

Australian Standard[®]

**METHODS FOR
THE CHEMICAL
EXAMINATION OF EGGS
AND EGG PRODUCTS**

METRIC UNITS

The following scientific, industrial and governmental organizations and departments were officially represented on the committee entrusted with the preparation of this standard:

Australian Department of Agriculture
Council of Egg Marketing Authorities
CSIRO, Division of Food Research
Department of Primary Industries, Queensland
Departments of Public Health
Department of Science
Egg Marketing Boards
Health Commission of N.S.W.
Royal Australian Chemical Institute

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**METHODS FOR
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PREFACE

These methods for the preparation and chemical examination of eggs and egg products were drawn up by the Association's Committee on Examination of Egg and Egg Products.

The section (Section 2) on preparation of eggs and egg samples for chemical examination describes the procedures for preparing samples of eggs and egg products received by the testing laboratory for chemical examination by the other sections of the standard. It is assumed for the purpose of this standard that the laboratory sample is representative of the bulk of the subject material.

Methods of examination described herein include those for total solids, protein, lipids, reducing substances, Haenni value, pH, flavour, alpha-amylase, chloride, yolk colour, Haugh Unit value and headspace gas composition.

In drawing up this standard, reference was made to the Official Methods of Analysis of the Association of Official Analytical Chemists, and acknowledgment is made of the assistance obtained therefrom.

This standard refers to the following standards:

BS 2648, Performance Requirements for Electrically Heated Laboratory Drying Ovens

AS*, Methods for Sampling and Transportation of Samples of Eggs and Egg Products

BS 2586, Glass Electrodes for Measurement of pH

ISO/R 383, Interchangeable Conical Ground Glass Joints.

Attention is drawn to the fact that this standard prescribes methods of examination only and should not be used or quoted as a specification defining limits of purity. Any reference to such a standard should be in the form of words indicating that the methods of examination used are those specified in AS 1383.

* In course of preparation.

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STANDARDS ASSOCIATION OF AUSTRALIA

Australian Standard
METHODS FOR THE CHEMICAL EXAMINATION
OF EGGS AND EGG PRODUCTS

SECTION 1. SCOPE AND GENERAL

1.1 SCOPE. This standard describes the preparation of samples for examination, and methods for the chemical examination of eggs and egg products.

1.2 DEFINITIONS. For the purposes of this standard, 'eggs' are those laid by domestic hens; 'egg products' are products consisting wholly or mainly of one or more of the constituents of eggs.

1.3 APPARATUS AND REAGENTS.

1.3.1 Apparatus. Volumetric glassware used shall comply, where appropriate, with the relevant Australian standard (Class A for primary standardization or Class B for other uses).

1.3.2 Reagents. All reagents used, including water, shall be of suitable purity to meet the requirements of the particular examination.

1.4 CLASSIFICATION. For the purposes of this standard, eggs and egg products are classified into the following groups:

- (i) Shell eggs
- (ii) Liquid egg products
- (iii) Frozen egg products
- (iv) Dried egg products.

1.5 SAMPLING PROCEDURE AND TRANSPORT TO LABORATORY. The eggs or egg products shall be sampled and transported to the testing laboratory in accordance with AS*.

* AS, Methods for Sampling and Transportation of Samples of Eggs and Egg Products (in course of preparation).

SECTION 2. PREPARATION OF SAMPLES

2.1 PREPARATION OF SAMPLES. Samples of eggs and egg products shall be prepared for chemical examination as described below according to their classification. It is necessary to prepare a uniform mixture of the sample.

NOTE: For some methods particular requirements for preparation of samples are necessary and are described in the relevant method.

2.2 APPARATUS. Where a blender is necessary it shall be cleaned and dried before use.

2.3 SHELL EGGS. Where examination cannot be made immediately, shell eggs shall be held in the laboratory at a temperature between 0°C and 10°C and, unless otherwise specified, shall be examined within 7 days. The sample shall be prepared for testing as follows:

- (i) Allow the eggs to equilibrate to room temperature.

NOTE: When shell eggs are transferred from one temperature/humidity condition to another, ensure that excess condensation does not form on the shell.

- (ii) If the eggs are soiled, wash them with warm running water (not more than 45°C) and dry rapidly with a clean tissue or in warm air.
- (iii) Transfer the contents to a clean dry wide-mouthed container fitted with a watertight and airtight closure. One or more eggs may be collected in each container, depending on the purposes of the examination.

If required, egg yolk and egg white may be separated.

- (iv) Thoroughly mix the sample taking care to avoid excessive foaming.

2.4 LIQUID EGG PRODUCTS. For the purposes of this standard this classification includes whole egg, egg white, egg yolk, salted yolk, sugared yolk and other liquid egg products.

Samples of liquid egg products shall be held in the laboratory at a temperature between 0°C and 5°C and, unless otherwise specified, shall be examined within 72 h of sampling.

The sample shall be prepared for testing by thoroughly mixing the sample, taking care to avoid excessive foaming.

2.5 FROZEN EGG PRODUCTS. For the purposes of this standard, this classification includes whole egg, egg yolk, egg white, salted yolk, sugared yolk and other frozen egg products.

Samples of frozen egg products shall be kept at a temperature below -10°C prior to sample preparation. Thawed samples arriving at the laboratory shall be rejected.

The sample shall be prepared for testing as follows:

- (i) Thaw the sample overnight in a refrigerator at a temperature of not more than 5°C.

Alternatively, the frozen sample may be thawed rapidly in a water bath at a temperature of not more than 45°C. Rotate or shake the sample container frequently during the thawing and remove from the water bath immediately thawing is complete.

- (ii) After thawing, thoroughly mix the sample taking care to avoid excessive foaming.

2.6 DRIED EGG PRODUCTS. Samples of dried egg products shall be held in the laboratory at a temperature lower than 10°C in an airtight container.

The sample shall be prepared for testing as follows:

- (i) Allow the sample to equilibrate to room temperature before opening the container.
- (ii) Thoroughly mix the sample.

SECTION 3. INTERPRETATION OF TEST RESULTS

3.1 ROUNDING OF NUMBERS. The following procedure* is to be used in rounding off numbers:

- (i) Choose the rounding (up or down) whichever is the nearer. In general this will be evident.
- (ii) If, despite the use of all the figures which can be obtained from the data, the figures to be discarded fall exactly between two alternative prospective round values, and there is no evidence to indicate in which direction to round, choose the even round value. This rule applies to both positive and negative values.

Rounding off should be carried out in one operation. If a value, e.g. of 99.253 percent, is to be rounded off to one place of decimals, the figures in the second and third decimal places should be considered together, the rounded-off value being 99.3 percent. If rounding off is carried out in two operations an incorrect figure may be obtained in the last place, e.g. if 99.253 percent is rounded off in two operations, it first becomes 99.25 percent and then 99.2 percent.

Where the figure next beyond the last figure or place to be retained is 5, or a 5 followed only by zeros, the figure in the last place retained shall be:

- (a) increased by one if it is odd, and
- (b) left unchanged if even.

NOTE: Zero would be regarded as an even number for this purpose.

3.2 EXAMPLES. As examples of the above rule:

0.0505 is rounded off to 0.050
0.15 is rounded off to 0.2
10.5 is rounded off to 10
99.84 is rounded off to 99.8
99.85 is rounded off to 99.8
99.851 is rounded off to 99.9
99.86 is rounded off to 99.9.

* Based on the provisions of BS 1957, Presentation of Numerical Values (Fineness of Expression: Rounding of Numbers).

SECTION 4. DETERMINATION OF TOTAL SOLIDS

4.1 SCOPE. This method describes the procedure for determining the total solids in eggs and egg products.

NOTE: Provision for expressing the result in terms of moisture content is also included.

4.2 DEFINITION. The total solids of eggs and egg products is defined as the percentage of material remaining when determined as described in Clause 4.5.

4.3 PRINCIPLE. The total solids are determined by drying the sample initially on a water bath and then in a vacuum oven at 100°C.

4.4 APPARATUS.

- (i) *Dishes.* Dishes shall be made of glass or aluminium and shall be flat bottomed, with readily removable but close fitting lids. A dish with a diameter between 50–80 mm and a depth of less than 40 mm is suitable.
- (ii) *Vacuum oven.* Capable of continuous operation at a temperature of $100 \pm 2^\circ\text{C}$ under a vacuum of 25 mm of mercury (approximately 3 kPa) or less.
- (iii) *Vacuum pump.*

NOTES:

1. Select a vacuum pump which is capable of maintaining the pressure in the oven at approximately 3 kPa. Adjust the rate of flow of the purge gas (dry air or dry nitrogen) through the oven to control the pressure at 3 kPa and to complete the drying operation in 5 h.
2. The temperature of operation of the vacuum oven is measured by means of a thermometer whose bulb protrudes into the oven near the samples.
3. To open the vacuum oven, disconnect the vacuum and shut off the supply of purge gas when the pressure reaches that of the atmosphere.

- (iv) *Boiling water bath.*

4.5 PROCEDURE.

- (a) Dry dish and lid in an oven at $100 \pm 2^\circ\text{C}$ for 30 min, and then transfer to a desiccator containing a suitable desiccant and allow to cool. Weigh to the nearest milligram.
- (b) Remove the lid and evenly spread about 5 g of the liquid egg sample or 2 g of the dried egg sample over the bottom of the dish. Replace the lid and rapidly determine the mass of the sample to the nearest milligram.
- (c) Place the uncovered dish on a boiling water bath until the sample is apparently dry.

NOTE: Omit this step for dried egg products.

- (d) Dry the bottom of the dish and transfer it, with its lid alongside, to a vacuum oven at $100 \pm 2^\circ\text{C}^*$ for 5 h. Slowly break the vacuum[†], cover the dish with its lid. Transfer to the desiccator and weigh after the necessary period of cooling.
- (e) Repeat the heating procedure described in step (d), but with a heating period of 1 h, until the change in mass between successive weighings does not exceed 1 mg.

4.6 CALCULATION AND REPORT.

4.6.1 Total Solids. Determine the mass of the sample after drying and express this figure as a percentage of the original mass of the sample. Report the result as percentage total solids (m/m).

4.6.2 Moisture. If so required, express the loss in mass of the sample after drying as a percentage of the original mass of the sample and report the result as percentage of moisture (m/m).

* Refer to Note 2 above.

† Refer to Note 3 above.

SECTION 5. DETERMINATION OF NITROGEN

5.1 PURPOSE AND SCOPE. This method describes the procedure for determining total nitrogen in liquid and dried eggs.

5.2 DEFINITION. For the purposes of this method the following definition applies:

Total protein — the percentage by mass calculated from the total nitrogen determined in accordance with the procedure described.

5.3 PRINCIPLE. The percentage by mass of total nitrogen in the sample is determined by a micro-Kjeldahl technique and the protein content calculated by multiplying the nitrogen content by a conversion factor.

5.4 APPARATUS, REAGENTS AND SOLUTIONS.

5.4.1 Apparatus.

- (i) *Kjeldahl flask*, 800 ml
- (ii) *Kjeldahl distillation unit*.

5.4.2 Reagents and Solutions.

Cupric sulphate, pentahydrate

Potassium sulphate or anhydrous sodium sulphate

Boric acid solution, aqueous 4 percent (*m/m*)

Hydrochloric acid solution, 0.1N standardized

Sulphuric acid concentrated, $d = 1.84$ g/ml at 20°C

Mixed methyl red-methylene blue indicator. Mix two volumes of 0.2 percent alcoholic solution of methyl red with 1 volume of 0.2 percent alcoholic solution of methylene blue.

Sodium hydroxide solution. Dissolve 450 g of sodium hydroxide in water and dilute to 1 litre, with due regard to the spray and heat evolved.

5.5 PROCEDURE.

5.5.1 Blank Test. Carry out a blank test using the analytical method below, but with 1 g of sucrose in place of the sample.

5.5.2 Analytical Method.

- (a) Weigh to the nearest milligram approximately 2–3 g of the liquid egg sample or 1 g of the dried egg sample, and transfer it to the Kjeldahl flask. Add 0.2 g of cupric sulphate and pour 25 ml of the sulphuric acid down the neck of the flask while gently rotating so as to wash any sample down into the body of the flask.

- (b) Gently rotate the flask so that the contents are well mixed and then place on a rack so that the neck is inclined at an angle of about 45 degrees to the horizontal. Heat the flask until it is gently boiling, and when frothing has ceased add 10 g of the potassium sulphate or sodium sulphate. Boil the contents of the flask gently until clear and free from yellowish colour, and then boil for a further hour.

NOTE: During the heating stages take care that the flask is not directly heated above the level of the contents.

- (c) Allow the digest to cool and carefully dilute with approximately 200 ml of water, mix, add a few glass beads or anti-bumping granules. Set up the distillation unit, with the tip of the condenser immersed in 100 ml of the boric acid solution containing four drops of indicator solution, in a 500 ml beaker flask.
- (d) Carefully add 100 ml of the sodium hydroxide solution to the Kjeldahl flask so as to form two layers. Seal the apparatus and mix gently. Heat steadily, so that boiling is maintained at a uniform rate, until all the ammonia has been distilled (about 150 ml). About 2 min before the end of the distillation, lower the beaker to bring the tip of the condenser above the acid solution and rinse the outside of the tip with water.

Sodium hydroxide solution must be present in excess; this is indicated by the blue solution turning black on heating.

- (e) Titrate the sample and blank distillate to the same end-point with the standardized hydrochloric acid solution.

NOTE: Occasionally it is not necessary to titrate the blank.

5.6 CALCULATION AND REPORT.

5.6.1 Calculation of Total Nitrogen. Calculate the difference between the sample and blank titrations to obtain the volume of standardized hydrochloric acid solution equivalent to the mass of nitrogen in the sample used.

1 ml of 0.1N hydrochloric acid solution = 1.401 mg nitrogen.

5.6.2 Calculation of Total Protein. Determine the percentage (*m/m*) total nitrogen in the sample and calculate the total protein content of the sample by multiplying this result by the appropriate factor* listed below:

6.68 for whole egg samples

6.70 for egg white samples

6.62 for egg yolk samples.

5.6.3 Report. The report shall state the percentage (*m/m*) of total nitrogen determined and the percentage (*m/m*) of the total protein calculated.

* 'The Chemical Analysis of Foods', D. Pearson, 6th Edition (1970), J. and A. Churchill.

SECTION 6. DETERMINATION OF LIPIDS

6.1 INTRODUCTION. Two tests for the determination of lipids are described. In Test A the lipids are separated from the acid-hydrolyzed sample by solvent extraction, while in Test B, the lipids are extracted from a mixed solvent solution of the sample by separating the solvent which contains the lipids.

6.2 TEST A—MOJONNIER TECHNIQUE.*

6.2.1 Scope. This method describes a procedure for determining lipids in liquid and dried eggs and eggs products.

6.2.2 Definition. For the purpose of this method the following definition applies:

Lipids — the percentage by mass of substances extracted and determined by the procedure described.

6.2.3 Principle. The sample is digested with hydrochloric acid and the lipids extracted from the acidic solution with diethyl ether and light petroleum. The solvents are evaporated and the lipid content determined gravimetrically.

6.2.4 Apparatus and Reagents.

6.2.4.1 Apparatus.

- (i) *Mojonnier flask* of borosilicate glass, having a bulb capacity of approximately 26 ml, an overall length of 180 ± 2 mm and a neck diameter above the bulb of 16 ± 1 mm. The flask shall be provided with a ground glass stopper, or a stopper of suitably inert material, the dimensions of which shall be such that the joint between the flask and the stopper will comply with the requirements given for a 19/26 joint in ISO/R 383, Interchangeable Conical Ground Glass Joints.

NOTE: A suitable flask is illustrated in Fig. 6.1.

- (ii) *A flat-bottomed flask*, 150–250 ml.
- (iii) *A drying oven*, electrically heated and complying with the requirements of BS 2648, Performance Requirements for Electrically Heated Laboratory Drying Ovens.

* This method is adapted from the AOAC Methods, 11th Ed., 1970.

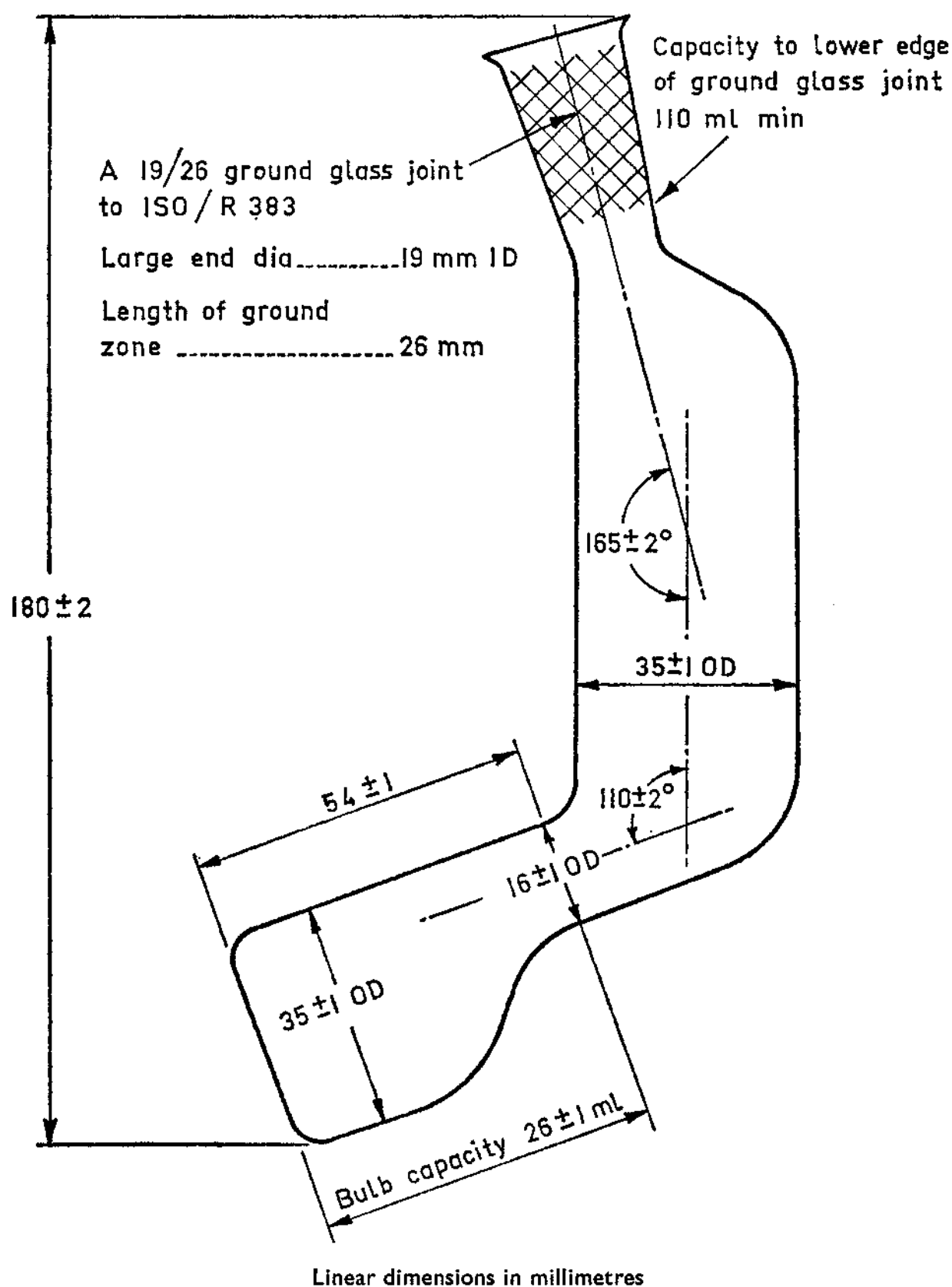


Fig. 6.1 MOJONNIER FLASK (Without Stopper)

6.2.4.2 Reagents.

Hydrochloric acid, concentrated; or

Hydrochloric acid. Dilute 4 vol. of the concentrated acid with 1 vol. of water.

Light petroleum (petroleum ether), recently fractionated, with any boiling range between 40 and 70°C.

NOTE: Hexane is suitable.

Diethyl ether, peroxide-free.

NOTES:

1. To test for peroxides, add to 10 ml of ether in a small glass stoppered cylinder, previously rinsed with ether, 1 ml of freshly prepared 10 percent potassium iodide solution. Shake and let stand for 1 min. No yellow colour should be observed in the ether layer.
2. Diethyl ether may be freed and maintained free from peroxides by adding prepared wet zinc foil that has been completely immersed in dilute acidified copper sulphate solution for 1 min and subsequently washed with water. As a result of this preparation some copper deposit should be observable on the foil. Use approximately 80 cm² of zinc foil per litre; cut in strips long enough to reach at least halfway up the container.

Mixed solvent, prepared shortly before use by mixing equal volumes of diethyl ether and light petroleum.

6.2.5 Procedure.

6.2.5.1 Blank test. At the same time as the determination of the lipid content of the sample, perform a blank determination on 5 ml of water using the same type of extraction apparatus, the same reagents in the same amounts and the same procedure as described hereafter, but omitting the addition of the egg sample. If the blank exceeds 0.001 g, the reagents should be checked and the impure reagent or reagents should be purified or replaced.

6.2.5.2 Pretreatment of sample.

- (a) *Liquid eggs.* Weigh, by difference, to the nearest milligram, approximately 2 g yolks, or 3 g whole egg or 5 g whites (as the case may be) into the Mojonnier flask. Slowly with vigorous shaking, add 10 ml of the concentrated hydrochloric acid.
- (b) *Dried eggs.* Weigh to the nearest milligram approximately 1 g of the sample and transfer to the Mojonnier flask. Slowly add 10 ml of the diluted hydrochloric acid, washing down any particles adhering to the sides of the flask.

6.2.5.3 Extraction of lipid.

- (a) Set the Mojonnier flask in a water bath heated to 70°C, bring the water bath to boiling and continue boiling for 30 m, shaking the Mojonnier flask carefully at 5 m intervals. Remove the Mojonnier flask from the water bath, add water to nearly fill the lower bulb and cool to room temperature.

- (b) Add 25 ml diethyl ether, stopper the flask and shake vigorously for 1 min. Remove the stopper and wash this and the neck of the flask with 25 ml of light petroleum so that the washings run back into the Mojonnier flask. Replace the stopper and shake vigorously for 30 s.
- (c) Allow the flask to stand (or alternatively centrifuge) until the ethereal layer is clear.
- (d) Draw off as much as possible of the ethereal layer into a tared flat-bottomed flask containing porcelain chips or broken glass. Add 10 ml of mixed solvent to the Mojonnier flask without shaking, then transfer the solvent to the flat-bottomed flask. Rinse the outside neck of the Mojonnier flask with 5 ml of mixed solvent, collecting the rinsings in the flat-bottomed flask. At this stage it is advisable to commence distillation of the solvents in the flat-bottomed flask.
- (e) Re-extract the liquid remaining in the Mojonnier flask twice, each time using only 30 ml of mixed solvent, shaking the flask after each addition.
- (f) Decant the clear upper layer into the same flat-bottomed flask used earlier. Before the final decantation add water carefully to raise the interface to the upper end of the neck of the Mojonnier flask. After the final decantation, rinse the inside and outside of the neck of the Mojonnier flask with mixed solvent, collecting the rinsings in the flat-bottomed flask.
- (g) Carefully evaporate the solvents in the flat-bottomed flask on a steam bath, finally assisting the process with a current of air. Place the flat-bottomed flask in an oven at $100 \pm 2^\circ\text{C}$ for 1 h.
- (h) Remove the flat-bottomed flask from the oven, allow to cool to the temperature of the balance room, either in an enclosed cabinet or in a desiccator charged with an efficient desiccant, and weigh. Avoid wiping or unnecessary handling of the flat-bottomed flask immediately before weighings to prevent static charge.
- (j) Repeat the drying procedure until the change in mass between successive weighings does not exceed 1 mg.

6.2.6 Calculation and Report. From the difference in mass of the flat-bottomed flask, corrected where necessary for the reagent blank, calculate and report as the percentage lipid by acid hydrolysis (*m/m*).

6.3 TEST B — MODIFIED BLIGH AND DYER TECHNIQUE*.

6.3.1 Purpose and Scope. This method describes a procedure for determining lipids in liquid and dried eggs and egg products.

* This method is based upon the principles elaborated by E.G. Bligh and W.J. Dyer in 'A Rapid Method for Total Lipid Extraction and Purification', *Can. J. Biochem. Physiol.*, 37, 911 (1959).

6.3.2 Definition. For the purposes of this method the following definition applies:

Total lipids — the percentage by mass of substances extracted and determined by the procedure described.

6.3.3 Principle. The lipids present in the sample are extracted into a miscible solvent mixture of methanol, chloroform and water. The addition of more chloroform and water causes a lower layer of chloroform, containing the extracted lipids, to separate. The lower layer is separated, the solvent evaporated and the lipid content determined gravimetrically.

6.3.4 Apparatus and Reagents.

6.3.4.1 Apparatus.

- (i) *Centrifuge tubes* of at least 50 ml capacity, fitted with screwcaps, with polytetrafluoroethylene (or equivalent) liners.
- (ii) *Pasteur pipette* and rubber bulb, pipette (15 ml), filter funnel and cotton wool.
- (iii) *A drying oven*, electrically heated and complying with the requirements of BS 2648, Performance Requirements for Electrically Heated Laboratory Drying Ovens.
- (iv) *Drying vessel*. A vessel, preferably glass, having a capacity of about 100 ml.

6.3.4.2 Reagents.

Methanol

Chloroform — redistilled, if necessary

Sodium chloride solution, 1N.

6.3.5 Procedure.

6.3.5.1 Blank test. Carry out a blank test corresponding to the procedure described below using 6.0 ml of the sodium chloride solution in place of the sample.

6.3.5.2 Pretreatment of sample.

- (a) *Liquid whole egg*. Weigh to the nearest milligram, approximately 3 g of the prepared sample into a centrifuge tube. Add 3.8 ml of the sodium chloride solution.
- (b) *Liquid yolk*. Weigh to the nearest milligram approximately 1 g of the prepared sample into a centrifuge tube. Add 5.5 ml of the sodium chloride solution.
- (c) *Dried whole egg*. Weigh to the nearest milligram, approximately 1 g of the prepared sample into a centrifuge tube. Add 6 ml of the sodium chloride solution and mix to reconstitute.