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Milk — Determination of fat content (Routine method)

Lait — Détermination de la teneur en matière grasse (Méthode de routine)

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FOREWORD

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Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 2446 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the Member Bodies in June 1975.

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It has been approved by the Member Bodies of the following countries :

Austria	Hungary	Poland
Belgium*	India	Romania
Canada	Iran	South Africa, Rep. of
Czechoslovakia	Ireland	Spain
Egypt, Arab Rep. of	Mexico	Thailand
France	Netherlands	Turkey
Germany	New Zealand	United Kingdom
Ghana	Peru	Yugoslavia

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* Belgium approved the International Standard with the exception of items b) and c) and notes 1 and 2 of clause 1, and of clauses 12 and 13.

The Member Body of the following country expressed disapproval of the document on technical grounds :

Israel

Milk — Determination of fat content (Routine method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a routine method (the Gerber method) for the determination of the fat content of milk and includes guidance on the determination of the appropriate capacity of the milk pipette and on the determination of the corrections to apply to the results if the milk is not of average fat content (see 6.1). The procedure for checking the capacity of the milk pipette is described in the annex.

The method is applicable to liquid milk, whole or partially skimmed, raw or pasteurized. With modifications, details of which are given, it is also applicable to

- a) milk containing certain preservatives (see clause 11);
- b) milk that has undergone the process of homogenization, in particular sterilized milk and ultra heat-treated (UHT) milk (see clause 12);
- c) skimmed milk (see clause 13).

NOTES

1 The result obtained by the procedure described in clause 12 (modified procedure for milk that has undergone the process of homogenization) may be slightly high. Further study to obtain a more reliable procedure for such milk is in progress.

2 Work is in progress on a procedure for testing skimmed milk with a special 0 to 0,5 % fat butyrometer. This butyrometer will be more suitable than the 0 to 4 % fat butyrometer currently used for skimmed milk (see clause 13).

2 REFERENCES

ISO/R 488, *Butyrometers for the determination of the fat content of milk by the Gerber method*.

ISO/R 707, *Milk and milk products — Sampling*.

ISO/R 1211, *Milk — Determination of fat content (Reference method)*.

NOTE — ISO/R 1211 is being revised. The revised version will specify a reference Röse-Gottlieb method applicable to certain milk products as well as to liquid milk.

3 DEFINITION

Gerber method: An empirical procedure which gives a value for fat content in grams of fat per 100 g of milk or per 100 ml of milk — depending on the capacity of the milk

pipette used — that is the same as, or has a known relationship to, the value obtained by the reference method (ISO/R 1211).

NOTE — Periodic comparative determinations by the Gerber method and by the reference method should be made to ensure that the Gerber method satisfies the above definition (see 10.4).

4 PRINCIPLE

Separation of the fat of the milk in a butyrometer by centrifuging after dissolving the protein with sulphuric acid, the separation being aided by the addition of a small quantity of amyl alcohol. The butyrometer is graduated to give a direct reading of fat content.

5 REAGENTS

5.1 Sulphuric acid

5.1.1 Requirements

The sulphuric acid shall have a density at 20 °C of $1,816 \pm 0,004$ g/ml, which corresponds to approximately $90,4 \pm 0,8$ % (m/m) H₂SO₄. The acid shall be colourless, or not darker in colour than pale amber, shall be free from suspended matter and shall be found suitable for use when tested as specified in 5.1.2.

5.1.2 Suitability test

5.1.2.1 PURPOSE OF TEST

A sulphuric acid may satisfy the specific requirements of 5.1.1 for density and appearance and yet be unsuitable for the Gerber method. Therefore, check the suitability of the acid before use by means of the following comparative test with a standard sulphuric acid.

5.1.2.2 STANDARD SULPHURIC ACID

Add sulphuric acid of analytical reagent quality (for example 98 % (m/m) H₂SO₄, ρ_{20} 1,84 g/ml) to distilled water, or water of at least equal purity, to obtain a solution with a density within the range specified in 5.1.1.

NOTE — Approximately 1 l of standard sulphuric acid is obtained by adding 908 ml of 98 % (m/m) sulphuric acid to 160 ml of water, checking the density of the diluted acid with a suitable hydrometer and adjusting the density, if necessary, by adding a small volume of water or 98 % (m/m) acid.

5.1.2.3 COMPARISON PROCEDURE

Determine in duplicate the fat content of four samples of whole milk with average fat content by the Gerber method described, using butyrometers whose scale errors are less than 0,01 % and standard amyl alcohol (5.2.6.2). In one of each pair of duplicates use 10 ml of the sulphuric acid under test and in the other use 10 ml of the standard sulphuric acid (5.1.2.2). Keep the butyrometers in a random order from the shaking stage onwards. Take the readings to the nearest 0,01 % fat (read by at least two persons). The mean fat content of the four milk samples obtained with the sulphuric acid under test shall not differ by more than 0,015 % fat from the mean value obtained using the standard sulphuric acid.

5.2 Amyl alcohol

5.2.1 Composition

At least 98 % (V/V) of the amyl alcohol shall consist of the primary alcohols 3-methylbutan-1-ol and 2-methylbutan-1-ol, the only permissible major impurities being 2-methylpropan-1-ol and butan-1-ol. It shall be free from secondary pentanols, 2-methylbutan-2-ol, 2-furaldehyde, gasoline (petrol) and derivatives of benzene. Not more than a trace of water shall be present.

5.2.2 Appearance

The amyl alcohol shall be clear and colourless.

5.2.3 Density

The amyl alcohol shall have a density at 20 °C of 0,808 to 0,818 g/ml.

5.2.4 2-furaldehyde and other organic impurities

When 5 ml of the amyl alcohol is added to 5 ml of the sulphuric acid (5.1), no more than a yellow or light-brown colour shall develop.

5.2.5 Distillation range

When the amyl alcohol is distilled at a pressure of 1 013 mbar*, not less than 98 % (V/V) shall distil below 132 °C and not more than 5 % (V/V) below 128 °C. There shall be no solid residue after distillation.

NOTE – If the atmospheric pressure during the distillation is lower or higher than 1 013 mbar, the specified temperatures should be respectively decreased or increased by 0,03 °C/mbar.

* 1 mbar = 0,1 kPa

5.2.6 Suitability test

5.2.6.1 PURPOSE OF TEST

An amyl alcohol may satisfy the requirements of 5.2.1 to 5.2.5 yet be unsuitable for the Gerber method. Therefore, check the suitability of the amyl alcohol before use by means of the following comparative test with a standard amyl alcohol.

5.2.6.2 STANDARD AMYL ALCOHOL

Distil an amyl alcohol satisfying the requirements of 5.2.1 to 5.2.5, using a suitable fractionation column, and collect a fraction within a boiling range of 2 °C between 128 °C and 131,5 °C (see note to 5.2.5). Apply the following tests to the fraction :

- a) When analysed by gas-liquid chromatography, at least 99 % (V/V) shall consist of 3-methylbutan-1-ol and 2-methylbutan-1-ol. Only traces of impurities other than 2-methylbutan-1-ol and butan-1-ol shall be present.
- b) When fractionally distilled, the first 10 % and the last 10 % collected, when compared using the procedure described in 5.2.6.3, shall give values for the fat content of milk that do not differ by more than 0,015 % fat.

If the fraction satisfies both these tests it can be regarded as standard amyl alcohol. The standard amyl alcohol can be used for several years, provided that it is kept in the dark in a cool place.

5.2.6.3 COMPARISON PROCEDURE

Determine in duplicate the fat content of four samples of whole milk with average fat content by the Gerber method described, using butyrometers whose scale errors are less than 0,01 % and standard sulphuric acid (5.1.2.2). In one of each pair of duplicates use 1 ml of the amyl alcohol under test, and in the other use 1 ml of the standard amyl alcohol (5.2.6.2).

Keep the butyrometers in a random order from the shaking stage onwards. Take the readings to the nearest 0,01 % fat (read by at least two persons).

The mean fat content of the four milk samples obtained with the amyl alcohol under test shall not differ by more than 0,015 % fat from the mean value obtained using the standard amyl alcohol.

NOTE – Instead of the specified amyl alcohol an artificial amyl alcohol or an amyl alcohol substitute, coloured if desired, may be used, provided that its use has been demonstrated by experiment not to lead to any significant differences in the results of the determination.

6 APPARATUS

6.1 Milk pipette

6.1.1 The milk pipette shall be of the single graduation line, bulb type, and its capacity shall be defined as the volume, in millilitres, of water at 20 °C (27 °C in tropical countries) delivered by the pipette when emptied as described in the annex.

The capacity of the pipette, determined by the method described in the annex, shall not differ from the nominal capacity, established according to 6.1.3, by more than 0,03 ml.

6.1.2 The capacity of the milk pipette shall be such that, when the pipette is used as described in 9.2 (i.e. using the top of the milk meniscus when adjusting the milk to the graduation line) and whichever method of expressing the result is adopted (see clause 3), the value for fat content obtained agrees with the value obtained by the reference method using whole milk having a fat content equivalent to the accepted average of the national milk supply.

NOTE — Some milk pipettes are available which allow the bottom of the milk meniscus to be observed during pipetting. If such pipettes are so used, their capacity should be such that when they are used with milk of average fat content the requirement of 6.1.2 is satisfied.

6.1.3 In each country, the appropriate capacity (see 6.1.1 and 6.1.2) of the milk pipette shall be established by carrying out comparative determinations using the Gerber method described and the reference method (ISO/R 1211) on a large number of whole milks covering a wide range of fat content. Statistical analysis of the results of these determinations taken in conjunction with a knowledge of the national average fat content of milk shall be used to establish the appropriate capacity of the milk pipette. These comparative determinations on whole milk, together with similar determinations on partially skimmed milk and skimmed milk, will also provide the corrections to be applied, if desired or if necessary, to Gerber values when milk is not of average fat content. For these comparative determinations, butyrometers whose scale errors are less than 0,01 % shall be used, and the butyrometers read to the nearest 0,01 % fat.

6.1.4 If the value for fat is to be expressed in grams of fat per 100 ml of milk, the basis of comparison with the reference method shall be stated.

6.2 Butyrometer and stopper, as described in ISO/R 488.

NOTES

1 Use a butyrometer whose scale range is appropriate for the expected fat content of the sample.

2 With corrugated-neck butyrometers, either lock stoppers or solid single-ended or double-ended rubber stoppers may be used.

With plain-neck butyrometers, lock stoppers should preferably be used.

6.3 Automatic measure, or safety pipette, to deliver $10,0 \pm 0,2$ ml of sulphuric acid (5.1).

6.4 Automatic measure, or safety pipette, to deliver $1,0 \pm 0,05$ ml of amyl alcohol (5.2).

6.5 Protected stand, for shaking the butyrometers (6.2).

6.6 Centrifuge, in which the butyrometers can be spun, provided with a speed indicator which indicates the number of revolutions per minute with a maximum tolerance of ± 50 rev/min, and preferably of the vertical-loading type rather than the horizontal-loading type.

The design of the centrifuge shall be such that the temperature of the butyrometer contents after the centrifuging (see 9.6) is between 30 and 50 °C.

The centrifuge shall be capable of producing within 2 min, when fully loaded, a relative centrifugal acceleration of $350 \pm 50 g$ at the outer end of the butyrometer stopper. This acceleration is produced by centrifuges with the following effective radius (horizontal distance between the centre of the centrifuge spindle and the outer end of the butyrometer stopper) operated at the speed indicated.

Effective radius mm	Revolutions per minute ± 70 rev/min
240	1 140
245	1 130
250	1 120
255	1 110
260	1 100
265	1 090
270	1 080
275	1 070
300	1 020
325	980

NOTE — The relative centrifugal acceleration produced in a centrifuge is given by the following formula :

$$1,12 \times 10^{-6} RN^2$$

where

R is the effective horizontal radius, in millimetres;

N is the speed, in revolutions per minute.

6.7 Water bath for butyrometers, capable of being maintained at 65 ± 2 °C and such that the butyrometers (6.2) can be supported in a vertical position with their scales completely immersed.

6.8 Thermometer, suitable for insertion in the water bath (6.7).

6.9 Water bath, if necessary, for the preparation of the sample (see clause 8).

7 SAMPLING

See ISO/R 707.

8 PREPARATION OF TEST SAMPLE

8.1 Adjust the temperature of the laboratory sample to 20 to 30 °C, using a water bath if necessary. Mix the milk thoroughly but gently by repeatedly inverting the sample bottle without causing frothing or churning of the fat. If there is difficulty in dispersing a cream layer, or if the milk shows evidence of slight churning, warm the milk slowly to 34 to 40 °C in a water bath and mix gently; if necessary, a suitable mixing device may be used to assist the dispersal of the fat. When a uniform distribution of the fat has been achieved, quickly adjust the temperature of the milk to approximately 20 °C (approximately 27 °C in tropical countries where the milk pipette is calibrated at this temperature). Allow the milk to stand after the final temperature adjustment, to let air bubbles rise. Normally 3 to 4 min is sufficient but, if a mixing device has been used, up to 2 h may be required, followed by a further temperature adjustment.

NOTE — If, after the preparation of the test sample, white particles are visible on the walls of the sample bottle, or liquid fat is visible on the surface of the sample, a reliable value for fat content cannot be expected.

8.2 Immediately after the preparation of the test sample, the procedure described in clause 9, 11, 12 or 13, as appropriate, should be started and completed without interruption.

9 PROCEDURE FOR WHOLE MILK AND PARTIALLY SKIMMED MILK

WARNING — Take suitable precautions, such as the wearing of a face-visor, against the accidental release of sulphuric acid.

9.1 Measure $10 \pm 0,2$ ml of sulphuric acid (5.1) into the butyrometer (6.2), using the automatic measure or safety pipette (6.3), in such a way that the acid does not wet the neck of the butyrometer or entrap any air.

9.2 Gently invert the bottle containing the prepared test sample (clause 8) three or four times and immediately measure the required volume of milk into the butyrometer in the following manner.

Suck the milk into the milk pipette (6.1) until the milk level is slightly above the graduation line and wipe the outside of the delivery jet free from milk. With the pipette held vertically, the graduation line at eye level and the tip of the jet touching the inside of the neck of the inclined sample bottle, allow the milk to flow from the pipette until the top of the milk meniscus (not the bottom of the meniscus, which is difficult to see) is coincident with the graduation line (see the note to 6.1.2).

Remove the jet from contact with the sample bottle and then, with the butyrometer in a vertical position and the pipette held at an angle of about 45° with the tip of the jet just below the bottom of the neck of the butyrometer, allow the milk to flow gently down the inside of the butyrometer to form a layer on top of the acid, preventing

as far as possible any mixing with the acid. When the out-flow has ceased, wait 3 s, touch the tip of the pipette against the bottom of the neck and then remove the pipette. Care should be taken not to wet the neck of the butyrometer with milk.

9.3 Measure $1 \pm 0,05$ ml of the amyl alcohol (5.2) into the butyrometer, using the automatic measure or safety pipette (6.4). Do not wet the neck of the butyrometer with the amyl alcohol and avoid at this stage mixing the liquids in the butyrometer.

9.4 Securely stopper the butyrometer without disturbing its contents. When a double-ended stopper is used, screw it in until the widest part is at least level with the top of the neck. When a lock stopper is used, insert it until the rim is in contact with the neck of the butyrometer.

9.5 Shake and invert the butyrometer, suitably protected (6.5) in case of breakage or loosening of the stopper, until its contents are thoroughly mixed, and until the protein is completely dissolved, i.e. until no white particles remain.

9.6 Immediately place the butyrometer in the centrifuge (6.6), bring the centrifuge to the operating speed required to give a relative centrifugal acceleration of $350 \pm 50 g$ within 2 min, and then maintain this speed for 4 min.

9.7 Remove the butyrometer from the centrifuge and, if necessary, adjust the stopper to bring the fat column on to the scale. Place the butyrometer, stopper downwards, in the water bath (6.7) at 65 ± 2 °C for not less than 3 min and not more than 10 min; the water level shall be above the top of the fat column.

9.8 Remove the butyrometer from the water bath and carefully adjust the stopper to bring the bottom of the fat column, with the minimum movement of the column, to the top edge of a graduation line, preferably a main graduation line. When a solid rubber stopper is used, the adjustment should preferably be done by slightly withdrawing the stopper and not by forcing it farther into the neck. When a lock stopper is used, insert the key and apply sufficient pressure to raise the fat column to the required position.

Note the scale reading coincident with the bottom of the fat column and then, taking care that the fat column does not move, as quickly as possible note the scale reading coincident with the lowest point of the fat meniscus at the top of the fat column. Take the reading at the top of the column to the nearest half of a smallest scale division. While readings are being taken, hold the butyrometer vertically with the point of reading at eye level. Record the difference between the two readings (see 10.1).

NOTE — If the fat is turbid or dark in colour, or if there is white or black material at the bottom of the fat column, the value for fat content will not be reliable.

9.9 If a check of the value obtained is desired, replace the butyrometer in the water bath (6.7) at $65 \pm 2^\circ\text{C}$ for not less than 3 min and not more than 10 min, and then remove it from the bath and again take readings as described in 9.8.

10 EXPRESSION OF RESULTS

10.1 Method of calculation

The fat content of the milk is

$$B - A$$

where

A is the reading at the bottom of the fat column;

B is the reading at the top of the fat column.

The fat content shall be expressed in grams of fat per 100 g of milk or in grams of fat per 100 ml of milk, according to whether the capacity of the milk pipette was chosen with the former or latter method of expressing the results in view.

10.2 Repeatability

If two determinations are carried out simultaneously, or in rapid succession, by the same analyst, the difference between the results should not exceed the value corresponding to one smallest scale division of the butyrometer. When butyrometers with scale errors less than 0,01 % are used (for example see 6.1.3) the difference between the results of two determinations obtained as described should not exceed the value corresponding to half a smallest scale division.

10.3 Correction of results

If the value obtained is outside the range in which the particular milk pipette used gives results in agreement with the reference method, the appropriate correction (see 6.1.3) may be applied if desired.

10.4 Special precision

For the comparative determinations mentioned in the note to clause 3 and for other special purposes which require a Gerber value for fat that is as precise as possible, use a butyrometer whose scale errors are less than 0,01 %, and read the butyrometer to the nearest 0,01 % fat. If necessary, apply a correction as described in 10.3.

11 MODIFIED PROCEDURE FOR MILK CONTAINING PRESERVATIVE

11.1 The following procedure is applicable in certain instances to whole milk and partially skimmed milk, raw or pasteurized, to which preservative (for example, potassium dichromate, mercury(II) chloride, or a mixture of the two) has been added. The procedure is applicable provided that the concentration of preservative in the milk and the duration and conditions of storage of the preserved milk are such that the result of the determination is the same as would have been obtained with the fresh milk not containing preservative.

If the milk containing preservative is milk that has gone through the process of homogenization, follow the procedure described in clause 12 but, if necessary, ensuring complete solution of the protein at the appropriate stage in the procedure as described in 11.4.

If the milk containing preservative is skimmed milk, follow the procedure described in clause 13 but, if necessary, ensuring complete solution of the protein at the appropriate stage in the procedure as described in 11.4.

11.2 Use the reagents and apparatus described in clauses 5 and 6 respectively.

11.3 Prepare the test sample as described in clause 8. Milk containing preservative will usually require slow warming to 35 to 40°C to ensure complete dispersal of the cream layer.

11.4 Follow the procedure described in clause 9. With milk containing preservative, there may be some difficulty in achieving complete solution of the protein (see 9.5). In this case, place the butyrometer, stopper downwards, in the water bath (6.7) at $65 \pm 2^\circ\text{C}$ with occasional shaking and inversion of the butyrometer until no white particles can be seen. Then proceed as described in 9.6 to 9.9 inclusive.

NOTE — If the time required in the water bath to dissolve the protein exceeds 10 min, the method will not give an accurate result and is not applicable to the sample.

11.5 Calculate the fat content as described in 10.1. The requirements of 10.2, 10.3 and 10.4 apply.

12 MODIFIED PROCEDURE FOR MILK THAT HAS UNDERGONE THE PROCESS OF HOMOGENIZATION (See note 1 to clause 1)

12.1 Use the reagents and apparatus described in clauses 5 and 6 respectively.

12.2 Prepare the test sample as described in clause 8.

12.3 Follow the procedure described in 9.1 to 9.8 inclusive and obtain a first value for fat content.

NOTE — When a number of samples is being examined simultaneously, begin to read the first of the series after 3 min. Replace each butyrometer in the water bath (6.7) at $65 \pm 2^\circ\text{C}$ after reading. The number of butyrometers should not be more than can be read within the time limit given in 9.7.

12.4 Repeat the procedure given in 9.6, 9.7 and 9.8, and obtain a second value for fat content. If the second value does not exceed the first value by more than half a smallest scale division, the second value shall be recorded as the fat content of the milk.

12.5 If the second value exceeds the first value by more than half a smallest scale division, repeat the procedure described in 9.6, 9.7 and 9.8 and obtain a third value for the fat content. If the third value does not exceed the second value by more than half a smallest scale division, the third value shall be recorded as the fat content of the milk.

12.6 If the third value exceeds the second value by more than half a smallest scale division, repeat the procedure described in 9.6, 9.7 and 9.8 and obtain a fourth value for the fat content. The fourth value shall be recorded as the fat content of the milk, but if this value exceeds the third value by more than half a smallest scale division, it should be regarded as of doubtful accuracy.

12.7 Calculate the fat content as described in 10.1. The requirements of 10.2, 10.3 and 10.4 apply.

NOTE — If, after the several centrifugings, the fat is turbid or dark in colour, or if there is white or black material at the bottom of the fat column, the value for fat content will not be accurate.

13 MODIFIED PROCEDURE FOR SAMPLES OF SKIMMED MILK

13.1 Use the reagents and apparatus described in clauses 5 and 6 respectively; the butyrometer with a range of 0 to 4 % fat should be used. (See note 2 to clause 1.)

13.2 Prepare the test sample as described in clause 8.

13.3 Follow the procedure described in 9.1 to 9.7 inclusive. Then remove the butyrometer from the water bath, immediately repeat the centrifuging (9.6) and temperature adjustment (9.7), and proceed as described in 9.8 and 9.9.

13.4 Calculate the apparent fat content as described in 10.1. Apply the appropriate correction as determined by the statistical analysis of the results of comparative determinations on skimmed milks of differing fat content by the Gerber method (clause 13) and the Röse-Gottlieb reference method ISO/R 1211 (see 6.1.3).

If there is insufficient fat in the butyrometer to enable scale readings to be taken, the fat content cannot be calculated as described in 10.1; in such a case, record the apparent fat content as (for example) : "nil", "trace", "fraction of meniscus".

14 TEST REPORT

The test report shall show the method used and the result obtained, including :

- a) its method of expression;
- b) the capacity of the milk pipette;
- c) the scale range of the butyrometer;
- d) indication of whether the result is uncorrected or corrected as described in 10.3 and whether the procedure of 10.4 has been followed;
- e) any observation that indicates that the result is of doubtful accuracy (for example, see notes to 8.1, 9.8, 11.4 and 12.7 and see 12.6).

The report shall also mention any operating conditions not specified in this International Standard, or conditions regarded as optional, and any circumstances that may have influenced the result.

The report shall include all details required for complete identification of the sample.

ANNEX

PROCEDURE FOR CHECKING THE CAPACITY OF THE MILK PIPETTE

A.1 Carry out the following operations at room temperature and with the water and the pipette at room temperature.

A.2 Suck distilled water into the thoroughly cleaned milk pipette until the water level is a few millimetres above the graduation line, then wipe the outside of the delivery jet free from water. With the pipette held vertically and the graduation line at eye level, allow water to flow from the pipette until the lowest point of the meniscus is coincident with the graduation line. Remove any water adhering to the tip of the jet by momentarily bringing the tip of the jet into contact with the inside of an inclined glass beaker.

A.3 With the pipette held vertically and the tip of the jet touching the inside of an inclined weighing bottle (previously weighed), allow the water to flow freely from the pipette until visible outflow ceases. Then, after 3 s, remove the weighing bottle from contact with the jet, stopper the bottle, weigh it and calculate the mass of water delivered by the pipette. Record the temperature of the water to the nearest 0,1 °C. Using appropriate tables for use in the calibration of volumetric glassware, calculate the capacity of the pipette as the volume, in millilitres, of water at 20,0 °C (27,0 °C in tropical countries) delivered by the pipette.

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