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Traditional Chinese medicine — Panax notoginseng seeds and seedlings

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National foreword

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Traditional Chinese medicine — *Panax notoginseng* seeds and seedlings

Médecine traditionnelle chinoise — Graines et plants de Panax notoginseng

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The committee responsible for this document is ISO/TC 249, *Traditional Chinese medicine*.

Traditional Chinese medicine — *Panax notoginseng* seeds and seedlings

1 Scope

This document specifies minimum requirements and test methods for seeds and seedlings of *Panax notoginseng* (Burk.) F. H. Chen. It is suitable for marketing of cultivated *Panax notoginseng* seeds and seedlings. It is also suitable to be used for quality assurance by cultivators of *Panax notoginseng*.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

International Seed Testing Association (ISTA): 2016, *International Rules for Seed Testing*

International Seed Testing Association (ISTA): 2003, *Working Sheets on Tetrazolium testing*

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:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
1 000-seed weight
average weight of every 1 000 pure seeds of a *test sample* (3.19)

3.2
composite sample
portion formed by combining and mixing all the *primary samples* (3.8) taken from the *lot* (3.6)

3.3
foreign matter
parts of *Panax notoginseng seeds* (3.11) or *seedlings* (3.15) other than those named with the limits specified for the herbal materials concerned

Note 1 to entry: Foreign matter is any organism, part or product of an organism, other than that named in the specification and description of *Panax notoginseng* seeds or seedlings.

Note 2 to entry: Foreign matter is mineral admixtures not adhering to *Panax notoginseng* seeds or seedlings, such as soil, stones, sand and dust.

3.4
hibernaculum
hibernated bud with bud scales

3.5
hibernaculum diameter
largest distance at the site of the *hibernaculum* (3.4)

Note 1 to entry: See Figure 2.

Note 2 to entry: It is expressed in centimetres.

3.6
lot
specified quantity of *seeds* (3.11) or *seedlings* (3.15) that is physically and uniquely identifiable

3.7
maturity
percentage of mature seeds, determined by number, in the *test sample* (3.19)

3.8
primary sample
portion taken from the *lot* (3.6) during one single sampling action

3.9
purity
weight of pure seed fraction over the total weight of the *test sample* (3.19)

Note 1 to entry: The pure seed refers to the species stated by the applicant or found to predominate in the test and includes all botanical varieties and cultivars of that species.

Note 2 to entry: It is expressed in per cent.

3.10
sealed
<container for seeds> closed in such a way that the container cannot be opened to get access to the seed and be closed again without either destroying the seal or leaving evidence of tampering

Note 1 to entry: This definition refers to the sealing of seed *lots* (3.6), as well as of seed samples.

3.11
seed
mature ovules produced by *Panax notoginseng* (Burk.) F. H. Chen, consisting of three basic parts: embryo, endosperm and seed coat

3.12
seed length
largest distance from the lower part to the top

Note 1 to entry: See Figure 1.

Note 2 to entry: It is expressed in millimetres.

3.13
seed thickness
largest distance from the side perpendicular to the ridge to the opposite side

Note 1 to entry: See Figure 1.

Note 2 to entry: It is expressed in millimetres.

3.14
seed width
largest distance from the side of the raphe to the opposite

Note 1 to entry: See Figure 1.

Note 2 to entry: It is expressed in millimetres.

3.15
seedling
young plant of *Panax notoginseng* (Burk.) F. H. Chen, which, after cultivation for one year, consists of *hibernaculum* (3.4), *tap root* (3.18), lateral root and fibrous root

3.16
seedling diameter
largest distance at the site of the taproot

Note 1 to entry: See Figure 2.

Note 2 to entry: It is expressed in centimetres.

3.17
seedling weight
average weight of *test samples* (3.19) of *seedlings* (3.15)

3.18
tap root
root generated from the one year development of the radicle after seed germination

3.19
test sample
portion of the *composite sample* (3.2) to which one of the test required in this standard is applied

Note 1 to entry: Test samples may be packed in different materials meeting conditions for specific tests [e.g. moisture or *purity* (3.9)].

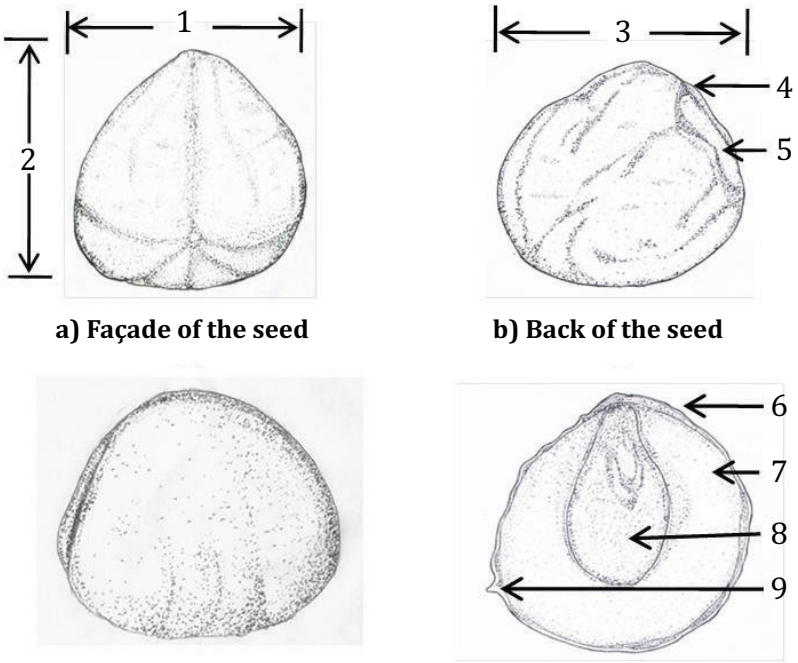
3.20
viability
potential ability of a seed to germinate, or capability of an embryo to live, as a percentage of stained seeds in the *test sample* (3.19)

Note 1 to entry: The percentage of stained seeds in the test sample shall be estimated by the method of Topographical Tetrazolium Test.

4 Descriptions

4.1 *Panax notoginseng* seed

In this document, *Panax notoginseng* seed is the dehydrated seed of plant *Panax notoginseng* (Burk.) F. H. Chen consisting of three basic parts: embryo, endosperm and the seed coat, as shown in Figure 1.



a) Façade of the seed

b) Back of the seed

c) Kernel, without seed coat

d) Section of the kernel of a stratified seed

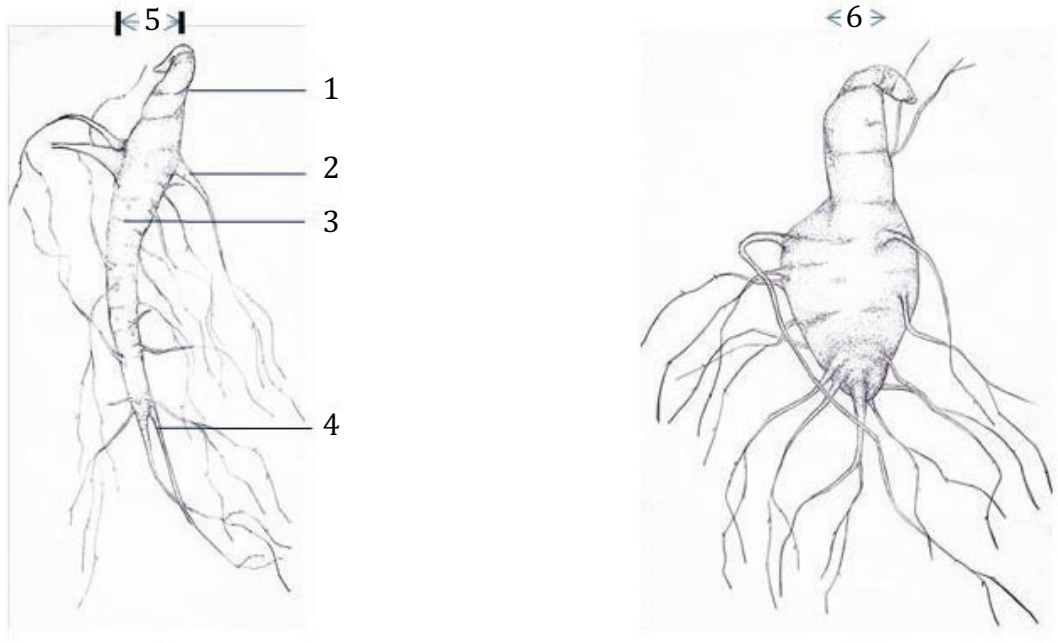
Key

1 seed width
2 seed length
3 seed thickness
4 circular water hole
5 raphe
6 seed coat
7 endosperm
8 embryo
9 cavity of seed

Figure 1 — Structure of *Panax notoginseng* seed

4.2 *Panax notoginseng* seedling

Panax notoginseng seedling is one-year-old seedling, consisting of four parts: hibernaculum, taproot, lateral root and fibrous root. For practical application, *Panax notoginseng* seedlings are classified into two groups on the basis of their morphology: long seedlings and short seedlings, as shown in Figure 2.



a) Long seedling **b) Short seedling**

Key

1 hibernaculum
2 lateral root
3 taproot
4 fibrous root
5 seedling diameter
6 hibernaculum diameter

Figure 2 — *Panax notoginseng* seedling

5 Requirements

5.1 General characteristics

The following requirements shall be met before separating the composite sample into test samples.

a) *Panax notoginseng* seeds shall be clean and free from foreign matter.

b) *Panax notoginseng* seedlings shall be healthy and intact.

c) The presence of mouldy seeds and external contaminants which are visible to the naked eye shall not be permitted.

5.2 *Panax notoginseng* seed

5.2.1 Morphological features of seed

The colour of the seed shall be white or yellowish white. The seed shall be hard-texture, without any unusual smell or mildew.

5.2.2 Moisture

The moisture content in percentage mass shall not be less than 60 %.

5.2.3 Purity

Seed purity shall not be less than 98 %.

5.2.4 Viability

Viability shall not be less than 90 %.

5.2.5 Maturity

Maturity shall not be less than 90 %.

5.2.6 Fungi

Neither *Fusarium* spp. nor *Alternaria* spp. shall be detected.

5.2.7 1 000-seed weight, seed width, seed thickness and seed length

The 1 000-seed weight, seed width, seed thickness and seed length shall comply with the requirements in Table 1.

Table 1 — Grading requirements of *Panax notoginseng* seed

Grade	1 000-seed weight[a] g	Seed width mm	Seed thickness mm	Seed length mm
First[b]	≥100	≥5,5	≥5,5	≥6,3
Second[c]	80 to <100	5,0 to <5,5	5,0 to <5,5	5,5 to <6,3
Third[d]	60 to <80	4,5 to <5,0	4,5 to <5,0	5,0 to <5,5
Unqualified	<60	<4,5	<4,5	<5,0

NOTE The above requirements were established on the basis of seeds collected from different regions.

[a] The 1 000-seed weight shall be determined when the moisture content of the seed is 60 % or slightly above.

[b] The seeds, of which the seed width is not less than 5,5 mm, shall not be less than 95 %. Otherwise, it shall be judged to be the second grade.

[c] The seeds, of which the seed width is not less than 5,0 mm, shall not be less than 95 %. Otherwise, it shall be judged to be the third grade.

[d] The seeds, of which the seed width is not less than 4,5 mm, shall not be less than 95 %. Otherwise, it shall be judged to be the unqualified grade.

5.3 *Panax notoginseng* seedling

5.3.1 Morphological features of seedling

a) The taproot and lateral root of seedling shall be intact, without diseases and insects. Taproot shall be solid.

b) The hibernaculum of seedling shall be intact and solid, without diseases and insects.

5.3.2 Fungi

Neither *Fusarium* spp. nor *Alternaria* spp. shall be detected.

5.3.3 Nematode

Nematode shall not be detected.

5.3.4 Seedling weight

The seedling weight shall comply with the requirements in Table 2.

Table 2 — Grading requirements of *Panax notoginseng* seedling

Grading	Seedling weight g	Seedling diameter cm	Hibernaculum diameter cm
First	≥2,5	≥1,2	≥0,6
Second	1,5 to <2,5	0,9 to <1,2	0,4 to <0,6
Third	1,0 to <1,5	0,6 to <0,9	0,2 to <0,4
Unqualified	<1,0	<0,6	<0,2

6 Sampling

6.1 Seed sampling

The seed sampling shall be carried out in accordance with ISTA, *International Rules for Seed Testing*: 2016, Chapter 2. The maximum weight of lot and minimum weight of sample are specified in Table 3.

Table 3 — Maximum weight of lot and minimum weight of sample

Maximum weight of seed lot (kg)	Minimum weight of test sample (g)		
	For measure of 1 000-seed weight, width, thickness and length	For purity analysis	For other tests
2 000	500	100	300
NOTE The establishment of the above requirement is based on seeds collected from different regions and different treatment.			

6.2 Seedling sampling

The maximum seedling lot shall be 300 000 seedlings and the minimum composite samples shall be 100 seedlings.

7 Test methods

7.1 Moisture content

Determination of the mass fraction of moisture shall be in accordance with the high constant temperature method specified in ISTA, *International Rules for Seed Testing*.

7.2 Seed width

Take 100 to 150 intact seeds in duplicate from the composite sample using the quartering method. The seed width shall be measured using a Vernier caliper in millimetres (to two decimal places). If the difference between measured values of the duplicate samples is less than 5 %, the seed width of the seed lot shall be the arithmetic mean of the two measured values; otherwise, it shall be re-measured.

7.3 Seed thickness

Take 100 to 150 intact seeds in duplicate from the composite sample by quartering method. The seed thickness shall be measured using a Vernier caliper in millimetres (to two decimal places). If the difference between measured values of the duplicate samples is less than 5 %, the seed thickness of the seed lot shall be the arithmetic mean of the two measured values; otherwise, it shall be re-measured.

7.4 Seed length

Take 100 to 150 intact seeds in duplicate from the composite sample using the quartering method. The seed length shall be measured using a Vernier caliper in millimetres (to two decimal places). If the difference between measured values of the duplicate samples is less than 5 %, the seed length of the seed lot shall be the arithmetic mean of the two measured values; otherwise, it shall be re-measured.

7.5 Maturity

Take duplicate samples of seeds using the quartering method, each containing 100 to150 seeds. The seed shall be cut into two parts along the raphe. The shape of its embryo is determined with a microscope. The seed is considered to be mature if its embryo is pyriform or saddle. Formula (1) is used to express the maturity of a seed lot:

$$M(\%) = \frac{A_{\mathrm{m}}}{A} \times 100 \tag{1}$$

where

M is maturity (%);

A_{m} is the number of mature seeds;

A is the number of seeds in the test sample.

If the difference between the measured maturities of the duplicate samples is less than 5 %, the maturity of the seed lot shall be the arithmetic mean of two measured values; otherwise, it shall be re-estimated.

7.6 Purity

Determination of the seed purity shall be in accordance with the purity analysis of ISTA, *International Rules for Seed Testing*.

7.7 Seed viability

Determination of the seed viability shall be in accordance with ISTA, *International Rules for Seed Testing* and ISTA, *Working Sheets on Tetrazolium Testing*.

7.8 1 000-seed weight

Determination of the 1 000-seed weight shall be in accordance with Weight Determination of ISTA, *International Rules for Seed Testing*.

7.9 Fungi testing

7.9.1 Preparation of seeds

Take 100 seeds into a sterilized flask and add 20 ml sterilized water and shake for 8 min. Centrifuge the suspension at 4 000 rpm for 20 min. Re-suspend the residue with 2 ml of sterilized water. Spot 100 µl of the final suspension on the blotter or filter paper in a plate for incubation. A total of five spots shall be included in the plate.

7.9.2 Preparation of seedlings

Take 500 ml sterilized water into a sterilized flask. Take 10 to 20 seedlings. Immerge the seedlings into the flask and shake for 8 min one by one. Centrifuge the suspension at 4 000 rpm for 20 min. Re-

suspend the residue with 2 ml of sterilized water. Spot 100 µl of the final suspension on the blotter or filter paper in a plate for incubation. A total of five spots shall be included in the plate.

7.9.3 *Alternaria* spp. testing

Alternaria spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*:2016, method 7-001a.

7.9.4 *Fusarium* spp. testing

Fusarium spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*:2016, method 7-009.

7.10 Nematode testing

Take 100 to 150 seedlings. Inspect nematode knots or lesions with the naked eye. Check the root knots or root lesions by nematode under microscope.

7.11 Seedling weight

Take three to five samples randomly, each one containing 100 seedlings. The seedlings shall be weighed one by one. If the relative standard deviation of the average weight of each sample is less than 6 %, the arithmetic mean of all samples shall be the average weight of the seedling lot.

The percentage of seedlings not meeting the minimum weight requirement of the grade shall not be more than 5 %. Otherwise, it shall be judged to be inferior grade and be tested accordingly.

7.12 Seedling diameter

Take 100 to 150 seedlings. Measure the top of the seedlings using a Vernier caliper (record the value to two significant figures). The arithmetic mean of all samples shall be the average seedling diameter of the seedling lot.

7.13 Hibernaculum diameter

Take 100 to 150 seedlings. Measure the top of the hibernaculum using a Vernier caliper (record the value to two significant figures). The arithmetic mean of all samples shall be the average hibernaculum diameter of the seedling lot.

8 Test report

For each test method, the test report shall specify the following items:

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

b) the sampling method used;

c) the test method used;

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.

9 Packaging, storage and transportation

The packaging shall not transmit any odour or flavour to the product and shall not contain substances which may damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

A sand moisture content of 20 % shall be required for seed storage. The temperature for seed storage shall be not higher than 20 °C. The seed storage time shall not exceed 90 days.

The temperature for seedling storage shall be 10 °C to 15 °C for long distance delivery.

10 Marking

The following items shall be marked or labelled on the packages:

a) grade of the product in accordance with 5.2 and 5.3;

b) all quality features, indicated in 5.2 and 5.3, determined in accordance with methods specified in Clause 7;

c) gross weight and net weight of the package;

d) country of origin and province/state of the seed;

e) expiration date of the seeds;

f) any items required by regulatory bodies of destination country.

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