Dye standards, Part I: terminology and general principles

EUROPEAN COMMITTEE FOR CLINICAL LABORATORY STANDARDS (ECCLS), SUBCOMMITTEE ON REFERENCE MATERIALS FOR TISSUE STAINS (SRMTS)*

Accepted by the SRMTS on 23 September 1990

This standard has been drafted by the SRMTS, which has been set up by the Board of ECCLS. The SRMTS consists of: H. Lyon (Chairman), E. Schulte (Secretary)*, A. de Leenheer, S. Lewis, V. Friemert, C. Struck, D. Gadsdon, R. Allison, U. Brunk, B. van Liederkerke, E. Hasselager, R. Horobin, O. Husain, D. Wittekind and H. Zschoch

1 Kobenhavns Kommunes Hvidovre Hospital, Department of Pathology, 134 Copenhagen, Denmark
2 Anatomische Anstalt, Ludwig-Maximilians-Universität, München, Germany
3 Laboratorium voor Medische Biochemie en voor Klinische Analyse, Gent, Belgium
4 Royal Postgraduate Medical School, London, UK
5 Sigma Diagnostics, Deisenhofen, Germany
6 Serva GmbH, Heidelberg, Germany
7 Centre for Biomedical Sciences, Liverpool Polytechnic, Liverpool, UK
8 Department of Oral Medicine, University of Wales, Cardiff, UK
9 Institute for Pathology, Hälsouniversitetet, Linköping, Sweden
10 Department of Veterinary Anatomy, Royal Veterinary and Agricultural University, Copenhagen, Denmark
11 Department of Biomedical Science, The University of Sheffield, Sheffield, UK
12 Department of Cytology, Charing Cross Hospital, London, UK
13 Anatomisches Institut, University of Freiburg, Germany
14 E. Merck, Darmstadt, Germany

The ECCLS standards for dyes and stains comprise two parts:

Part I. Terminology and general principles. This part defines dyes, stains, stock solutions, working solutions and chromogenic reagents. In addition, the essential principles are given for standardized tests of dyes, stains and chromogenic reagents. These comprise physico-chemical descriptions of the compounds and, in addition, staining tests designed to show how the compounds behave in practice.

Part II. Compounds. This part consists of a series of subsections, each dealing with one individual dye, stain or chromogenic reagent, and giving a physico-chemical description of the compound with appropriate biological test(s).

* Correspondence and reprint requests to Dr E. Schulte, Anatomische Anstalt, Ludwig-Maximilians-Universität, Pettenkoferstraße 11, D-8000 München 2, Germany.

Introduction

The ECCLS Subcommittee notes the excellent work done by the Biological Stain Commission in the USA, and the subcommittee will refer extensively to the experience of the Commission, as reported in Lillie (1977) and Clark (1981) and relevant announcements in Stain Technology (since 1991 titled Biotechnic & Histochemistry).

The need for fully standardized dyes, stains and chromogenic reagents is becoming increasingly important both in routine methods in clinical laboratories and as histochemical and cytochemical tools in research. The reproducibility of staining patterns is a prerequisite for diagnostic work and for interlaboratory comparisons of staining results. This is especially true when high-resolution image analysis is used for the investigation of cell and tissue features. Experimental evidence already shows that automated cell pattern recognition cannot be performed unless variations in the staining patterns are eliminated, or at least considerably reduced, by the
introduction of standardized preparatory techniques. Standardization of staining results depends not only on the availability of standardized dyes, stains and chromogenic reagents, but also requires that all steps before and after the actual staining process be standardized.

It is anticipated that quantitative cytochemical and histochemical methods will play an increasingly important role. In this context, the importance of precisely standardized dyes, stains and chromogenic reagents is obvious. It has been a major goal of the Subcommittee to give both the user and the producer of dyes, stains and chromogenic reagents guidelines as to which specifications the compounds should comply with, and in which manner the compounds should be used to achieve reproducible results.

A further incentive has been to furnish technical staff with sufficient physico-chemical information on dyes, stains and chromogenic reagents to enable adequate and suitable precautions to be taken whereby the chemical hazards and toxic potentials associated with the preparation and handling of the compounds may be kept to a minimum. It is common knowledge that small changes in the chemical composition of a compound, such as the removal or introduction of a methyl group, can make all the difference between the compound being carcinogenic or not.

1. Terminology

In this section simple definitions of frequently used terms in this field are given.

1.1. DYE

In general, dyes are coloured organic molecules with large systems of delocalized electrons (conjugated \( \pi \)-electronic systems). The physical origin of colour is selective absorption (or emission, or both) in the visible region of the electromagnetic spectrum between about 400 and 800 nm. The absorption of light in the visible part of the spectrum is electronic in origin. The light absorption characteristics of dyes are displayed by absorption spectra, obtained by plotting absorption of light by a dye against wavelength. The shape of the spectra depends on the chemical structure of the dye and on the conditions of the spectral measurements.

Dyes are available as crystals or as powders which, on solution in a suitable solvent, impart colour to a substrate.

1.2. STAIN

A stain is a solution of a dye in a suitable solvent. Stains may be subdivided into stock solutions and working solutions.

1.2.1. Stock solution

A stock solution is a stable solution of one or more dyes at a concentration which is higher than that usually employed for staining. When more than one dye is present the composition of the solvent must sometimes be chosen so as to hinder interaction between the dyes themselves. The solvent must provide the maximum of stability to the dissolved dyes and avoid precipitation. Elaboration of standard tests for stability of stock solutions is highly desirable.

1.2.2. Working solution

A working solution is a solution of one or more dyes in a suitable solvent at concentration(s) adapted to staining purposes. The working solution may be prepared by directly dissolving the dye in the solvent or by dilution of a stock solution with suitable agents. The most important solvent of working solutions is water.

1.3. CHROMOGENIC AND RELATED REAGENTS

A chromogenic reagent is a reagent which can react with suitable groups present or induced in the biological substrate (cells and tissue) with the formation of a dye in situ. Examples are diazonium salts and Schiff's reagent.

1.4. BUFFER

A buffer is a chemical system that can provide resistance to change in pH. It is composed of a weak acid and its corresponding weak conjugate base. When a buffer is used, it has to be defined in terms of chemical composition, pH and molar strength.

2. General principles

The general principles used in the characterization of dyes, stains and chromogenic reagents in the present documents will include a physico-chemical description (2.1) of the compound, and a staining test (2.2) designed to show how the compound behaves in actual staining practice. Additionally, section 2.3 gives a list of applications; section 2.4 provides information to be given on the label; and section 2.5 contains references.

2.1. PHYSICO-CHEMICAL DESCRIPTION

The purpose of the physico-chemical description of dyes, stains and chromogenic reagents is to make it possible to identify these compounds unequivocally and to determine the purity of a sample. The following data should be presented.

2.1.1. General description

The general description includes the preferred name (Lillie, 1977; Clark 1981), the number in the Colour Index (1971), the generic name, dye class, synonyms, systematic name, molecular formula and structural formula, and the molecular weight of the coloured or colour-generating species. In addition, relevant information on solubility may be given and, when necessary, information on synthesis and purification is noted.