeldstrasse 5, CH-9014 St. Gallen) or another lab. The parcel shall be sent as express delivery and should arrive in the lab within 24 hours. In summertime, the parcel shall be kept cool by cooling elements.*
- 7. Results: The microbial reduction is reported per biomonitor as well as an average microbial reduction rate inclusive the standard deviation per test germ.*

Quality:

A quality control certificate is enclosed to every biomonitor shipment with the following information: microbial load of the biomonitors and reduction rate in a reference washing test, ATCC strains, date of expiry, and lot-number.

Shipment:

Within 2–3 working days, in an isolation box, cooled.

Storage:

In sealed vials at 4–7 °C.

Shelf life:

6 weeks after production date.

Price:

Please take the current prices at www.swissatest.ch

Publications:

Amberg, C., Faeh, D. and Frey, F (2010). Novel Method to Assess the Sanitization Efficacy of Laundry Processes (HyWa-Check). Poster Presentation AOCS Montreux 2010.

Amberg, C. (2011). HyWa-Check – Novel Screening tool to assess the process hygiene of household washing cycles. Presentation at IDC Conference in Düsseldorf, Mai 2011.

Amberg, C. (2011). Hygiene performance of household washing machines – New protocols to assess process hygiene and biofilm removal. Presentation at Sepawa Austria in Salzburg, Mai 2011.

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