INTERNATIONAL STANDARD

ISO 16604

First edition
2004-04-01

Clothing for protection against contact with blood and body fluids — Determination of resistance of protective clothing materials to penetration by blood-borne pathogens — Test method using Phi-X174 bacteriophage

Vêtements de protection contre les contacts avec le sang et les fluides corporels — Détermination de la résistance à la pénétration par des pathogènes véhiculés par le sang des matériaux entrant dans la fabrication des vêtements de protection — Méthode d'essai utilisant le bactériophage Phi-X174

Reference number
ISO 16604:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 16604 was prepared by Technical Committee ISO/TC 94, *Personal safety — Protective clothing and equipment*, Subcommittee SC 13, *Protective clothing*. It is based on ASTM F1671-97b.

Introduction

Workers, primarily those in the health care profession, involved in treating and caring for individuals injured or sick, can be exposed to biological liquids capable of transmitting disease. These diseases, which may be caused by a variety of microorganisms, can pose significant risks to life and health. This is especially true of blood-borne viruses which cause hepatitis [hepatitis B virus (HBV) and hepatitis C virus (HCV)] and acquired immune deficiency syndrome (AIDS) [human immunodeficiency viruses (HIV)]. Since engineering controls cannot eliminate all possible exposures, attention is placed on reducing the potential of direct skin contact through the use of protective clothing.

This International Standard is concerned with protective clothing and related protective devices designed to protect against the penetration of blood or body fluids.

Given the variety of health care settings, activities, and the potential for exposure to blood or body fluids, the barrier requirements for protective clothing materials will change with the application.

This International Standard describes a hydrostatic pressure test for measuring the viral penetration resistance of clothing materials to a surrogate virus. The choice of an appropriate test method depends on the specific application of protective clothing and its intended use. A risk assessment should be performed to determine the level of risk for determining the appropriate test method.[1]

This test method does not apply to all forms or conditions of blood-borne pathogen exposure. Users of this test method should review modes for worker/clothing exposure and assess the appropriateness of this test method for their specific applications. This test method has been specifically defined for modelling the viral penetration of hepatitis (B and C) and human immunodeficiency viruses transmitted in blood and other potentially infectious body fluids. The surrogate microbe, Phi-X174 bacteriophage, used in this test method, is similar to HCV in size and shape but also serves as a surrogate for HBV and HIV. Inferences for protection from other pathogens should be assessed on a case-by-case basis.

This test method addresses only the performance of materials or certain material constructions (e.g. seams) used in protective clothing. This test method does not address the design, overall construction and components, or interfaces of garments or other factors which may affect the overall protection offered by the protective clothing. It is emphasized that the test does not necessarily simulate conditions that clothing materials are likely to be exposed to in practice. The use of test data should therefore be restricted to broad comparative assessment of such material according to their viral penetration resistance characteristics.

Testing prior to degradation by physical, chemical, and thermal stresses which could negatively impact the performance of the protective barrier, could lead to a false sense of security. Consider tests which assess the impact of sterilization, storage conditions, and shelf life on the penetration resistance for disposable products, and the effects of laundering and sterilization on the penetration resistance for reusable products. The integrity of the protective barrier can also be compromised during use by such effects as flexing and abrasion.[1] It is also possible that pre-wetting by contaminating materials such as alcohol and perspiration also compromises the integrity of the protective barrier. If these conditions are of concern, evaluate the performance of protective clothing materials for Phi-X174 bacteriophage penetration following an appropriate preconditioning technique representative of the expected conditions of use.

Medical protective clothing materials are intended to be a barrier to blood, body fluids, and other potentially infectious materials. Many factors can affect the wetting and penetration characteristics of body fluids, such as surface tension, viscosity, and polarity of the fluid, as well as the structure and relative hydrophilicity or hydrophobicity of the materials. The surface tension range for blood and body fluids (excluding saliva) is approximately 0,042 N/m to 0,060 N/m.[2] In order to help simulate the wetting characteristics of blood and body fluids, the surface tension of the Phi-X174 bacteriophage challenge suspension is adjusted to approximate the lower end of this surface tension range. The resulting surface tension of the Phi-X174 bacteriophage challenge suspension is $(0{,}042 \pm 0{,}002)$ N/m.

Part of this method for exposing the protective clothing material specimens with Phi-X174 bacteriophage challenge suspension involves pressurization of the test cell to 14,0 kPa (in Procedures A and B). This hydrostatic pressure has been documented to produce test results that correlate with visual penetration results that are obtained with a human factors validation.[3] Some studies, however, suggest that mechanical pressures exceeding 345 kPa can occur during clinical use.[4] [5] Therefore, it is important to understand that this test method does not simulate all the physical stresses and pressures that are exerted on protective clothing garments during actual use. Procedures C and D use a stepped pressurization approach with pressures up to 20,0 kPa. These procedures simulate a range of possible pressures for ranking material performance.

Clothing for protection against contact with blood and body fluids — Determination of resistance of protective clothing materials to penetration by blood-borne pathogens — Test method using Phi-X174 bacteriophage

1 Scope

This International Standard describes a laboratory test method for measuring the resistance of materials used in protective clothing to penetration by blood-borne pathogens. This test method uses a surrogate microbe under conditions of continuous liquid contact. Protective clothing “pass/fail” determinations are based on the detection of viral penetration at a specific hydrostatic pressure using the ISO 13994 test apparatus.

This test method is not always effective in testing protective clothing materials having thick, inner liners which readily absorb the challenge fluid.

This test method involves a sensitive assay procedure. Because of the length of time required to complete this test method, it might not be suitable for use as a material or protective clothing quality control or assurance procedure.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 304, *Surface active agents — Determination of surface tension by drawing up liquid films*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 3801, *Textiles — Woven fabrics — Determination of mass per unit length and mass per unit area*

ISO 5084, *Textiles — Determination of thickness of textiles and textile products*

ISO 13994, *Clothing for protection against liquid chemicals — Determination of the resistance of protective clothing materials to penetration by liquids under pressure*

ISO 16603, *Clothing for protection against contact with blood and body fluids — Determination of the resistance of protective clothing materials to penetration by blood and body fluids — Test method using synthetic blood*

3 Terms and definitions

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

3.1
agar
semi-solid culture medium used to support the growth of bacteria and other microorganisms

3.2
assay
analysis of a mixture to determine the presence of or concentration of a particular component

NOTE In this test method, the component being analysed is a microorganism, Phi-X174 bacteriophage.

3.3
assay fluid
sterile liquid used to wash the test material surface to determine microbiological penetration

NOTE In this test method, the assay fluid is nutrient broth and the bacterial virus is the Phi-X174 bacteriophage. The assay fluid is used to wash the Phi-X174 bacteriophage from the normal inside material surface of the test specimen.

3.4
bacteriophage
type of virus which infects bacteria

NOTE In this test method, the bacteriophage is Phi-X174. The Phi-X174 bacteriophage is not pathogenic to humans, but serves to simulate viruses that are pathogenic to humans.

3.5
blood-borne pathogen
infectious secreted or excreted bacterium, virus, or other disease-inducing microbe carried in blood or other body fluids

NOTE For the purpose of this International Standard, the primary blood-borne pathogens include hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Other microorganisms should be considered on a case-by-case basis.

3.6
body fluid
any liquid produced (secreted or excreted) by the body

NOTE For the purpose of this International Standard, body fluids include those liquids potentially infected with blood-borne pathogens, including, but are not limited to, blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, and any body fluid that is visibly contaminated with blood, and all body fluids in situation where it is difficult or impossible to differentiate between body fluids.

3.7
body fluid simulant
liquid which is used to act as a model for human body liquids

NOTE In this test method, the body fluid simulant is bacteriophage nutrient broth, which is intended as a model for human body liquids as its approximates the lower end of the surface tension range for blood and body fluids (excluding saliva), (0,042 ± 0,002) N/m.

3.8
challenge suspension
liquid containing an agent that is used to test the penetration resistance of materials

NOTE In this test method, the challenge suspension is the bacteriophage challenge suspension; a nutrient broth containing the Phi-X174 bacteriophage.

3.9
lawn
⟨microbiology⟩ cloudy, uniform growth of bacteria in a thin layer of top agar in a petri dish

NOTE In this test method, *Escherichia coli C. (E. coli C)* has been selected as the bacterium used to produce the lawn.

3.10
lysis
disintegration or destruction of whole bacterial cells

NOTE In this test method, the lysis of the host bacteria, *E. coli C.,* is caused by Phi-X174 bacteriophage.

3.11
medium
media
nutrient system for the cultivation of cells or organisms

NOTE In this test method, the term media is used to describe mixtures compounded to support the growth of specific microorganisms, for example, bacteriophage nutrient broth and top agar.

3.12
morphology
form and structure of a particular organism

3.13
nutrient broth
liquid medium

NOTE In this test method, the nutrient broth is the bacteriophage nutrient broth which is used to culture the host bacteria, *E. coli C.*, and to aid in manipulating the Phi-X174 bacteriophage through the various stages of the procedure, such as suspending the Phi-X174 bacteriophage for challenging the test material in the penetration cell, assaying the normal inside test material surface, and if required, making dilutions of assay fluid for plating.

3.14
penetration
flow of a liquid through closures, porous materials, seams and holes or other imperfections in a protective clothing material on a non-molecular level

3.15
plaque
⟨virology⟩ visible, clear area which is theoretically the result of the infection and lysis of host cells by a single viable virus

NOTE In this test method, the term plaque is used to describe a visible, clear area, in the lawn of *E. Coli C.* in top agar which is theoretically the result of a single viable Phi-X174, where the bacteria have been destroyed by bacteriophage infection and lysis.

3.16
plaque-forming unit
PFU
virus particle capable of producing plaques by infecting and lysing bacteria in a lawn in top agar

3.17
plate
⟨microbiology⟩ Petri dish containing culture medium

3.18
protective clothing
item of clothing that is specifically designed and constructed for the intended purpose of isolating all or part of the body from a potential hazard; or, isolating the external environment from contamination by the wearer of the clothing.

3.19
surrogate microbe
microorganism which is used to act as a simulant for other microorganisms which are pathogenic to humans

NOTE In this test method, the surrogate microbe is the Phi-X174 bacteriophage, intended as a model for HCV and to simulate both HBV and HIV.

3.20
titre
quantity of a substance required to react with, or to correspond to, a given amount of another substance

NOTE In this test method, titre is used to describe the concentration of viable bacteriophage as measured in plaque-forming units per millilitre (PFU/ml).

3.21
virus
minute infectious agent, which lacks independent metabolism and is only able to replicate within a living host cell

3.22
viral penetration
penetration of a material by a virus

NOTE In this test method, the physical translocation of Phi-X174 bacteriophage through closures, seams, pores, and pinholes or other imperfections in materials used in protective clothing.

3.23
viral resistant
referring to materials which impede viral penetration under specified laboratory test conditions and detection methods

NOTE In this test method, protective clothing materials which demonstrate “pass” results are considered to be resistant to viral penetration.

4 Principle

A specimen is subjected to a nutrient broth containing a virus in a test apparatus as specified in ISO 13994 for a specified time and pressure sequence. Visual detection of penetration is supplemented with an assay procedure that will detect viable viruses which penetrate the material even when liquid penetration is not visible. Any evidence of viral penetration for a test specimen constitutes failure.

This test method requires a working knowledge of basic microbiological techniques.

5 Microorganisms and reagents

5.1 Bacteriophage Phi-X174 (ATCC 13706-B1), used at a challenge titre of at least $1{,}0 \times 10^8$ PFU/ml (plaque-forming units per millilitre).

NOTE The surrogate microbe, Phi-X174 bacteriophage, was selected as the most appropriate model for blood-borne pathogens because of its small size, spherical (icosahedral) morphology, environmental stability, non-human infectivity, high assay sensitivity, rapid assay, and high titre. The Phi-X174 bacteriophage has no envelope and is one of the smallest known viruses (0,027 µm in diameter).

5.2 ***Bacteria E. coli*** (ATCC 13706).

5.3 Purified water, grade 3 in accordance with ISO 3696:1987.

5.4 Nutrient broth.

5.5 Calcium chloride ($CaCl_2$).

5.6 Potassium chloride (KCl).

5.7 Sodium hydroxide (NaOH), 2,5 mol/l solution.

5.8 Surfactant, Polysorbate 80.

5.9 Bacto-agar.

6 Apparatus and materials

6.1 Penetration test cell, as specified in ISO 13994, to restrain the specimen during contact with the pressurized challenge fluid.

In the test cell, the specimen acts as a partition separating Phi-X174 bacteriophage challenge suspension from the view side of the test cell. It consists of a cell body that is fastened to a cell support. The cell body has a capacity of approximately 60 ml for the Phi-X174 bacteriophage challenge suspension. A flange cover, with an open area to allow visual observation, and a transparent cover are included. The cell body has a top port for filling and a drain valve for draining the penetration test cell. Other items, such as a fitting to allow attachment of the air line to the top port in the cell body, gaskets, and the retaining screen are also required. A diagram of the penetration test cell and apparatus are provided in Figures 1 and 2.

6.2 Other equipment

6.2.1 Thickness gauge, suitable for measuring thickness to the nearest 0,02 mm.

6.2.2 Retaining screen, comprising a smooth finish plastic or metal square mesh screen to support extensible or elastomeric materials, meeting the following specifications:

a) open area of > 50 %,

b) deflection of the test specimen is limited to $\leqslant 5{,}0$ mm.

6.2.3 Air pressure source, capable of providing air at $\left(20{,}0\,^{+2}_{0}\right)$ kPa.

6.2.4 Incubator, capable of sustaining a temperature range of (36 ± 1) °C.

6.2.5 Water bath, capable of achieving a temperature range of (45 ± 2) °C.

6.2.6 Balance, with a precision of 0,001 g.

6.2.7 Vortex mixer.

6.2.8 Refrigerator, capable of maintaining a temperature range of (5 ± 3) °C.

6.2.9 Autoclave, capable of maintaining (122 ± 1) °C and (214 ± 7) kPa absolute.

6.2.10 Stopwatch, or electronic timer.

6.2.11 Orbital shaker.

6.2.12 pH meter, sensitive to 0,1 pH units.

6.2.13 Inoculating loop.

6.2.14 Torque wrench, capable of applying a torque of 13,6 N·m.

6.2.15 Spectrophotometer, capable of measuring absorbed light at 640 nm.

6.2.16 Centrifuge, capable of an acceleration of 10 000 g.

6.3 Laboratory glassware

6.3.1 Petri dishes, sterile, 15 mm × 100 mm.

6.3.2 Pipettes, sterile, of 1 ml, 5 ml, 10 ml capacity.

6.3.3 Test tubes, 13 mm × 100 mm.

6.3.4 Test tube rack.

6.3.5 Glass bottles, sterile, with a capacity of 100 ml to 500 ml.

6.3.6 Micropipettes, capable of delivering 2 µl accurately and consistently.