SRI LANKA STANDARD 623:1983

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METHODS FOR TESTING THE RESISTANCE OF LEATHER TO SURFACE FUNGAL GROWTH

BUREAU OF CEYLON STANDARDS

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SLS 623:1983

Gr. 6

BUREAU OF CEYLON STANDARDS
53, Dharmapala Mawatha,
Colombo 3,
Sri Lanka.

SRI LANKA STANDARD

METHODS FOR TESTING THE RESISTANCE OF LEATHER TO SURFACE FUNGAL GROWTH

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Bureau of Ceylon Standards on 1983-12-20, after the draft, finalized by the Drafting Committee on Tanned Leather, had been approved by the Chemicals Divisional Committee.

Surface fungal growth does not physically weaken the leather but is objectionable for aesthetic reasons or for its adverse effects on surface properties.

All standard values given in this specification are in SI units.

In reporting the results of a test or analysis made in accordance with this specification, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with **CS 102**.

In the preparation of this specification, considerable assistance derived from the publications of the Australian Standards Association is gratefully acknowledged.

1 SCOPE

This method describes the procedure to be adopted for determining the resistance of leather to surface-fungal growth.

2 REFERENCES

CS 102 Presentation of numerical values

SLS 403 Leather - Laboratory samples, location and identification.

3 PRINCIPLE

Test specimens are inoculated with a mixed spore suspension and then incubated for a period of 14 days. At the end of the incubation period the fungal growth on the specimens and on the test control medium is assessed.

4 APPARATUS AND TEST ORGANISMS

4.1 Apparatus

4.1.1 Apparatus as specified in Appendices A, B and C for the following;

a) Leaching of *test specimens*; (Appendix A)

b) Preparation of the *test medium*; (Appendix B)

c) Preparation of the *spore suspension*; (Appendix C).

4.1.2 A forced *air circulating oven*, capable of continuous operation at 50 ± 2 °C.

4.1.3 *Test vessels*, petri dishes having an internal diameter of approximately 90 mm are satisfactory, but other suitable vessels providing sufficient air above the test specimen may be used.

4.1.4 *An incubator*, capable of continuous operation at a temperature of 30 ± 2 °C at a relative humidity in excess of 90 per cent.

4.1.5 Where *sterile equipment* is specified, it shall be sterilized either;

- in an oven at 170 °C to 175 °C for not less than 1 hour; or
- in an autoclave at 121 ± 1 °C for not less than, 20 min.

4.2 Test medium

The test medium shall be prepared using the procedure and reagents described in Appendix B.

4.3 Test organisms

The test organisms used for the preparation of the spore suspension is *aspergillus niger*.

5 TEST SPECIMENS

5.1 Sampling

Four representative samples shall be selected from the batch of leather being examined in accordance with SLS 403.

5.2 Selection of test specimens

Six test specimens having a minimum dimension of 50 mm shall be cut from each of the four samples of leather. The thickness of any test specimen shall be approximately the same as that of the sample from which it was obtained.

5.3 Test control medium

The test control medium shall be prepared using the procedure and reagents described in Appendix B, together with 10 g of sucrose per litre. The test control medium shall then be sterilized and transferred to sterile petri dishes. Two petri dishes containing the test control medium shall be prepared for this test.

6 PROCEDURE

6.1 General

Two test specimens from each sample of leather are leached, two are heated and the remaining pair are not pre-treated in any way. The specimens are then pressed onto the test medium, inoculated, incubated and the fungal growth assessed on the grain and flesh sides of the leather.

6.2 Leaching

Two test specimens from each of the four samples of leather shall be leached in water for 48 h as described in Appendix A and allowed to dry in moving air for five days.

6.3 Heating

Two test specimens from each of the four samples of leather shall be heated for seven days at 50 $\pm$ 2 ^{o}C using forced air circulation and allowed to cool in air.

6.4 Transfer of specimens to test vessels

The test specimens shall be transferred under aseptic conditions into the prepared petri dishes containing the test medium, one test specimen being placed centrally in each petri dish. The test specimens shall be so placed in the petri dishes that one of each pair will be grain-side up and one flesh-side up. The test specimens shall be pressed firmly onto the medium using a sterile instrument.

6.5 Inoculation

The test control media and the exposed surfaces of the test specimens shall be inoculated by spraying or pipetting thereon a mixed spore suspension of the test organism specified in 4.3 and prepared as in Appendix C. The petri dishes shall then be re-covered.

6.6 Incubation

The petri dishes shall be incubated for 14 days at 30 ± 2 °C at a relative humidity in excess of 90 per cent.

To obtain reasonable control of the temperature, the use of a water-jacketed incubator is recommended. The relative humidity can usually be maintained within the test vessels by virtue of the water in the medium. However, to reduce the drying rate of the medium, it is a customary precaution to have open trays of water in the incubator.

6.7 Assessment of surface fungal growth

Fungal growth on test specimens and test control medium shall be assessed, immediately after the incubation period, on a scale of 0 to 5, by comparing each specimen with the reference prints in Fig. 1. A rating of 0 represents complete absence of fungal growth and a rating of 5 represents complete coverage of the surface in the affected areas. If any trace of fungal growth occurs the minimum assessment for that specimen shall be 1.

The reference prints show maximum contrast in colour between the fungal growth and the specimen. In using them for assessment, due allowance shall be made for the colours of the specimens and of the fungal growth; for the dampness of the specimens; and for surface irregularities of the specimen, all of which may give less visible contrast than the prints. Assessment should be based on completeness of surface coverage and density of growth, rather than on degree of discoloration.

Where the surface of the specimen shows two or more regions having sharply differentiated and non-intergrading densities of growth, the report shall state the assessment of fungal growth separately for all major areas affected together with a brief description of the shape and position of the areas covered.

Any additional observations on the fungal growth on or around the outside of the specimens shall be reported.

All specimens shall be sterilized as soon as possible after assessment. The specimens may then be used for evaluating the change in some property or may be discarded.

7 REPORT

The report shall state the individual fungal growth assessment for each test specimen and for each test control medium. The pH of the water used for leaching shall also be reported.

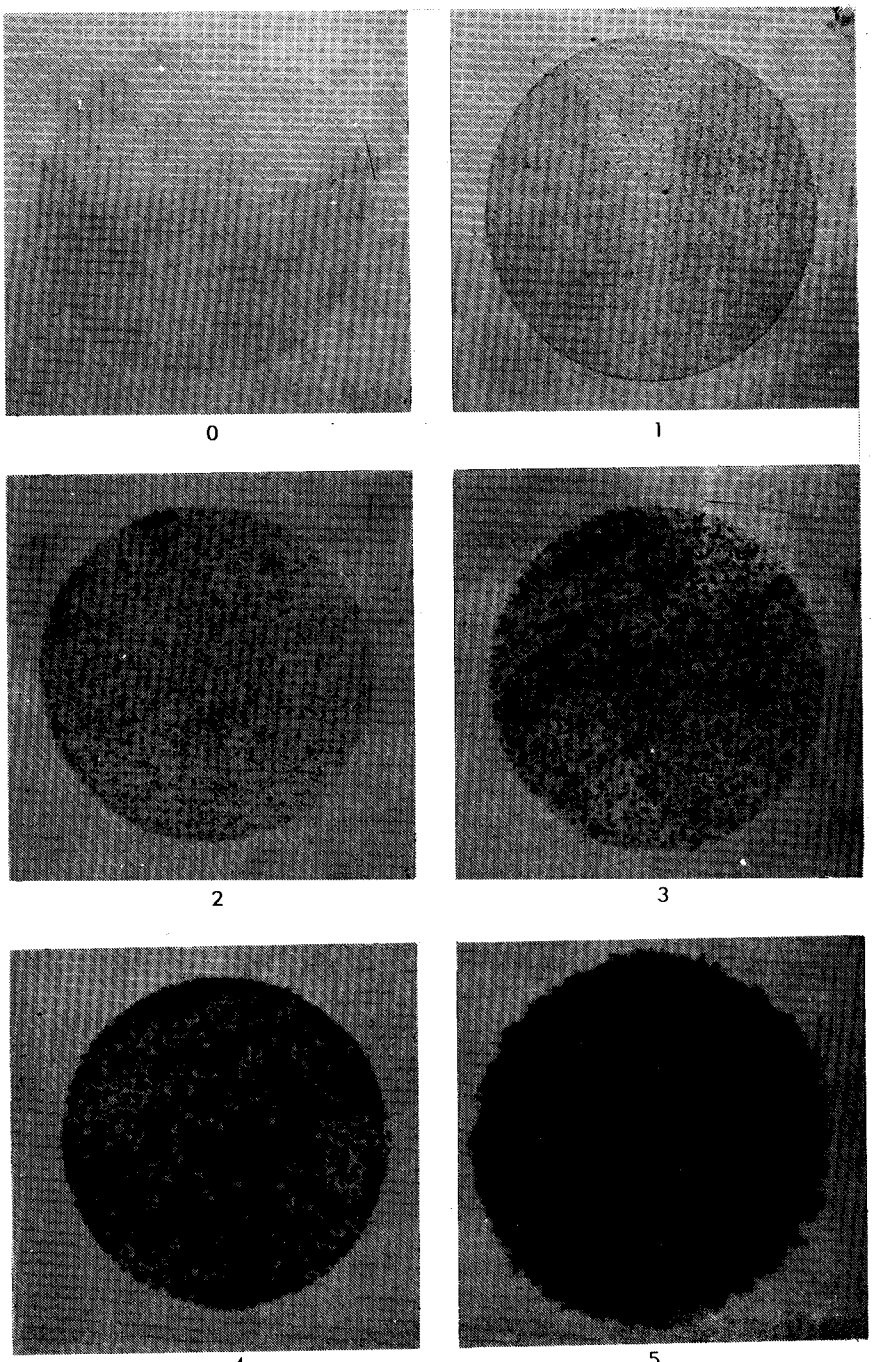

Fig. 1. REFERENCE PHOTOGRAPHS FOR RATING THE EXTENT AND INTENSITY OF FUNGAL GROWTH

APPENDIX A

METHOD FOR LEACHING TEST SPECIMENS

A.1 PRINCIPLE

The test specimens are immersed in a vessel through which water flows continuously.

A.2 APPARATUS

A.2.1 *Vessel*, preferably of glass, of such shape and size that the specimens can be completely immersed in water when the vessel is full, with the water making contact with all surfaces of each specimen.

A.2.2 Means for suspending specimens in the water so that they are completely immersed without touching one another or any part of the container.

A.2.3 Means for supplying a continuous flow of filtered water to the bottom of the container at a temperature of 27 ± 2 °C.

A.3 SPECIMENS

The specimens shall be as specified in the standard.

A.4 PROCEDURE

The leaching vessel shall be filled with water and the specimens mounted in it. Only specimens of the same kind shall be placed in the vessel at one time and care shall be taken to ensure that the water makes contact with all parts of all specimens. The rate of water flow shall be adjusted so that a mass of water equivalent to 100 times the mass of the specimens flows through the container per hour or to a rate of 2 l/h, whichever is the greater. Leaching shall be continued for the period specified in the particular method, after which the specimens shall be removed from the vessel and allowed to dry in air.

NOTE - Some methods require that the pH value of the water used for leaching be reported.

APPENDIX B

METHOD FOR PREPARATION OF SOLID TEST MEDIA

B.1 PRINCIPLE

An agar medium containing the necessary nutrient compounds is prepared and sterilized by heating.

B.2 APPARATUS AND REAGENTS

B.2.1 *Autoclave* to operate at a steam pressure equivalent to 121 °C (approximately 105 KPa gauge steam pressure).

B.2.2 *Erlenmeyer flasks* of capacity 200 ml to 250 ml with sterile cotton wool plugs.

B.2.3 Large *beakers*.

B.2.4 The medium specified below shall be used.

a)	*Ammonium nitrate*	2.7 g;
b)	*Potassium dihydrogen phosphate*	0.9 g;
c)	*Dipotassium hydrogen phosphate*	0.7 g;
d)	*Magnesium sulphate heptahydrate*	0.5 g;
e)	*Potassium nitrate*	0.5 g;
f)	*Agar*	15.0 g; and
g)	*Water*	1 litre.

B.2.5 For test control medium (see **5.3**), 10 g of *sucrose* per litre shall be added.

B.3 PROCEDURE

The quantities of chemical reagents and agar as specified shall be added to the cold water in a large beaker. After allowing to stand for 15 minutes, the mixture shall be stirred and heated to near boiling until the agar dissolves. It shall then be poured into bottles or flasks, so that they are not more than two-thirds full. The caps shall be screwed onto the bottles (or the cotton plug placed in the flask) and these containers placed in the autoclave.

The media so prepared shall be sterilized in an autoclave at a temperature of 121 °C for 15 minutes. Transfer the media to sterile petri dishes.

APPENDIX C

METHOD OF PREPARATION OF SPORE SUSPENSIONS

C.1 PRINCIPLE

Spores are transferred from the surface of actively sporing fungus cultures into sterile water, and the resulting suspension filtered.

C.2 APPARATUS, REAGENTS AND TEST ORGANISMS

C.2.1 *Sterile glass rod* or *sterile glass beads.*

C.2.2 *Sterile muslin gauze* and a *sterile filter funnel.*

C.2.3 *Sterile spray bottle* or other *container* for the suspension.

C.2.4 *Sterile water,* or a *sterile aqueous solution* of *wetting agent.* (see Note 2).

C.2.5 Culture of the fungus specified in the standard (see **4.3**). The cultures shall be on agar shapes, freely-sporing and not more than 21 days old, but preferably 10 to 15 days.

C.3 PROCEDURE

Add a small quantity of sterile water to a culture of the fungus, for example: 5 ml to 10 ml for a standard size (that is 150 mm^2 x 160 mm) test tube. The spore shall be detached from the surface of the culture by rubbing with a sterile glass rod or by shaking with sterile glass beads, and the suspension so produced shall be filtered into a sterile spray bottle through a sterile muslin gauze taking the usual aseptic precautions.

The suspension shall be diluted not more than ten-fold with sterile water. If a wetting agent would assist the *wetting* of the specimen by the spore suspension, this final dilution may be made with a sterile solution of a surfactant which does not inhibit or encourage fungal growth.

CAUTION - Care should be taken when handling spores and spore suspensions as the inhalation of spores, may cause serious illness.

NOTES

1 Spore suspensions must be used within 8 hours of their preparation and destroyed when they are no longer require.

2 A 0.1 per cent (active ingredient) solution of nonyl phenol ethylene oxide condensate has been satisfactorily used as a wetting agent in these tests.

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