
**Water quality — Sampling —
Part 3:
Preservation and handling of water
samples**

Qualité de l'eau — Échantillonnage —

Partie 3: Conservation et manipulation des échantillons d'eau

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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 voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html. (standards.iteh.ai)

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods)*.
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This fifth edition cancels and replaces the fourth edition (ISO 5667-3:2012), of which it constitutes a minor revision. The changes compared to the previous edition are as follows:

- updated references in [Table A.1](#);
- clarification in the Introduction concerning use of the preservation times and conditions set out in [Table A.1](#).

A list of all parts in the ISO 5667 series can be found on the ISO website.

Introduction

This document is intended to be used in conjunction with ISO 5667-1, which deals with the design of sampling programmes and sampling techniques.

Where possible this document has been brought into line with current standards. Where new research or validation results have provided new insights, the latest knowledge has been used.

Guidance on validation protocols can be found in ISO 17034.

ISO 5667-3 provides in [Table A.1](#) validated preservation times and/or conditions as well as descriptions of best practice. [Table A.1](#) also refers, for each analyte, to those ISO standards available at the date of publication of this ISO 5667-3. This is however not an exhaustive list. Other methods may be used when they have been validated. However, it is strongly recommended that where a method validation is not available, the preservation times for the analyte as listed in [Table A.1](#) for ISO test methods be followed.

The preservation and storage conditions and maximum storage times per analyte as listed in [Table A.1](#) should be regarded as default conditions to be applied in the absence of any other information.

However, if validation of preservation techniques and holding times has been carried out, relative to specific circumstances and matrices, by a laboratory, then, provided that it can produce evidence of this validation where they differ from those set out in [Table A.1](#) of this standard, these validated preservation and storage conditions and maximum storage times are deemed acceptable for use by the validating laboratories.

Attention is drawn to the proposed development of a new part in the ISO 5667 series, which further elaborates on ISO 5667-3:2018, Annex C, and which will contain guidelines and the elaboration of the required techniques of how to validate new storage times or preservative methods and details of the techniques described.

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Water quality — Sampling —

Part 3: Preservation and handling of water samples

NOTICE — This document and the analytical International Standards listed in [Annex A](#) are complementary. Where no analytical International Standard is applicable, the technique(s) described in [Tables A.1](#) to [A.3](#) take(s) normative status.

When new or revised analytical standards are developed with storage times or preservative techniques differing from those in [Tables A.1](#) to [A.3](#), then the storage times or preservative techniques should be validated and presented to ISO/TC 147/SC 6/WG 3 for incorporation into the next revision of this document.

1 Scope

This document specifies general requirements for sampling, preservation, handling, transport and storage of all water samples including those for biological analyses.

It is not applicable to water samples intended for microbiological analyses as specified in ISO 19458, ecotoxicological assays, biological assays and passive sampling as specified in the scope of ISO 5667-23.

This document is particularly appropriate when spot or composite samples cannot be analysed on site and have to be transported to a laboratory for analysis.

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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.

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

ISO 5667 (all parts), *Water quality — Sampling*

ISO 19458, *Water quality — Sampling for microbiological analysis*

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 <https://www.iso.org/obp>

3.1

integrity

property that the parameter(s) of interest, information or content of the sample container has not been altered or lost in an unauthorized manner or subject to loss of representativeness

3.2

sample preservation

any procedure used to stabilize a sample in such a way that the properties under examination are maintained stable from the collection step until preparation for analysis

Note 1 to entry: Different analytes may require several samples from the same source that are stabilized by different procedures.

[SOURCE: ISO 11074:2015, 4.4.20, modified — Note 1 to entry has been added.]

3.3

sample storage

process, and the result of keeping a sample available under predefined conditions, usually for a specified time interval between collection and further treatment of a sample

Note 1 to entry: Specified time is the maximum time interval.

[SOURCE: ISO 11074:2015, 4.4.22, modified — Note 1 to entry has been added; “soil sample” has been changed to “sample”.]

3.4

storage time

period of time between filling of the sample container and further treatment of the sample in the laboratory, if stored under predefined conditions

Note 1 to entry: Sampling finishes as soon as the sample container has been filled with the sample. Storage time ends when the sample is taken by the analyst to start sample preparation prior to analysis.

Note 2 to entry: Further treatment is, for most analytes, a solvent extraction or acid destruction. The initial steps of sample preparation can be steps complementary to the storage conditions for the maintenance of analyte concentrations.

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4 Sampling and chain of custody

If there is a need to take samples, this is done according to a sampling programme. The first step is to design a sampling programme. Guidance on this topic is given in ISO 5667-1.

Depending on the sample type and matrix, the guidelines found in ISO 19458 and in the relevant part(s) of ISO 5667 shall be consulted.

The process of preservation and handling of water samples consists of several steps. During this process, the responsibility for the samples might change. To ensure the integrity of the samples, all steps involving the sample shall be documented.

All preparation procedures shall be checked to ensure positive or negative interferences do not occur. As a minimum, this shall include the analysis of blanks (e.g. field blank or sample container) or samples containing known levels of relevant analytes as specified in ISO 5667-14.

5 Reagents and materials

WARNING — Certain preservatives (e.g. acids, alkalis, formaldehyde) need to be used with caution. Sampling personnel should be warned of potential dangers, and appropriate safety procedures should be followed.

The following reagents are used for the sample preservation and shall only be prepared according to individual sampling requirements. All reagents used shall be of at least analytical reagent grade and water shall be of at least ISO 3696, grade 2. Acids referred to in this document are commercially available “concentrated” acids.

All reagents shall be labelled with a “shelf-life”. The shelf-life represents the period for which the reagent is suitable for use, if stored correctly. This shelf-life shall not be exceeded. Any reagents that are not completely used by the expiry of the shelf-life date shall be discarded.

NOTE Often the shelf-life of reagents is supplied by the receiving laboratory.

Check reagents periodically, e.g. by field blanks, and discard any reagent found to be unsuitable.

Between on-site visits, reagents shall be stored separately from sample containers and other equipment in a clean, secure cabinet in order to prevent contamination.

Each sample shall be labelled accordingly, after the addition of the preservative. Otherwise, there could be no visible indication as to which samples have been preserved, and which have not.

5.1 Solids.

5.1.1 Sodium thiosulfate pentahydrate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, $w(\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}) > 99 \%$.

5.1.2 Ascorbic acid, $\text{C}_6\text{H}_8\text{O}_6$, $w(\text{C}_6\text{H}_8\text{O}_6) > 99 \%$.

5.1.3 Sodium hydroxide, NaOH , $w(\text{NaOH}) > 99 \%$.

5.1.4 Sodium tetraborate decahydrate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, $w(\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}) > 99 \%$.

CAUTION — Sodium tetraborate decahydrate is known to be a carcinogen, mutagen and reproductive toxin (CMR). (standards.iteh.ai)

5.1.5 Hexamethylenetetramine (hexamine, urotropine), $\text{C}_6\text{H}_{12}\text{N}_4$, $w(\text{C}_6\text{H}_{12}\text{N}_4) > 99 \%$.

5.1.6 Potassium iodide, KI , $w(\text{KI}) > 99 \%$.
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5.1.7 Iodine, I_2 , $w(\text{I}_2) > 99 \%$.

5.1.8 Sodium acetate, $\text{C}_2\text{H}_3\text{NaO}_2$, $w(\text{C}_2\text{H}_3\text{NaO}_2) > 99 \%$.

5.1.9 Ethylenediamine, $\text{C}_2\text{H}_8\text{N}_2$, $w(\text{C}_2\text{H}_8\text{N}_2) > 99 \%$.

5.2 Solutions.

5.2.1 Zinc acetate solution $\text{C}_4\text{H}_6\text{O}_4\text{Zn}$ (10 g/l).

Dissolve 10,0 g of zinc acetate in ~100 ml of water. Dilute to 100 ml with water. Store the solution in a polypropylene or glass bottle for a maximum period of 1 a.

5.2.2 Orthophosphoric acid ($\rho \approx 1,7 \text{ g/ml}$), H_3PO_4 , $w(\text{H}_3\text{PO}_4) > 85 \%$, $c(\text{H}_3\text{PO}_4) = 15 \text{ mol/l}$.

5.2.3 Hydrochloric acid ($\rho \approx 1,2 \text{ g/ml}$), HCl , $w(\text{HCl}) > 36 \%$, $c(\text{HCl}) = 12,0 \text{ mol/l}$.

5.2.4 Nitric acid ($\rho \approx 1,42 \text{ g/ml}$), HNO_3 , $w(\text{HNO}_3) > 65 \%$, $c(\text{HNO}_3) = 15,8 \text{ mol/l}$.

5.2.5 Sulfuric acid ($\rho \approx 1,84 \text{ g/ml}$), H_2SO_4 (freshly prepared).

Dilute concentrated sulfuric acid (H_2SO_4), $\rho \approx 1,84 \text{ g/ml}$, $w(\text{H}_2\text{SO}_4) \approx 98 \%$ 1 + 1 by carefully adding the concentrated acid to an equal volume of water and mix.

WARNING — Adding the concentrated acid to the water can give violent reactions because of an exothermic reaction.

5.2.6 Sodium hydroxide solution ($\rho \approx 0,40$ g/ml), NaOH.

5.2.7 Formaldehyde solution (formalin), CH_2O , $\varphi(\text{CH}_2\text{O}) = 37\%$ to 40% (freshly prepared).

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

5.2.8 Disodium salt of ethylenediaminetetraacetic acid (EDTA) ($\rho \approx 0,025$ g/ml), $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$, $w(\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}) > 99\%$.

Dissolve 25 g EDTA in 1 000 ml of water.

5.2.9 Ethanol $\text{C}_2\text{H}_5\text{OH}$, $\varphi(\text{C}_2\text{H}_5\text{OH}) = 96\%$.

5.2.10 Alkaline Lugol's solution, 100 g potassium iodide (5.1.6), 50 g iodine (5.1.7), and 250 g sodium acetate (5.1.8) in 1 000 ml water to pH 10.

5.2.11 Acidic Lugol's solution, 100 g potassium iodide (5.1.6), 50 g iodine (5.1.7) and 100 ml glacial acetic acid (5.2.17) in 1 000 ml water to pH 2.

5.2.12 Neutralized formaldehyde solution, formaldehyde solution (5.2.7) neutralized with sodium tetraborate (5.1.4) or hexamethylenetetramine (5.1.5). Formalin solution at 100 g/l gives a final solution of $\varphi(\text{CH}_2\text{O}) = 3,7\%$ to $4,0\%$.

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

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5.2.13 Ethanol preservative solution.

Ethanol (5.2.9), formaldehyde solution (5.2.7) and glycerol (5.2.18) (100 + 2 + 1 parts by volume, respectively).

5.2.14 Sodium hypochlorite NaOCl, $w(\text{NaOCl}) = 10\%$. Dissolve 100 g sodium hypochlorite (NaOCl) in 1 000 ml of water.

5.2.15 Potassium iodate KIO_3 , $w(\text{KIO}_3) = 10\%$. Dissolve 100 g potassium iodate (KIO_3) in 1 000 ml of water.

5.2.16 Methanoic acid (formic acid) CH_2O_2 , $\varphi(\text{CH}_2\text{O}_2) > 98\%$.

5.2.17 Glacial acetic acid $\text{C}_2\text{H}_4\text{O}_2$, $w(\text{C}_2\text{H}_4\text{O}_2) > 99\%$.

5.2.18 Glycerol (glycerin, glycerine) $\text{C}_3\text{H}_5(\text{OH})_3$.

5.3 Materials.

5.3.1 Container and cap, types as specified in [Tables A.1](#) to [A.3](#).

5.3.2 Filter, pore size $0,40\ \mu\text{m}$ to $0,45\ \mu\text{m}$, unless a different filter size is specified in the analytical International Standard.

6 Containers

6.1 Container selection and preparation

The choice of sample container (5.3.1) is of major importance and ISO 5667-1 provides some guidance on this subject.

Details of the type of container used for the collection and storage of samples are given in Tables A.1 to A.3. The same considerations given to this selection of suitable container material shall also be given to the selection of cap liner materials.

Sample containers shall be made of a material appropriate for preserving the natural properties of both the sample and the expected range of contaminants. Suitable types of containers for each analyte to be measured are given in Tables A.1 to A.3.

NOTE For very low concentrations of metals, containers prescribed can be different from those used for higher concentrations. Details can be found in Table A.1 or in the analytical International Standards.

If the samples are to be frozen, suitable containers, such as polyethylene (PE) or polytetrafluoroethylene (PTFE), shall be used to prevent breakage.

The use of disposables is preferred. Some manufacturers supply containers with a certificate of cleanliness. If such a certificate of cleanliness is supplied, it is not necessary to clean or rinse the containers before use.

6.2 Filtration on site

Filtration on site is required in some cases.

- Groundwaters shall be filtered on site if dissolved metals need to be analysed.
- Waters shall be filtered (5.3.2) on site, if this is required according to Annex A. Unless specified otherwise, a filter pore size 0,40 µm to 0,45 µm shall be used.

If immediate filtration on site is impossible, then the reason and the time between sampling and filtration shall be added to the test report.

6.3 Filling the container

The container (5.3.1) shall be filled completely unless prescribed differently in Tables A.1 to A.3 or the analytical International Standard used. If the samples are to be frozen as part of their preservation, sample containers shall not be completely filled. This is in order to prevent breakage which may arise from expansion of ice during the freezing and thawing process.

If no preservatives are present in the bottle, then prerinsing the bottle may be advisable. Guidance on prerinsing can be found in ISO 5667-14.

7 Sample handling and preservation

7.1 Sample handling and preservation for physical and chemical examination

Waters, particularly fresh waters, waste waters and groundwaters, are susceptible to changes as a result of physical, chemical or biological reactions which may take place between the time of sampling and the commencement of analysis. The nature and rate of these reactions are often such that, if precautions are not taken during sampling, transport and storage (for specific analytes), the concentrations determined are different to those existing at the time of sampling.

The extent of these changes is dependent on the chemical and biological nature of the sample, its temperature, its exposure to light, the type of the container in which it is placed, the time between

sampling and analysis, and the conditions to which it is subjected, e.g. agitation during transport. Further specific causes of variation are listed in a) to f).

- a) The presence of bacteria, algae and other organisms can consume certain constituents of the samples. These organisms can also modify the nature of the constituents to produce new constituents. This biological activity affects, for example, the concentrations of dissolved oxygen, carbon dioxide, compounds of nitrogen, phosphorus and, sometimes, silicon.
- b) Certain compounds can be oxidized either by dissolved oxygen present in the samples, or by atmospheric oxygen [e.g. organic compounds, Fe(II) and sulfides].
- c) Certain substances can precipitate out of solution, e.g. calcium carbonate, metals, and metallic compounds such as Al(OH)₃, or can be lost to the vapour phase (e.g. oxygen, cyanides, and mercury).
- d) Absorption of carbon dioxide from air can modify pH, conductivity, and the concentration of dissolved carbon dioxide. Passage of compounds like ammonia and silicon fluoride through some types of plastics may also affect pH or conductivity.
- e) Dissolved metals or metals in a colloidal state, as well as certain organic compounds, can be irreversibly adsorbed on to the surface of the containers or solid materials in the samples.
- f) Polymerized products can depolymerize, and conversely, simple compounds can polymerize.

Changes to particular constituents vary both in degree and rate, not only as a function of the type of water, but also, for the same water type, as a function of seasonal conditions.

These changes are often sufficiently rapid to modify the sample considerably in a short time. In all cases, it is essential to take precautions to minimize these reactions and, in the case of many analytes, to analyse the sample with a minimum of delay. If the required precaution for changes is filtration on site, then a filter (5.3.2) shall be used.

Details of the sample preservation are given in [Table A.1](#).

7.2 Sample handling and preservation for biological examination

The handling of samples for biological examination is different from that for samples requiring chemical analysis. The addition of chemicals to the sample for biological examination can be used for either fixation and/or preservation of the sample. The term “fixation” is defined as the protection of morphological structures, while the term “preservation” is defined as the protection of organic matter from biochemical or chemical degradation. Preservatives, by definition, are toxic, and the addition of preservatives may lead to the death of living organisms. Prior to death, irritation may cause the most delicate organisms, which do not have strong cell walls, to collapse before fixation is complete. To minimize this effect, it is important that the fixation agent enter the cell quickly.

IMPORTANT — Acidic Lugol’s solutions (5.2.11) can lead to the loss of structures in organisms or also lead to the loss of small organisms (e.g. some flagellates); in this case, use an alkaline Lugol’s solution (5.2.10), e.g. during the summer, when the appearance of silico-flagellates is frequently observed.

The fixing and/or preservation of samples for biological examination shall meet the following criteria:

- a) the effect of the fixative, and/or preservative, on the loss of the organism shall be known beforehand;
- b) the fixative or preservative shall effectively prevent the biological degradation of organic matter at least during the storage period of the samples;
- c) the fixative, and/or preservative, shall enable the biological analyte (e.g. organisms or taxonomical groups) to be assessed during the storage period of the samples.

Details of the preservation of samples are given in [Table A.2](#).

7.3 Sample handling and preservation for radiochemical analysis

WARNING — Radioprotection such as shielding may be necessary, depending on the activity of the sample.

There is little difference between the handling of samples for radiochemical analysis and the handling of samples for physicochemical analysis.

The delay between sampling and measurement has to be consistent with the radioactive half-life of the radionuclides of interest. The conditions to be taken for adequate storage are independent of the radioactive half-life, but identical to those required for the corresponding stable isotope.

NOTE Cooling radiological samples is primarily used to prevent algal growth and biological spoilage. It is not a necessary preservation step for radiochemical analyses. These samples are often combined with those for physical, chemical or biological analysis.

8 Sample transport

Cooling or freezing procedures shall be applied to samples to increase the time period available for transport and storage and if required by [Tables A.1](#) to [A.3](#). When transport takes place, the sampling plan (e.g. ISO 5667-1) shall consider:

- the time between sampling and start of transport;
- transport time;
- starting time of analysis in the laboratory.

This sum of these three periods is limited to the maximum storage times according to [Tables A.1](#) to [A.3](#).

If the maximum storage time cannot be met, then the sampling plan shall be reformulated to allow these requirements to be accommodated.

A cooling temperature of the device during transport of $(5 \pm 3) ^\circ\text{C}$ has been found suitable for many applications. Cooling and freezing procedures applied shall be in line with instructions from the analytical laboratory. Freezing especially requires detailed control of the freezing and thawing process in order to return the sample to its initial equilibrium after thawing.

Containers holding samples shall be protected and sealed during transport in such a way that the samples do not deteriorate or lose any part of their content. Container packaging shall protect the containers from possible external contamination, particularly near the opening, and should not itself be a source of contamination.

Glass containers shall be protected from potential breakage during transport by appropriate packaging. Samples shall be transported as soon as possible after sampling and with cooling if necessary according to [Tables A.1](#) to [A.3](#).

Laboratory samples for dispatch or transport by third parties and preserved laboratory samples should be sealed in such manner that the integrity of the sample can be maintained.

Samples required for (potential) regulatory investigations should be sealed to a level that meets the requirements of the authorities or other organization(s) concerned with the transport of the sample.

During transportation samples shall be stored in a cooling device capable of maintaining a temperature of $(5 \pm 3) ^\circ\text{C}$. For proper evaluation of the conditions during transport, a device capable of recording the (maximum) temperature of the air surrounding the sample may be used.

NOTE Devices capable of logging of the air temperature during the transportation are available, but their use and adequate calibration can be costly.

9 Identification of samples

Container labels should withstand wetting, drying and freezing without detaching or becoming illegible. The labelling system shall be waterproof to allow use on site.

The exact information given in the sampling report and on the sample labels depends on the objectives of the particular measurement programme. In all cases, an indelible label shall be secured to the sample container.

For each sample, at least the following information shall be available.

- A unique identifier, traceable to
 - date, time and location of sampling;
 - sample number;
 - description of sample;
 - name of sampling personnel;
 - details of sample preservation, or fixation used;
 - details of sample storage used;
 - any information regarding integrity and manipulation of the sample;
 - any other information, as necessary.
- A unique identifier, traceable to sample date, location, and sample number shall appear on the label of the sample container.

All other information is supplementary and should be detailed in the sample report.

10 Sample reception

All information regarding sample, handling and storage shall be included in a sampling report.

Laboratory staff shall receive and check information on sample preservation and sample transport conditions.

In all cases, and especially when a “chain of custody” process needs to be established, the number of sample containers received in the laboratory shall be verified against the number of sample containers submitted.

11 Sample storage

The storage duration of the water samples within the laboratory is specific to the analyte(s) to be analysed. Samples shall be stored no longer than the maximum storage period given in [Tables A.1](#) to [A.3](#). The maximum storage time includes the time of transport to the laboratory ([3.4](#)).

The refrigeration conditions within the laboratory shall be $(3 \pm 2) ^\circ\text{C}$. Where samples are frozen for preservation, unless otherwise specified, the temperature shall be maintained below $-18 ^\circ\text{C}$. Exceptions to these refrigeration conditions are listed in [Tables A.1](#) to [A.3](#).

When thawing frozen samples, it is recommended that each sample container be placed in a separate secondary container to minimize the risk of liquid loss, should a split become apparent during the thawing process or a rupture occur during initial freezing and storage. A mild impact can cause splitting of some plastics at low temperatures.

With respect to thawing, it is recommended that this be done under ambient conditions, unless specified otherwise in [Tables A.1](#) to [A.3](#) or the analytical International Standard being used.

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