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# Traditional Chinese medicine — Microscopic examination of medicinal herbs

*Médecine traditionnelle chinoise — Examen microscopique des herbes médicinales*



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# Foreword

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



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# Traditional Chinese medicine — Microscopic examination of medicinal herbs

## 1 Scope

This document specifies the methods for microscopic examination of medicinal herbs. It covers the equipment, sampling, preparation and observation methods. This document is applicable to medicinal herbs used in traditional Chinese medicine, including Chinese materia medica (whole medicinal materials) and decoction pieces derived from plants. It is not applicable to medicinal materials derived from animals or minerals.

## 2 Normative references

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**
**microscopic examination**
examination of a test specimen by microscope with a magnification of generally × 50 to × 500

[SOURCE: ISO 17639:2003, 3.2, modified.]

**3.2**
**medicinal herbs**
raw materials derived from various parts of plants for drugs used in herbal medicine

Note 1 to entry: Herbal medicine includes traditional Chinese medicine, Korean medicine and Kampo.

**3.3**
**slide**
flat rectangular plate of glass on which an object is mounted for microscopic examination

[SOURCE: ISO 10934-1:2002, 2.133]

**3.4**
**cover glass**
rectangular or circular piece of thin glass used to cover a microscopical preparation

[SOURCE: ISO 10934-1:2002, 2.34, modified — Note 1 to entry removed.]

**3.5**
**micrometer**
device for measuring small lengths

[SOURCE: ISO 10934-1:2002, 2.96]

## 4 Sampling

Small-sized, cut or powdered material (50 g to 250 g) samples shall be taken after mixing thoroughly. Large-sized or whole material (250 g to 500 g) samples shall be taken after mixing thoroughly. After that, select a representative sample of the material. If necessary, the samples should be preserved in airtight containers.

## 5 Apparatus

Use the usual laboratory apparatus and, in particular, the following:

**5.1 Optical microscope or slide scanner**.

**5.2 Optical or in-software micrometer**.

**5.3 Imaging devices** such as drawing attachments, embedded camera or digital imaging sensor for the microscope.

**5.4 Slides and cover glasses**.

**5.5 Botanical dissecting instruments** such as tweezers, surgical knife, razor blade, microtome.



## 6 Preparation for microscopic examination

### 6.1 Cross-section or longitudinal-section slides



a) According to the sample condition, moisturizing, fixation or maceration process can be added. See A.1 to A.3 for additional information.

b) Select representative pieces of the material being examined and cut into suitable lengths.

c) After softening, cut the material with a razor blade or a microtome to a thickness of 10 µm to 20 µm.

d) Place a section on a slide glass, add two or three drops of a mounting agent or chloral hydrate solution and place a cover glass over it, taking precautions against the inclusion of bubbles.

e) Embed the material in hard paraffin for cutting, if necessary. See A.4.

### 6.2 Powder slides

a) Place about 0,1 g of powdered sample in a watch glass containing two or three drops of a swelling agent or chloral hydrate solution, stir well with a small rod to prevent the inclusion of bubbles and allow to stand for more than 10 min to swell the sample.

b) Using a small glass rod, smear the slide glass with a small amount of the swollen sample, add one drop of the mounting agent and place a cover glass on it so that the tissue sections spread evenly without overlap, taking precautions against the inclusion of bubbles.

### 6.3 Mounting and swelling agents

Mounting and swelling agents may be made of a mixture of glycerine and water (1:1) or a mixture of glycerine, 95 % ethanol and water (1:1:1) as mounting and swelling agents. Other agents which have characteristics of mounting and swelling agents can be used.

## 7 Observation of components

Observation can be conducted in the order of the outer portion, inner portion and cell contents. In case of a powdered sample, observation can be made in the order of characteristic component, matter present in large amounts, rarely existing matter and cell contents. For histochemical detection of the sample, see A.6.

## 8 Test report

The test report shall include the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used;

c) the test method used, with reference to this document, i.e. ISO/TS 21310:2020;

d) the test result(s) obtained;

e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

g) the date of the test.

# Annex A
(informative)

# Preparation methods for microscopy

## A.1 Moisturizing

Dried parts of a plant may require softening before preparation for microscopy, preferably by soaking in water. Use a desiccator for larger quantities of material, placing water into the lower part instead of the drying agent.

Bark, wood and other dense and hard materials need to be soaked in water or in equal parts of water, ethanol and glycerol for a few hours or overnight until they are soft enough to be cut. Boiling in water for a few minutes can sometimes be necessary.

Any water-soluble contents can be removed from the cells by soaking in water. Starch grains can be gelatinized by heating in water. In certain cases, material can be moistened with water for a few minutes to soften the surfaces and allow sections to be cut.

## A.2 Fixation



Fixation is the process of preserving the tissue by placing the tissue in fixatives. The permeation of fixatives into the tissue can be dependent upon the size of the sample. Before fixation, it is recommended that samples be cut smaller than 6 mm × 6 mm × 6 mm.



The following water solutions are used as fixatives:

— ethanol: 50 % to 70 %

— formalin: under 5 %

— acetic acid: approximately 100 %

— chromic acid: approximately 1 %

— F.A.A. solution: formalin 5 ml, acetic acid 5 ml, 50 % to 70 % ethanol 90 ml

— Craf III solution: 1 % chromic acid 30 ml, 10 % acetic acid 20 ml, formalin 10 ml, water 40 ml.

## A.3 Maceration

### A.3.1 General

For maceration, cut or slice the sample into small pieces about 2 mm in thickness. Depending on the feature of the material, one of the following three methods can be used. For medicinal herb samples with only a few or scattered woody tissues or with parenchyma tissues, use the potassium hydroxide method. For hard materials mainly composed of woody tissues or woody tissues grouped into bundles, use the chromic-nitric acid or potassium chlorate method.

### A.3.2 Potassium hydroxide method

Place the sample in a test tube, add an adequate quantity of aqueous potassium hydroxide solution (a volume fraction of 5 %), then heat until the residue can be easily separated when pressed with a glass rod. Decant the alkaline solution and wash the residue with water. Transfer a small amount of

macerated material onto a slide and tease it out with a needle. Mount in dilute glycerine and examine under a microscope.

### A.3.3 Chromic-nitric acid method

Place the sample in a test tube and add an adequate quantity of chromic-nitric acid test solution, then leave to stand until the material can be easily separated when pressing with a glass rod. Decant the acidic solution, wash the residue with water and prepare the slide as directed in A.3.2.

### A.3.4 Potassium chlorate method

Place the sample in a test tube, add dilute nitric acid (volume fraction of 50 %) and a few crystals of potassium chlorate. Warm gently until the effervescence subsides, then add a few crystals of potassium chlorate to maintain a slight effervescence. When the tissue shows a tendency to disintegrate, break the material with a glass rod. Decant the acidic solution, wash the macerated material with water and prepare the slide as directed in A.3.2.

## A.4 Sectioning

### A.4.1 Thin section (free hand section)

Grasp the sample to be sectioned between the top of the thumb and forefinger of one hand. Holding a single-edge razor blade or a microtome blade with the thumb and forefinger of the opposite hand, rapidly draw the blade through the plant material in a smooth continuous sliding motion (not sawing). The sections will accumulate on the flat part of the razor blade. After cutting a few sections, dip the razor blade into water standing in a watch glass and the sections will float off the blade. Select several of the best sections to observe.



### A.4.2 Paraffin embedding section



#### A.4.2.1 Dehydration

The series of tert-butanol (TBA) is the most common method for dehydration, but a lot of other methods can be applied. If the case of using TBA series, keep pure TBA in a warm place before use (e.g. on the top of an incubation oven) as it freezes below 25 °C. An example of TBA series is shown in Table A.1.

**Table A.1 — Example of TBA series**

| Stage | t-butanol<br>% | 95 % ethanol<br>% | Water<br>% |
|---|---|---|---|
| 1 | 10 | 40 | 50 |
| 2 | 20 | 50 | 30 |
| 3 | 35 | 50 | 15 |
| 4 | 55 | 45 | 0 |
| 5 | 75 | 25 | 0 |
| 6 | 100 | 0 | 0 |
| Dehydrate tissue at each stage for 1 h to 1 d, depending on tissue size (e.g. root tips for 1 h, anthers or leaves for 2 h at each stage). Stages 1 to 5 are processed at room temperature and stage 6 in the incubation oven at 56 °C to 60 °C. | | | |

#### A.4.2.2 Embedding section

a) Keep molten paraffin wax and plastic pipettes in incubation oven.

b) Add paraffin wax in vials half-filled with TBA. Alternatively, add an equal volume of molten wax to the volume of TBA in the vials.