PD ISO/TS 17518:2015

BSI Standards Publication

# Medical laboratories — Reagents for staining biological material — Guidance for users

bsi.

...making excellence a habit.™

## National foreword

This Published Document is the UK implementation of ISO/TS 17518:2015.

The UK participation in its preparation was entrusted to Technical Committee CH/212, IVDs.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.


Published by BSI Standards Limited 2015

ISBN 978 0 580 89925 6
ICS 11.100.01

**Compliance with a British Standard cannot confer immunity from legal obligations.**

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 May 2015.

## Amendments/corrigenda issued since publication

| Date | Text affected |
|---|---|

TECHNICAL SPECIFICATION

**ISO/TS 17518**

First edition
2015-04-15

# Medical laboratories — Reagents for staining biological material — Guidance for users

*Laboratoires médicaux — Réactifs pour coloration du matériel biologique — Directives pour les utilisateurs*

Reference number
ISO/TS 17518:2015(E)

## COPYRIGHT PROTECTED DOCUMENT



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.

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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

The committee responsible for this document is ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems.*

This Technical Specification addresses the need to use reagents in staining in biology that fulfill the criteria of ISO 19001, *In vitro diagnostic medical devices — Information supplied by the manufacturer with in vitro diagnostic reagents for staining in biology*. This Technical Specification states the requirements for these reagents when used for diagnostic work in medical laboratory fields such as microbiology, molecular biology, cytology, histopathology, and haematology.

# Introduction

This Technical Specification is based on ISO 19001, *In vitro diagnostic medical devices – Information supplied by the manufacturer with in vitro diagnostic reagents for staining in biology*. It is written for medical laboratories that prepare their own *in vitro* diagnostic examination procedures from commercially available reagents that are not specifically intended for *in vitro* diagnostic use, as well as medical laboratories that use commercially prepared *in vitro* diagnostic reagents that are specifically intended for performing *in vitro* diagnostic examinations.

This Technical Specification describes the information that laboratories performing *in vitro* diagnostic staining in biology need to receive from the suppliers and vendors of dyes, stains, chromogenic reagents and other reagents used for staining in biology. It also provides specific guidance for use of this information, which is a prerequisite for professional users in medical laboratories to achieve reproducible and comparable results in all fields of staining in biology.

# Medical laboratories — Reagents for staining biological material — Guidance for users

## 1 Scope

This Technical Specification provides requirements and guidance for selecting and assessing the quality of reagents to be used for *in vitro* diagnostic staining in biology.

This Technical Specification applies to the professional use of reagents for staining in biology by medical laboratories, and in particular, to those who are responsible for the requisition and evaluation of these reagents in medical laboratory disciplines such as clinical cytology, haematology, histopathology, microbiology, and molecular biology.

## 2 Normative references

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

ISO 15189:2012, *Medical laboratories — Requirements for quality and competence*

ISO 19001, *In vitro diagnostic medical devices — Information supplied by the manufacturer with in vitro diagnostic reagents for staining in biology*

## 3 Terms and definitions

For the purposes of this Technical Specification, the following terms and definitions apply:

**3.1**
**batch**
**lot**
defined amount of material that is uniform in its properties and has been produced in one process or series of processes

[SOURCE: ISO 18113-1]

**3.2**
**batch code**
**lot number**
distinctive set of numbers and/or letters that specifically identifies a batch and permits its manufacturing, packaging, labelling and distribution history to be traced

[SOURCE: ISO 18113-1]

**3.3**
**blocking reagent**
reagent that is used before staining to reduce the inherent background of a sample

[SOURCE: ISO 19001:2013, 3.2, Definition has been reworded to improve clarity.]

**3.4**
**chromogenic reagent**
reagent that reacts with certain chemical groups, present or induced, in cells and tissues with the formation of a coloured compound in situ

EXAMPLE Diazonium salt; Schiff's reagent.

[SOURCE: ISO 19001]

**3.5**
**component**
part of a finished, packaged and labelled IVD medical device

EXAMPLE raw material, substance, piece, part, software, firmware or labelling.

Note 1 to entry: Typical kit components include antibody solutions, buffer solutions, calibrators, and/or control materials.

[SOURCE: ISO 18113-1]

**3.6**
**control material**
substance, material or article intended by its manufacturer to be used to verify the performance properties of an IVD medical device

Note 1 to entry: For staining in biology, control material may also include previously diagnosed patient samples (cellular or tissue).

[SOURCE: ISO 18113-1:2009, 3.13, The term *performance characteristics* has been changed to *performance properties*.]

**3.7**
**dye**
coloured organic compound that, when dissolved in a suitable solvent, can impart colour to a material

[SOURCE: ISO 19001]

**3.8**
**examination**
set of operations having the purpose of determining the value or characteristics of a property

Note 1 to entry: In some disciplines (e.g. microbiology), an examination can be the total activity of several examinations.

[SOURCE: ISO 18113-1:2009, 3.16, Note 1 to entry has been modified for clarity.]

**3.9**
**expiry date**
**expiration date**
upper limit of the time interval during which the performance properties of a material stored under specified conditions can be assured

[SOURCE: ISO 18113-1:2009, 3.17, The term *performance characteristics* has been changed to *performance properties*.]

**3.10**
**fluorochrome**
reagent that emits visible light when irradiated with excitation light of a shorter wavelength

[SOURCE: ISO 19001]

**3.11**
**hazard**
potential source of harm

[SOURCE: ISO/IEC Guide 51:1999]

**3.12**
**information supplied by the manufacturer**
**labelling**
written, printed, or graphic matter

— affixed to an IVD medical device or any of its containers or wrappers, or

— provided for use with an IVD medical device, related to identification, technical description, and use of the IVD medical device, but excluding shipping documents

EXAMPLE Labels, instructions for use.

[SOURCE: ISO 18113-1]

**3.13**
**instructions for use**
information supplied by the manufacturer to enable the safe and proper use of an IVD medical device

Note 1 to entry: Includes warnings, precautions, and directions supplied by the manufacturer for the use, maintenance, troubleshooting and disposal of an IVD medical device.

[SOURCE: ISO 18113-1]

**3.14**
**intended use**
**intended purpose**
objective intent of an IVD manufacturer or the laboratory user regarding the use of a product, process or service as reflected in the specifications, instructions and information supplied by the IVD manufacturer or specified by the laboratory user

[SOURCE: ISO 18113-1:2009, 3.31, Definition has been expanded to include intent of laboratory users.]

**3.15**
***in vitro* diagnostic medical device**
**IVD medical device**
device, whether used alone or in combination, intended by the manufacturer for the *in vitro* examination of primary samples derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes including reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles

[SOURCE: ISO 18113-1]

**3.16**
**in vitro diagnostic reagent**
**IVD reagent**
chemical, biological or immunological components, solutions or preparations intended by the manufacturer to be used as an IVD medical device

[SOURCE: ISO 18113-1]

**3.17**
**kit**
set of components that are packaged together and intended to be used to perform one or more specific IVD examinations

Note 1 to entry: Kit components can include reagents (such as antibodies, enzymes, buffer and diluents), calibrators, controls and other articles and materials.

[SOURCE: ISO 18113-1]

**3.18**
**label**
printed, written, or graphic information placed on a medical device or its container

[SOURCE: ISO 18113-1]

**3.19**
**lectin**
protein of non-immunogenic origin with two or more binding sites that recognize and bind to specific saccharide residues

[SOURCE: ISO 19001]

**3.20**
**manufacturer**
natural or legal person responsible for the design, manufacture, fabrication, assembly, packaging or labelling of a medical device, assembling a system, or adapting a medical device before it is placed on the market and/or put into service, regardless of whether these operations are carried out by that person or on his or her behalf by a third party

[SOURCE: ISO 18113-1]

**3.21**
**medical device**
instrument, apparatus, implement, machine, appliance, implant, *in vitro* reagent or calibrator, software, material or other similar or related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purposes of

— diagnosis, prevention, monitoring, treatment or alleviation of disease,

— diagnosis, monitoring, treatment, alleviation of or compensation for an injury,

— investigation, replacement, modification or support of the anatomy or of a physiological process,

— supporting or sustaining life,

— control of conception,

— disinfection of medical devices,

— providing information for medical purposes by means of *in vitro* examination of primary samples derived from the human body,

and which does not achieve its primary intended action in or on the human body by pharmacological, immunological or metabolic means, but which can be assisted in its intended function by such means

Note 1 to entry: The concept medical device includes *in vitro* diagnostic medical device.

[SOURCE: ISO 18113-1:2009, 3.47, Note 1 to entry has been added for emphasis.]

**3.22**
**precaution**
statement that alerts users to special care or activities necessary for safe and effective use of an IVD medical device or to avoid damage to the IVD medical device that could occur as a result of use, including misuse

Note 1 to entry: The distinction between warnings and precautions is a matter of degree, considering the likelihood and seriousness of the hazard. See the definition of *warning* (3.38).

[SOURCE: ISO 18113-1]

**3.23**
**primary sample**
**specimen**
discrete portion of a body fluid or tissue taken for examination, study or analysis of one or more quantities or properties to determine the property of the whole

[SOURCE: ISO 18113-1:2009, 3.54, Non-relevant notes to entry have been deleted.]

**3.24**
**product certification**
third-party attestation that specified requirements relating to a product are fulfilled

[SOURCE: ISO/IEC 17000:2004, 5.5, Definition has been made specific for the product.]

**3.25**
**product certification body**
third-party organization that performs conformity assessment services and provides attestation related to products

[SOURCE: ISO/IEC 17000:2004, 2.5 and 5.5]

**3.26**
**product qualification**
process of demonstrating whether a product is capable of fulfilling specified requirements

[SOURCE: ISO/IEC 12207:2008, 4.22, Definition has been made specific for the product.]

**3.27**
**professional use**
designation that an IVD medical device is intended for personnel who are qualified to perform IVD examinations through special education and training

[SOURCE: ISO 18113-1]

**3.28**
**safety data sheet**
**SDS**
**material safety data sheet**
**MSDS**
document prepared in accordance with regulatory requirements for occupational safety to convey information about a hazardous chemical substance

Note 1 to entry: A safety data sheet typically describes physical properties, health hazards, toxicity, fire and reactivity properties, and provides storage and handling precautions.

Note 2 to entry: Safety data sheets are not considered part of IVD medical device labelling.

Note 3 to entry: A globally harmonized system of classification and labelling of chemicals (GHS) contains classification criteria and hazard communication elements. [20, 21]

[SOURCE: ISO 18113-1:2009, 3.38, The preferred term "*safety data sheet*" and Note 3 to entry have been added.]

**3.29**
**sample**
one or more representative parts taken from a system that are intended to provide information on the system

[SOURCE: ISO 18113-1]

**3.30**
**shelf life**
period of time until the expiry date, during which an IVD reagent in its original packaging maintains its stability under the storage conditions specified by the manufacturer

Note 1 to entry: *Stability* (3.31) and *expiry date* (3.9) are related concepts.

[SOURCE: ISO 18113-1]

**3.31**
**stability**
ability of an IVD medical device to maintain its performance properties within the limits specified by the manufacturer

Note 1 to entry: Stability applies to

— IVD reagents, calibrators and controls, when stored, transported and used in the conditions specified by the manufacturer,

— reconstituted lyophilized materials, working solutions and materials removed from sealed containers, when prepared, used and stored according to the manufacturer's instructions for use, and

— measuring instrument or measuring system after calibration

Note 2 to entry: Stability of an IVD reagent or measuring system is normally measured with respect to time

— in terms of the duration of a time interval over which a metrological property changes by a stated amount, or

— in terms of the change of a property over a stated time interval

[SOURCE: ISO 18113-1]

**3.32**
**stain**
solution of one or more dyes at defined concentrations in a defined solvent used for staining

Note 1 to entry: The stain can be prepared by directly dissolving the dye in the solvent or by dilution of a stock solution with suitable agents.

[SOURCE: ISO 19001]

**3.33**
**staining**
impartment of colour to a material by means of reaction with a stain or chromogenic reagent

[SOURCE: ISO 19001]

**3.34**
**stock solution of stain**
stable defined solution of one or more dyes at a higher concentration or content than that used for staining

Note 1 to entry: Stability refers to constant properties of the dye even in the presence of other dyes.

[SOURCE: ISO 19001:2009, 3.14, The phrase "*or content*" has been added to the definition.]

**3.35**
**validation**
verification, where the specified requirements are adequate for an intended use

Note 1 to entry: ISO 9000:2005, 3.8.5 defines validation as confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled.[1]

[SOURCE: ISO/IEC Guide 99:2007 (VIM), 2.45]

**3.36**
**verification**
provision of objective evidence that a given item fulfils specified requirements

EXAMPLE 1 Confirmation that a given reference material as claimed is homogeneous for the quantity value and measurement procedure concerned, down to a measurement portion having a mass of 10 mg.

EXAMPLE 2 Confirmation that performance properties or legal requirements of a measuring system are achieved.

Note 1 to entry: The item can be, e.g. a process, measurement procedure, material, compound or measuring system.

Note 2 to entry: The specified requirements can be, e.g. that a manufacturer's claims or specifications are met.

Note 3 to entry: Verification should not be confused with *validation* (3.35).

Note 4 to entry: In chemistry, verification of identity of entity involved, or of activity, requires a description of the structure or properties of that entity or activity.

[SOURCE: ISO/IEC Guide 99:2007 (VIM), 2.44]

**3.37**
**use error**
act or omission of an act that has a different IVD medical device response to that intended by the manufacturer or expected by the operator

Note 1 to entry: Use error includes slips, lapses and mistakes

Note 2 to entry: IEC 62366:2007, Annex B and D.1.3 give a discussion and examples of use errors.

[SOURCE: IEC 62366:2007]

**3.38**
**warning**
statement that alerts users about a situation that, if not avoided, could result in hazards or other serious adverse consequences from the use of an IVD medical device

Note 1 to entry: The designation of a hazard alert as a warning is reserved for the most significant consequences.

Note 2 to entry: The distinction between a warning and a *precaution* (3.22) is a matter of degree, considering the likelihood and seriousness of the *hazard* (3.11).

Note 3 to entry: Use includes *use errors* (3.37) and reasonably foreseeable misuse. See ISO 14971 and IEC 62366 for discussions of these concepts.

[SOURCE: ISO 18113-1]

**3.39**
**working solution of stain**
prepared solution including part stock stain and part solvent, used for staining biological materials

[SOURCE: ISO 19001]

# 4 Acquiring reagents for *in vitro* diagnostic staining in biology

## 4.1 General principles

The user shall ensure that the staining reagents received from a vendor fulfill the necessary criteria for obtaining reliable and reproducible results. The requirements of ISO 15189:2012, 5.3.2 of apply to the reception, storage, acceptance testing and inventory management of reagents for *in vitro* diagnostic staining in biology.

The information supplied by the manufacturer accompanies the reagent and can usually be found in the following places:

— Outer container label;

— Immediate container label;

— Instructions for use.

Users shall evaluate reagents used for *in vitro* diagnostic staining in biology against the criteria of ISO 19001. The user may obtain information for the assessment from the manufacturer's labelling, or may need to assess the quality of staining prior to performing testing. A checklist is available in Annex A for the purpose of performing the assessment.

*In vitro* diagnostic reagents used for staining shall meet the requirements of ISO 19001, which includes the information required by the user to evaluate the reagent. Reagents that do not meet the requirements of ISO 19001 shall not be utilized for *in vitro* diagnostic staining.

To achieve fully identical dye solutions repeatedly, the amount of dye shall be given as pure dye and not dye powder.

NOTE Dyes and stains sold for staining in biology do not always fulfill the in vitro diagnostic criteria specified in ISO 19001. Some dyes and stains have been sold under an incorrect name, while other dyes and stains have either been contaminated or have significant variation in the content of the dye.

As the use of dyes and stains in medical laboratories is intended for diagnostic work, it is essential that these reagents fulfill the criteria for *in vitro* diagnostic reagents. Furthermore, to ensure the desired results are achieved, validated laboratory quality control procedures are required.

## 4.2 Manufacturer name

The name of the manufacturer shall be documented.

## 4.3 Identification of kit components

In the case of a kit, the identification of the kit components shall be documented. Each component should be identified by name, letter, number, symbol, colour or graphics in the same manner on all labels and in the instructions for use.

# 5 Information associated with the *in vitro* diagnostic reagent

## 5.1 Intended use

For *in vitro* diagnostic medical devices, the intended use(s) are specified by the manufacturer in the information supplied by the manufacturer. The intended use statement may be broad and nonspecific, or it may also indicate the property to be identified, the type of sample material, and whether the test is for monitoring, diagnosis, or screening.

For non-*in vitro* diagnostic medical devices, the laboratory shall confirm or define the intended use(s) for the reagent. The intended use may be indicated by the name of the reagent, or a general or specific

intended use statement may be indicated by the manufacturer in the instructions for use. If the intended use is not indicated, the laboratory shall define its intended use for the reagent.

If a laboratory uses a reagent for a purpose other than the intended use stated by the manufacturer, the laboratory shall define and validate the intended use.

EXAMPLE Whether cell or tissue samples or both can be used; whether frozen or chemically-fixed material or both can be used; protocol for tissue processing; which embedding media can be used.

## 5.2 Instructions for use

The laboratory shall follow the manufacturer's instructions for use for staining of biological material. The instructions for use should address the following information:

a) handling and treatment of the primary sample before staining,

b) details of a suitable reaction procedure used by the manufacturer for testing the reactivity of the dye, stain, chromogenic reagent, fluorochrome, antibody or lectin used for staining of biological material,

c) result(s) expected when using the reaction procedure on the suggested type(s) of material in the way specified by the manufacturer,

d) notes on suitable positive and negative control tissue and on interpretation of the result(s),

e) references to published results obtained using the product in the way specified by the manufacturer, and

f) benefits and limitations of the IVD medical reagent with respect to the intended use may be described, where appropriate.

NOTE 1 In ISO 19001, A.2 and A.3, detailed examples are given of how guidelines of this kind could appear for the procedure methyl green – pyronin Y (a combination of two dyes for the differential staining of DNA and RNA) and the procedure for the Feulgen – Schiff reaction (a chromogenic reagent used for both qualitative and quantitative staining of DNA)].

NOTE 2 In Reference [14, 15] further details are given on the use of antibodies for staining in biology.

## 5.3 Identification of the reagent for staining of biological samples

### 5.3.1 Reagent name

The name of the reagent shall be documented.

When the name does not uniquely identify the reagent, an additional means of identification shall also be documented.

EXAMPLE Catalogue number, Chemical Abstracts Service (CAS)-Registry number or Colour Index (C.I.) number and name.

NOTE 1 CAS-Registry numbers are unique numerical code numbers assigned to chemical substances indexed by Chemical Abstracts.[17]

NOTE 2 The Colour Index gives a 5-digit number, the C.I. number and a specially constructed name to most dyes.[19]

NOTE 3 Colour Index numbers and names can for most dyes are found in Conn's Biological Stains.[18]

#### 5.3.2 Description of IVD reagent for staining of biological samples

For reagents designated by the manufacturer for *in vitro* diagnostic testing according to ISO 19001, the information supplied by the manufacturer should include appropriate physico-chemical data accompanied by relevant data sheets for each batch. The data may contain the following information:

a) Name;

b) Chemical formula including solvent;

c) Concentration or content;

d) Colour index number;

e) Expiry date;

f) Warnings and precautions.

#### 5.3.3 Batch code

A batch code, if provided, should be located on the reagent outer label.

If a kit contains different compounds bearing different batch codes, the batch code indicated on the outer container should enable the individual batch code of each component to be traced in the manufacturer's production record.

#### 5.3.4 Contents

The mass, purity, volume, volume or mass after reconstitution and/or the number of examinations obtainable may be indicated.

### 5.4 Storage and handling conditions

The user shall store the reagent according to the storage conditions necessary to maintain the stability of the reagents, calibrators and control materials. Information for proper storage should be provided by the manufacturer. If not, the user shall determine the storage and handling conditions that are adequate for its intended use.

EXAMPLE 1 2 °C to 8 °C or 2…8 °C or graphical symbol;

–18 °C or below, ≤ –18 °C, or graphical symbol

Other conditions that affect stability may be indicated in the manufacturer's instructions.

EXAMPLE 2 Light, humidity.

Any other conditions that affect the handling or storage of the reagents, calibrators and control materials may be specified in the manufacturer's instructions.

EXAMPLE 3 Fragile.

### 5.5 Expiry date

The user shall ensure that reagents used for *in vitro* diagnostic examination are within their expiry date. An expiry date based upon the stated storage instructions may be indicated by the manufacturer. If not, the user shall ensure that the stability of the reagent is adequate for its intended use.

Expiry dates should be expressed by the year, month and, where relevant, the day (see ISO 8601).[2]

EXAMPLE "YYYY-MM-DD" or ""YYYY-MM"

If only the year and month are given, the expiry date is the last day of the month indicated.

The label of the outer container indicates the expiry date of the component having the earliest expiry date, or an earlier date, where appropriate.

Reagents may have shorter expiry dates after opening (stock and in-use reagents). The user shall ensure that any opened reagent used for *in vitro* diagnostic testing is suitable for use.

## 5.6 Warnings and precautions

Information regarding whether an IVD reagent is considered hazardous may be found in the manufacturer's instructions or in the safety data sheet.

EXAMPLE Chemical, radioactive and biological hazards.

Statements or warning symbols for specific hazards may be required by local, national or regional regulations.

## 5.7 Additional information for specific kinds of reagents

### 5.7.1 Fluorochromes

Independent of the type of application, fluorochromes offered for staining in biology shall be accompanied by the following information:

a) selectivity, i.e. a description of the target(s) which may be demonstrated using the conditions specified;

b) excitation and emission wavelengths;

c) for fluorochromes conjugated to antibodies, the ratio of fluorochrome/protein (F/P).

### 5.7.2 Metal salts

When offering metal compounds for use in metal uptake procedures in staining in biology, the following additional information shall be included:

a) systematic name;

b) purity.

### 5.7.3 Additional required equipment

Any special equipment required for proper performance and safe use but not provided by the manufacturer shall be listed

Information necessary to enable special equipment to be identified and connected for proper use shall be given.

### 5.7.4 Reagent preparation

All steps required for the preparation of the reagent(s) shall be described

EXAMPLES Reconstitution, mixing, incubation, dilution.

### 5.7.5 Control procedure

Adequate information about the performance of the reagent and a means to verify that it is performing within specifications shall be provided

Users are responsible for determining the appropriate quality control procedures for their laboratory and for complying with applicable laboratory regulations.

EXAMPLE Identification of acceptable control materials, frequency of examination of control materials.

### 5.7.6 Interpretation

Where appropriate, criteria for acceptance or rejection of IVD examination results shall be specified, as well as whether additional examinations are required if a particular result is obtained.

1) Requirement to repeat an examination if the initial result is indeterminate.

If the examination procedure is intended to provide either positive or negative results, the criteria for positive and negative results shall be clearly defined, with cut-off values specified.

The diagnostic value of the examination results obtained shall be explained.

2) Information regarding the degree to which a negative result excludes or does not exclude the possibility of exposure to, or infection with, a particular organism.

If the IVD examination procedure requires the interpretation of visual observations, a clear description of the criteria shall be included, which may be a representation or reproduction of the possible results.

3) A colour chart may be given for colourimetric reactions.

### 5.7.7 Limitations of the examination procedure

Any limitations of the examination procedure shall be described, including information regarding

a) known clinically relevant interfering substances,

b) the examination of inappropriate primary samples and potential consequences, if known,

c) factors and circumstances that can affect the result, together with precautions to avoid incorrect results, and

d) potential for carryover.

NOTE Information provided by the manufacturer that enables users to reduce a risk is called "information for safety". ISO 14971[3] and ISO/TR 24971[7] contain discussions of information for safety and disclosure of residual risk.

### 5.7.8 Literature references

Pertinent literature references shall be given.

EXAMPLE Measurement method, biological reference intervals

## 6 Validation and verification of biological stains

### 6.1 General Information

*Validation* (3.35) requires defining the intended use(s). *Verification* (3.36) requires defining the product specifications. The requirements of clause 5.3.2 of ISO 15189:2012 apply to validation and verification of biological stains

For *in vitro* diagnostic reagents including biological stains, the intended use and the product specifications are determined by the manufacturer for particular conditions of use.

If a user changes the intended use and/or conditions of use for an *in vitro* diagnostic reagent, then the user is responsible for validating the new intended use and for redefining and verifying the specifications for the modified conditions.

For laboratory-developed reagents using biological dyes, the laboratory is responsible for determining a biological dye's intended use and the specifications for its use, e.g. purity, concentration or content, conditions of use.

## 6.2 Validation for the intended use

*Validation* (3.35) of biological stains or dyes involves testing of the biological stain or dye for the laboratory's intended use by staining positive and negative patient cellular materials or tissues previously known to be positive or negative for each disease state or condition intended to be identified by the staining procedure. Commercial cellular materials or tissues may also be used.

Obtaining appropriate positive responses checks the diagnostic sensitivity of the stain and appropriate negative responses checks the diagnostic specificity of the stain. The dynamic interval of the sensitivity and specificity of a stain can be checked by testing samples from different disease states, as applicable for the particular stain, and may be referred to as the "selectivity" of the stain. Selectivity is the ability of the prepared reagent to stain a particular marker associated with the condition of interest, and to not stain when the particular marker is absent, under specified testing conditions.

## 6.3 Verification of the specifications

*Verification* (3.36) of biological dyes and stains is the confirmation of the manufacturer's specifications for the dye or stain, e.g. purity, concentration or content, colour, batch-to-batch consistency, or determination of these parameters if not provided.

For *in vitro* diagnostic reagents, the user should check the manufacturer's information for use to ensure that the specifications of the reagent are appropriate for the laboratory's intended use. For laboratory-developed dyes, the user should test the dye to ensure it meets the desired specifications.

The user should verify the reagents' performance by testing previously known positive and negative samples of cellular materials and tissues for each disease state or condition included in the intended use.

NOTE A checklist is provided in Annex A to assist with this determination.

## 6.4 Certification of biological dyes

Certification of a dye is the verification of the manufacturer's information for use performed by an independent organization [*product certification body* (3.25)].

EXAMPLE The Biological Stain Commission, Rochester, New York, USA is an independent organization that performs certification of dyes for biological staining.

NOTE For methods for testing biological dyes, see chapter 28 of Reference [18].

## 6.5 Acquisition of biological stains and dyes

Biological stains and dyes should be acquired from reputable sources that provide information regarding the stain, such as concentration, content, or purity. For biological dyes, the laboratory is responsible for determining the dye's intended use.

Care should be taken to properly validate the use of each dye for each intended use.

# Annex A
(informative)

# Checklist for establishing or verifying specifications for biological stains and dyes

1) Obtain control materials representative of positive tissue (known normal or pathologic), providing appropriate representation of positive and negative samples, including known details of the tissue processing. It is of importance, particularly when performing immunohistochemical staining, to specifically state the type of fixative used and the following procedures up to the slides ready for staining, e.g. formaldehyde (mass fraction 3,6 %) buffered with phosphate (pH = 7,0) used at room temperature (18 °C to 28 °C) for 12 h to 24 h; routine dehydration, clearing, paraffin infiltration and embedding; routine preparation of microtome sections.

2) For dyes, physical and chemical characteristics should be provided on the product label, as applicable.

   a) Chemical composition (name, formula)

   b) Colour index and/or Chemical Abstracts Service registry number

   c) Mass concentration or mass content of dye in stain

   d) Purity or content of dye

   e) Expiry date

   f) Precautions and warnings

   g) Storage conditions

   For stains, check the package insert for the manufacturer's specifications. These will generally include

   h) Name of the reagent,

   i) Chemical composition including solvent and concentration or content of dye,

   j) Expiry date,

   k) Precautions and warnings, and

   l) Storage conditions.

3) Establish the allowable specification intervals by determining the uncertainty that is allowed for each property, ensuring that reagents prepared within the allowable range provide the desired colour intensities.

4) For each new reagent batch, verify that the reagent meets the originally established specifications.

If a new batch of reagent does not meet the established specification ranges, adjustments may be made within pre-determined limits, if established, for certain properties. For example, if the stock dye concentration or content is different from one batch to another, adjustments should be made when preparing the concentration of the working solution of the dye.

Any modifications (e.g. procedural timing or reagent volumes) not previously validated require validation using the control materials.

# Bibliography

[1] ISO/IEC Guide 99: 2007 International vocabulary of metrology – Basic and general concepts and associated terms (VIM). http://www.iso.org

[2] ISO 8601, *Data elements and interchange formats — Information interchange — Representation of dates and times*

[3] ISO 14971:2000, *Medical devices — Application of risk management to medical devices*

[4] ISO 15223-1:2012, *Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements*

[5] ISO 18113-1, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 1: Terms, definitions and general requirements*

[6] ISO 18113-2, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 2: In vitro diagnostic reagents for professional use*

[7] ISO/TR 24971:2013, Medical devices -- Guidance on the application of ISO 14971

[8] ISO 80000-1, *Quantities and units — Part 1: General*

[9] ISO 80000-9, *Quantities and units — Part 9: Physical chemistry and molecular physics*

[10] ISO/IEC Guide 51:1999, *Safety aspects — Guidelines for their inclusion in standards*

[11] ISO/IEC 17065:2012, *Conformity assessment — Requirements for bodies certifying products, processes and services*

[12] IEC 62366:2007, *Medical devices — Application of usability engineering to medical devices*

[13] Directive 98/79/EC of the European Parliament and the Council of 27 October 1998 on in vitro diagnostic medical devices. Off. J. Eur. Union L. 1998 December, **331** p. 7

[14] CLSI Quality assurance for design control and implementation of immunohistochemistry assays; Approved Guideline. 2nd ed.

[15] CLSI document I/LA 28-A2, Wayne, PA 19087 USA, Clinical and Laboratory Standards Institute, 2011

[16] The International Medical Device Regulators Forum (IMDRF) archive for the work of the Global Harmonization Task Force (GHTF) at www.imdrf.org/ghtf/ghtf-archives.asp

[17] Chemical Abstracts Service (CAS) Registry Database at

[18] Horobin R.W., & Kiernan J.A. Conn's Biological Stains: A handbook of dyes, stains and fluorochromes for use in biology. Published for the Biological Stain Commission by BIOS Scientific Publishers, Oxford, UK, Tenth Edition, 2002

[19] Colour Index International. 4th Edn. on line. www.colour-index.com

[20] United Nations, Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 1st edition (2003) at

[21] A Guide to The Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 4 edition. SG/.AC/AC 10/30 Rev. 4, at

**ICS 11.100.01**
Price based on 15 pages

*This page deliberately left blank*

*This page deliberately left blank*

# British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

## About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards -based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

## Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

## Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

## Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

**PLUS** is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

**BSI Group Headquarters**

389 Chiswick High Road London W4 4AL UK

## Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

## Copyright



## Useful Contacts:

**Customer Services**
**Tel:** +44 845 086 9001
**Email (orders):** orders@bsigroup.com
**Email (enquiries):** cservices@bsigroup.com

**Subscriptions**
**Tel:** +44 845 086 9001
**Email:** subscriptions@bsigroup.com

**Knowledge Centre**
**Tel:** +44 20 8996 7004
**Email:** knowledgecentre@bsigroup.com

**Copyright & Licensing**
**Tel:** +44 20 8996 7070
**Email:** copyright@bsigroup.com

bsi.

...making excellence a habit.™