
**Traditional Chinese medicine —
Determination of microorganisms in
natural products**

*Médecine traditionnelle chinoise — Détermination des micro-
organismes dans les produits naturels*



COPYRIGHT PROTECTED DOCUMENT

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	v
Introduction	vi
1 Scope	1
2 Normative reference	1
3 Terms and definitions	1
4 Symbols and abbreviated terms	1
5 Test methods	2
5.1 General	2
5.2 Strains	2
5.3 Test for sterility	2
5.3.1 General	2
5.3.2 Culture media and incubation temperatures	2
5.3.3 Growth promotion test for aerobes, anaerobes and fungi	3
5.3.4 Method suitability test	3
5.3.5 Test for sterility of the product to be examined	4
5.4 Microbiological examination of non-sterile products: microbial enumeration tests	7
5.4.1 General	7
5.4.2 Growth promotion test, suitability of the counting method and negative controls	7
5.4.3 Testing of products	13
5.4.4 Interpretation of the results	14
5.5 Microbiological examination of non-sterile products: tests for specified microorganisms	14
5.5.1 General	14
5.5.2 Growth-promoting and inhibitory properties of the media, suitability of the test and negative controls	15
5.5.3 Testing of products	17
6 Acceptance criterion of test methods	20
6.1 Acceptance criterion of test for sterility	20
6.1.1 Acceptance criterion of sterility in test for culture medium	20
6.1.2 Acceptance criterion of growth promotion test of aerobes, anaerobes and fungi	21
6.1.3 Acceptance criterion of method suitability test	21
6.1.4 Acceptance criterion of test for sterility	21
6.2 Acceptance criterion of microbial enumeration tests in microbiological examination of non-sterile products	21
6.2.1 Acceptance criterion of preparation of test strains in microbiological examination of non-sterile products: microbial enumeration tests	21
6.2.2 Acceptance criterion of negative control in microbiological examination of non-sterile products	21
6.2.3 Acceptance criterion of media suitability in microbiological examination of non-sterile products	22
6.2.4 Acceptance criterion of the method suitability in microbiological examination of non-sterile products	22
6.2.5 Acceptance criterion of the validity of the results in microbiological examination of non-sterile products	22
6.3 Acceptance criterion of tests for specified microorganisms in microbiological examination of non-sterile products	22
6.3.1 Acceptance criterion of preparation of test strains in microbiological examination of non-sterile products: tests for specified microorganisms	22
6.3.2 Acceptance criterion of negative control in microbiological examination of non-sterile products: tests for specified microorganisms	22

6.3.3	Acceptance criterion of media suitability in microbiological examination of non-sterile products: tests for specified microorganisms	22
6.3.4	Acceptance criterion of the method suitability in microbiological examination of non-sterile products: tests for specified microorganism	23
6.3.5	Acceptance criterion of the validity of the results in microbiological examination of non-sterile products	23
Annex A (normative) Microbiological quality of natural products		24
Annex B (informative) Recommended solutions and culture media		35
Bibliography		40

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Natural products used in traditional Chinese medicine are widely used around the world due to their high medicinal values and mild side effects. It is a common phenomenon that natural products are contaminated by microorganisms which not only impact their quality and efficacy, but also restrict the international trade in them and related products. Although the Pharmacopeia of the People's Republic of China, the British Pharmacopoeia, the Japanese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopeia have stipulated the microbial limits of natural products, there is no International Standard for microorganism detection methods, which adversely affects communication and trade between researchers and factories in different countries. Furthermore, microorganism levels on or in natural products usually exceed the maximum limit levels set by many international organizations and countries due to the lack of an International Standard.

Traditional Chinese medicine — Determination of microorganisms in natural products

1 Scope

This document specifies test methods to determine microorganisms in natural products. It is applicable only to natural products used in traditional Chinese medicine, including raw materials, herbal pieces and preparations.

2 Normative reference

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

sterility

state of being free from viable microorganisms

Note 1 to entry: In practice, no such absolute statement regarding the absence of microorganisms can be proven.

[SOURCE: ISO 11139:2018, 3.274]

3.2

microbial enumeration test

quantitative counting of mesophilic bacteria and fungi which may grow under aerobic conditions

4 Symbols and abbreviated terms

ATCC	American Type Culture Collection
CMCC	National Center for Medical Culture Collections
CIP	Collection de Bactéries de l'Institut Pasteur
IMI	International Mycological Institute
IP	Institut Pasteur
MPN	most-probable-number
NBRC	Biological Resource Center, National Institute of Technology and Evaluation
NCIMB	National Collection of Industrial and Marine Bacteria Ltd