



Growing competition from lower cost suppliers in the Far East has forced the predominantly small- and medium-sized enterprises that comprise Europe's footwear industry to adopt innovative features and optimise comfort. One area to receive increased attention is the microbial resistance of upper leathers and, as a consequence, the test methods to ensure their quality in this respect.

Microbiological testing of leather

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The inclusion of anti-microbial active agents in shoe upper materials is relevant to comfort. The main aim is to protect finished leather against infection by micro-organisms and any resulting change in colour and deterioration in quality. Mould is the biggest danger. These agents also reduce undesirable odours caused by microbial decomposition of perspiration and the growth of micro-organisms in the damp environment found in footwear. If critical germs are present, bacterial infections or even Athlete's Foot may result. Agents can also prevent dispersion of pathogens.

Shoe manufactures, supported by increasingly hygiene-aware consumers, are therefore demanding anti-microbial properties. There are also critics. Some say the use of anti-microbial substances is inappropriate, as their effect is not fully proven. Others even say the agents could have a toxic effect or even cause skin damage. Whatever your stance, unlike for textiles, there are no established standards or test methods to assess the activity of anti-microbial finishes in leather. As a result, those designed for the textile sector have been adapted and improved for use on leather by PFI Pirmasens, Germany, in a project funded by the Stiftung Rheinland-Pfalz für Innovation.

Targets

The efficiency of added agents was investigated with regard to the effective germ spectrum as well as intensity. An adequate method is now available for the leather and footwear industry which is able to supply information on:

- Material characteristics
- Anti-bacterial effectiveness against bacteria and micro-fungi
- Intensity and inhibition of growth (biostatic and sterilisation/death of germ cells such as biozid)
- Quality assurance of end products

Method

Sample leathers—with and without added anti-microbial agents—were examined. These included chrome and vegetable tanned samples of suede, nubuck, smooth, patent and other types of upper leather, plus lining and soling leathers. Different anti-microbial finishes included chemicals, silver and hydrophobing agents.

To cover a representative spectrum of micro-organisms, the gram-positive bacterium *Klebsiella pneumoniae* and the gram-

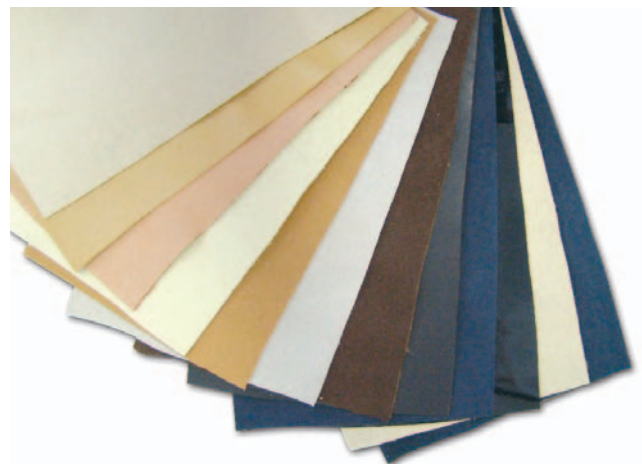


Figure 1: Leather samples with different anti-microbial properties

negative bacterium *Staphylococcus aureus* were chosen as test germs. Amongst fungi, dermatophyte *Trichophyton mentagrophytes* (known as Athlete's Foot), the yeast *Candida albicans* and the mould *Aspergillus niger* were examined. This wide spectrum made it possible to test both anti-bacterial and anti-mycotic (anti-fungal) effects. The selection of the test-organisms was based on potential material damage and possible health risks to humans, including established reference strains. Several approaches were taken.

A qualitative agar diffusion test, two further quantitative test systems (micro and flask) based on the challenge method, plus corresponding anti-mycotic methods. The latter was adapted to the requirements of micro-fungi. The respective tests were based on existing standards for textiles, adapted and standardised separately for specific leather features, and for each reference strain.

Tests

An agar diffusion test to quantify the anti-microbial effect is the first priority. For this, it is necessary to have a minimal diffusion ability of the effective agent into the nutrient agar. Leather samples are applied flat on to a plate that have been previously inoculated with a determined bacterium or fungus and then incubated for a defined length of time at a certain ➤

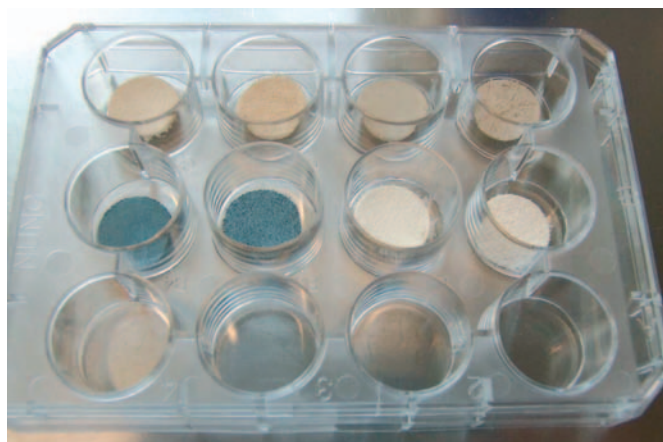


Figure 2: Micro test

temperature. Afterwards the halo (an inhibition-zone around the sample) is measured. (Samples should always be tested on both sides.)

If the anti-microbial active agent is not known, this test can indicate a negative result due to missing diffusion or immobilisation of the agent. This does not necessarily exclude anti-microbial effectiveness, so this test on its own is not sufficient to prove an anti-microbial effect. It should therefore only be regarded as a pre-test, providing a relatively quick qualitative evaluation. (Full verification by a challenge test is essential.)

Two further quantitative challenge tests were therefore developed. These examine the anti-microbial features of a leather and state its intensity and efficiency. During a challenge test, samples are contaminated with a specific test germ suspension. Immediately after inoculation and, at different defined points, the germs are washed off. After inactivation of the active agent, the total number of viable cells is determined as a colony forming unit (cfu). Over a defined period the percentage germ reduction and anti-microbial effects (AE) are quantified.

The micro test makes it possible to differentiate between the upper surface and the underside, as the samples are merely wetted with germ solutions. Contamination is thus effectively simulated. Samples are cut to a size of $16\text{mm} \pm 1\text{mm}$ diameter to fit micro-titerplates and make for easier handling, simplified engineering and a reduced health risk for technicians.

With the flask test, the sample size is $25\text{mm} \pm 5\text{mm}$ in diameter. Test pieces are placed in 250ml Erlenmeyer flasks with 50ml working solution containing a defined cell number of the test strain or fungal spores respectively. They are then incubated in these liquid cultures for 24 hours at a temperature dependent on the strain under test. 37°C is used for bacteria and 25°C or 30°C for different fungi. The advantages of this method are the direct comparison with already established procedures for textiles and the small amount of leather required.

The downside is the impossibility to differentiate between the upper surface and underside. To test the anti-mycotic character of a leather, these test methods were extended and altered to meet the needs of fungi. Mould stability of an agent-treated leather was also examined according to standard ASTM D 4576 (1996) over a three-week period. Tests revealed a susceptibility to mould on the underside in 50% of the samples, while some did not resist the formation of mould on the grain surface.

The influence of perspiration was also studied, as it is responsible for undesirable odours after microbial decomposition. Here, the leathers were saturated in technical



Figure 3: Flask test

perspiration (acidic and alkaline) to simulate an appropriate burden and subsequent colonisation by micro-organisms. The presence or absence of technical perspiration did not lead to any change in results using the methods described.

Conclusion

Anti-microbial finishes are often not specified and their formulae not revealed, so it is difficult to determine which work the best. It would assist all parties concerned with development, manufacturing, testing or selling, if this information was made more available. In the meantime, any agents used should be biostatic with respect to bacteria and mould but have no biocidal effects. They should also be capable of reducing germ numbers from an initial inoculation of 10,000 cells or spores to only 10 to 100 that are viable after 24-hours of incubation.

Some tanners and leather finishers already use anti-microbial agents and the number is growing as demand increases. In any event, all tanners need to include fungicides during the tanning process to remove and avoid mould. This sometimes results in an overlay of effects thanks to residuals being combined with additional anti-microbial agents that will then be active in the finished retail product. Mould is most likely to appear during the tanning and finishing processes. These should be carefully regulated to avoid the risk of microbial infection—especially bacterial and dermatophytes—in the case of lining materials or any that are in direct contact with bare skin, such as in summer sandals.

Investigations of worn shoes have shown a high concentration of germs and bacteria, dermatophyta, yeasts and moulds both inside and outside, which clearly demonstrates a need for treatment. Some leathers sold with anti-microbial finishes still show surprisingly poor anti-microbial performance. This may be due to a low concentration of the agents, poor anchorage within the leather or a masking of the effect through later manufacturing processes. On the other hand, some without specific treatment are shown to be, at least partially, active. This might be as a result of tanning residues or a passive anti-microbial effect from factors such as hydrophobation.

It is therefore important that consistent test methods are used to assess the activity of anti-microbial agents in or on any particular leather. On the one hand it is necessary to avoid over-spill or a lack of anti-microbial agents. On the other, it is essential to exclude a possible masking of anti-microbial efficacy in the end product through later overlays. Moreover, where this factor is required, an on-going guarantee of biocompatibility should be provided and this calls for regular investigations. 🌐