THE DAIRY PRACTICES COUNCIL®

GUIDELINES FOR CHEMICAL DETERMINATION OF BUTTERFAT IN VARIOUS DAIRY PRODUCTS

Publication: DPC 34

JANUARY 2016

First Edition – October 1979
First Revision – October 1988
Second Revision – December 2009
Third Revision – January 2016

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ABSTRACT

This Guideline provides the most commonly used chemical tests for the determination of butterfat in various dairy products. It provides detailed testing procedures for the Gerber and updated Babcock method, and the modified Mojonnier extraction method. It also provides advice on equipment and reagents to be used in the above testing methods. The intent of this guideline is to provide to the dairy industry a quick reference of the most commonly used testing methods for the determination of the butterfat content of various dairy products. The procedures contained in detail in this guideline are those recommended by the Dairy Practices Council as being the most practical and accurate as well as conforming to AOAC procedures. A lack of uniformity within the dairy industry has prompted the concern for uniform butterfat determinations of the various dairy products. Regulatory, farmer interests, handler interests, consumer acceptance, and the overall economic aspects suggest that this uniformity is needed now.

PREFACE

The first edition and first revision was sponsored jointly by the Quality Assurance Task Force and the Plant Equipment and Procedures Task Force with Charles W. Johnson Assistant to the Market Administrator Federal Order No. 1 as Subcommittee Chair. Members of this Subcommittee were: Bernice Belanski, Idelnot Farm Dairy Inc., N. Springfield, VT; David P. Brown, Dept. of Food Science, Cornell University, Ithaca, NY; James Fitts, Lab. Evaluation Officer, NY State Dept. of Ag. & Mkts., Homer, NY; Donald F. George, Director, Animal & Dairy Industries Div., VT Dept. of Ag., Montpelier VT; Gordon Hawkins, Federal Milk Mkt Admin., Boston MA; Laurie W. Justice, Lab. Evaluation Officer, VT Dept. of Ag., Montpelier, VT; John T. O'Conner, West Lynn Creamery, Inc., West Lynn, MA; August R. Peters, Garelick Farms, Franklin, MA; Donald Shields, Milk Quality Control Spec., CT. Dept. of Ag., Guilford, CT; Susan Valley, AgriMark, Inc., W. Springfield, MA; and Albert F. Zimmermann, Q.C. Labs, Inc., Southampton, PA.

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GUIDELINE PREPARATION AND REVIEW PROCESS

The Dairy Practices Council (DPC) Guideline development and update process is unique and requires several levels of peer review. The first step starts with a *Task Force* subcommittee made up of individuals from industry, regulatory and educational institutions interested in and knowledgeable about the subject to be addressed. Drafts, called "*white copies*," are circulated until all members of the subcommittee are satisfied with the content. The final "*white copy*" may be further distributed to the entire Task Force; DPC Executive Board; state and federal regulators; educational and industry members; and anyone else the Task Force Director and/or the DPC Executive Vice President feel would add strength to the review. Following final "*white copy*" review and corrections, the next step requires a "*yellow cover*" draft to be circulated to representatives of participating Regulatory Agencies referred to as "*Key Sanitarians*." Key Sanitarians may suggest changes and insert footnotes if their state standards and regulations differ from the text. After final review and editing, the Guideline is distributed in the distinctive DPC "*green cover*" to DPC members and made available for purchase to others. These guidelines represent our state of the knowledge at the time they are written. Currently, DPC Guidelines are primarily distributed electronically in pdf format without colored covers, but the process and designation of the steps remains the same. Contributors listed affiliations are at the time of their contribution.

DISCLAIMER

The DPC is not responsible for the use or application of the information provided in this Guideline. It is the responsibility of the user to ensure that the information addresses their needs and that any action taken complies with appropriate regulations and standards.

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SPECIFICATIONS FOR BABCOCK TESTING EQUIPMENT, GLASSWARE, AND REAGENTS

A. Equipment

1. Centrifuge

The centrifuge must be capable of rotating at the necessary speed, which will deliver the appropriate centrifugal force for separating butterfat from the acid-skim-milk mixture. . It must have a thermostatically controlled interior temperature of 46-51°C (114.8-123.8°F) and a thermometer, which measures the interior temperature. It must be frequently checked with a speed indicator to assure proper revolutions per minute under full load.

Centrifuge wheels of different sizes require different revolutions per minute to achieve the proper centrifugal force for butterfat separation. The correct speed for wheels of different diameters is listed below. The diameter of a wheel should be measured as the extreme distance between the inside of the bottoms of opposite cups extended in a horizontal position

Speed of Centrifuge

Diameter in Inches	10	12	14	16	18	20	22	24
Revolutions per Minute	1074	980	909	848	800	759	724	693

2. Mechanical Shaker

A mechanical shaker which mixes the acid and milk is recommended. The shaker should have the capacity to mix the maximum number of tests that may be contained in the centrifuge.

3. Hot Water Reservoir

For a supply of hot water to be added to tests after first and second centrifuging periods, a container equipped with a thermostat is recommended to deliver a supply of distilled water at 50-52°C (122-125.6°F) into Babcock bottles.

4. Hot Water

A supply of 60°C ±1°C DI water to add to cheese samples prior to the addition of sulfuric acid. A hot plate with a beaker of DI water is useful for this purpose.

5. Hot Water Bath for Tempering Finished Tests

The hot water bath shall be maintained at 48±1°C (116.6 -120.2±1.8°F) with sufficient depth that the entire fat column is below the water level. It is desirable for the water bath be equipped with an automatic device to maintain the correct water level and temperature.

6. Hot Water Bath for Tempering Milk Samples

A water bath capable of adjusting and maintaining temperature for heating liquid dairy samples to 38°C±1°C (98.6 –102.2°F) prior to pipetting.

7. Reading Light

A reading light is recommended as a background when measuring butterfat columns. The light shall be diffused and provide illumination from angles above and below the level of the butterfat column. It is helpful if the reading light is of soft green color. Attached magnification devices are suggested.

8. Sulfuric Acid Dispenser

Any method of adding acid to the test bottle, which will give the technician a means of accurately measuring desired volumes will be acceptable. (Under no circumstances shall sulfuric acid (H_2SO_4) be pipetted by mouth) It is also recommended that safety glasses and a rubber apron be provided when handling all acids and other chemicals. An eye wash device and safety shower are required by OSHA.

9. Calipers (Dividers)

A good quality caliper equipped with a friction adjusting device, which is resistant to corrosion and has two sharp points, is necessary. The needles on calipers must be kept sharpened at all times.

10. Thermometer

Use a digital thermometer for measurement of milk-acid reaction temperature. Digital thermometer that reads to the nearest degree in the range of 100°-120°C (212°-248°F). Use an acid resistant probe, with a small diameter (≤0.5mm) to ensure a rapid response time. The length of the probe should be such that its tip is approximately 1 cm above the bottom of the bottle when fully inserted.

Use thermometers to verify sample, water bath and DI water temperatures.

B. **Glassware and Weighing Devices**

All pipettes, test bottles, balances, cream weighing scales, and weights used shall meet AOAC specifications and State regulations. All glassware shall be clean and dry.

1. Balance: Sensitivity 0.01g

2. Standard Babcock milk-test bottle. - 8% milk-test bottle, total height 160-170mm (6.3-6.7"). Bottom of bottle is flat, and axis of neck is vertical when bottle stands on level surface. Quantity of milk for bottle is 18g.

a. Bulb. - Capacity of bulb to junction with neck must be ≥ 45ml. Shape of the bulb may be either cylindrical or conical. If cylindrical, OD (outside diameter) of base must be between 34 and 36mm; if conical, OD of base must be between 31 and 33 mm, and maximum diameter between 35 and 37mm.

b. Neck. - Cylindrical and of uniform diameter from ≥5mm below lowest graduation mark to ≥5mm above highest mark. Top of neck is flared to diameter of ≥10mm. Graduated portion of neck has length ≥75.0mm and is graduated in whole %, 0.5%, and 0.1%, respectively, from 0.0 to 8.0%. Graduations may be etched, with black or dark pigment annealed to graduation or may be an unetched black or dark line permanently annealed to the glass. Graduation line widths ≤0.2mm. Tenths % graduations are ≥3mm long; 0.5% graduations are ≥4 mm long and project 1 mm to left; and whole % graduations extend at least halfway around the neck but no more than three quarters of the way around and project ≥2mm to left of tenths % graduations. Each whole % graduation is numbered, with number placed to left of scale. A vertical line may be etched and annealed with black or dark pigment or may be an unetched black or dark line permanently annealed to the glass located 1 mm to the right of the 0.1% graduation marks and extends ≥1mm above the 8% line and ≥1mm below the 0% line. The zero line must be etched, annealed with black or dark pigment and be ≤ 0.2 mm wide. Capacity of neck for each whole % on scale is 0.200ml. Maximum error of total graduation or any part thereof must not exceed 0.008ml (.04% fat). Each bottle must be constructed so as to withstand stress to which it will be subjected to in the centrifuge.

c. Testing. - Accuracy of each bottle shall be determined (usually by the manufacturer or a certified laboratory). Bottle calibration accuracy is determined by placing the bottle upside down on a Babcock bottle calibration apparatus (modified NAFIS tester) that is capable of delivering known volumes of mercury into the Babcock bottle neck. Bottle calibration apparatus delivery is calibrated and the volume of mercury contained between the 8% and 4% (0.800ml), 4% and 0% (0.800ml), and 8% and 0% (1.600ml) marks are determined. Accuracy of any bottle can also be determined by calibration with Hg (13.5471g clean, dry Hg at 20°C (68°F) to be equal to 5% on scale of 18g milk bottle and 10% on scale of 9g cream bottle, bottle having been previously filled to 0 mark with Hg.

3. Cream bottle: 0 - 50%: 9g short-neck, 155 to 170mm total height; 0 - 50% 9g long-neck, 210-229mm total height; 0 – 50%, 18g long-neck, 210-229mm total height. See AOAC Official Method 995.18 for more detailed requirements of bottles.

4. **Paley bottle for cheese**: 0 – 10%, 0 – 20% or 0 -50%, 9g, 155 to 170mm total height with side opening for addition of sample.

5. **Skim milk bottle**: 0 – 0.5%, 18g 152 – 170 mm total height, double necked, with 0.01% divisions. For use with samples containing less than 0.5% fat.

6. **Ice Cream bottle**: 0 – 20%, 9g, 152 – 170mm total height, with 0.2% divisions. For use with samples containing 5 – 20% fat.

7. **Other bottles** exist with the general purpose of providing ease of use and a range of fat levels that is appropriate for various products. All bottles shall meet AOAC calibration specifications.

8. **Pipet**. - Standard milk pipet conforms to following specifications;

Total length.....≤350 mm
OD of suction tube..... 6-8 mm
Length of suction tube .....130 mm
OD of delivery tube (must fit into bottle (a)).4.5-5.0 mm
Length of delivery tube ..... 105-125 mm
Distance of graduation mark above bulb .....15- 45 mm
Nozzle parallel with axis of pipet, but slightly constricted so as to discharge in 5-8 seconds when filled with H_2O.
Graduation, marked to contain 17.6ml H_2O at 20°C (68°F) when the bottom of the meniscus coincides with the mark on the suction tube. Maximum error in graduation, ≤0.05ml.

Testing the pipet. Place the tip of the pipet against a firm rubber surface, clamp the pipet in a vertical position, and fill the pipet to the graduation mark with H_20 at 20°C (68°F) using a burette (Class A - graduations ≤0.05ml).

9. **Acid measure**. - Device used to measure sulfuric acid should be capable of delivery in the range from 10 to 20ml and can be set to consistently deliver the appropriate amount of acid to obtain the desired milk acid reaction temperature.

C. Reagents

1. Sulfuric Acid (H_2SO_4)

Sulfuric acid shall be of good quality, standardized to 1.820-1.830 specific gravity at 20°C (68°F) for use with milk, cream and cheese samples. (This should be checked frequently using an accurate acid hydrometer.) The reaction temperature obtained when the sulfuric acid is added to the milk must reach 108°±2°C (224°-228°F). The reaction temperature when sulfuric acid is added to cream must be 93°-103°C. Sulfuric acid containers shall be kept tightly closed to prevent weakening of the acid.

Dilute sulfuric acid standardized to 1.73±0.01 (20/20°C) specific gravity is used in Pennsylvania method for flavored milk, yogurt, cottage cheese, sour cream, cultured milk, ice cream and frozen desserts. Acid can be purchased already diluted to 1.73 sp. gr, or it can be diluted from the 1.825 sp. gr. acid by adding 3.5 parts acid to one part water. (For example, place 200 mL cold DI water in a 200mL beaker. Slowly add 700mL 1.825 sp. gr. acid. The solution will become extremely hot. Allow the water-acid solution to cool and then pour it into the acid dispensing apparatus. CAUTION: Never add water to acid or you will cause a violent reaction, always add acid to water.

a. Measurement of product-acid reaction temperature and determination of amount of sulfuric acid to use. Prior to testing a group of samples, determine the correct amount of acid to be used by measuring product-acid reaction temperature.

i. For milk, start by adding 17.5 ml of 21° ± 1°C (68°-72°F), Babcock sulfuric acid to a bottle containing 18g of milk of the same temperature. Add the 17.5ml of acid in one delivery that washes all traces of milk into bulb and cleanly layers the acid under the milk. Fully insert the digital thermometer probe down the bottle neck, immediately shake by hand rotation until all traces of curd disappear. The peak reaction temperature should be 108±2°C (224°-228°F).[6] Adjust the amount of sulfuric acid added, until the reaction temperature is within this range and the color of the fat columns is a translucent golden-yellow to amber. The

amount of acid required may be different for different technicians and different batches of acid.

ii. For cream add 9mL 21° ± 1°C sulfuric acid to 9g cream bottle or 16mL 21° ± 1°C sulfuric acid to 18g cream bottle. Add acid in one delivery that washes all traces of cream into bulb and cleanly layers acid under cream. Fully insert digital thermometer probe down bottle neck and immediately shake by hand rotation until all traces of curd disappear. Peak reaction temperature should be within 93° - 103°C for 30-45% cream. Adjust the amount of sulfuric acid added until reaction temperature is within this range and the fat column is translucent golden-yellow to amber upon completion of the analysis.

iii. For other types of samples, a product-acid reaction temperature and determination of amount of sulfuric acid to be used will need to be performed for each different sample type, for each run, for each analyst. The lower the fat content, the higher the reaction temperature. Specific guidelines for peak reaction temperature have not been published, however using the milk and cream reaction temperatures, and knowing approximately what fat level is in the product to be tested, the analyst should be able to come up with an approximate amount of acid to use for each different product type. By analyzing several replicates of each of the various product types to be tested at an individual lab, and noting the peak reaction temperature and amount of acid used to achieve a fat column that is translucent golden-yellow, with not char, and clear liquid under the fat column, the analyst should be able to determine the peak reaction temperature and amount of acid to be used for each product type tested at the lab.

2. Ammonium hydroxide (NH_4OH): 28 to 29% ammonia.

3. Normal butyl alcohol: Boiling point 117°C

BABCOCK BUTTERFAT TESTING OF VARIOUS DAIRY PRODUCTS

A. Preparation of Test Sample

1. Temper milk, cream and any other liquid samples to 38°C ±1°C (98° to100°F.) Once the samples have reached 38°C, do not leave them in water bath for more than 15 minutes before pipetting.

2. Cheese: Shred cheese using a grater or blender. Blend for a total of approximately 15 seconds. Turn blender off and shake sample in blender jar. Blend for an additional few seconds. Avoid prolonged mixing as the heat generated can cause fat separation and degradation of the sample. Warm sample to room temperature (21° ±1°C).

3. Cottage Cheese: Blend thoroughly a full retail carton of cottage cheese or a representative sample of larger containers until the product in uniform and pourable. The final temperature of the mixed sample should not be more than 25°C. Warm sample to room temperature (21° ±1°C).

4. Yogurt, Ice Cream, Sour Cream and like products: Warm sample to room temperature (21° ±1°C) and mix sample thoroughly.

B. Obtaining Test Portion

1. Liquids: Mix the 38°C (100°F) sample until homogeneous by gently inverting the sample container for ten full inversions (do not shake the sample). Pipet or weigh the sample immediately after mixing.

   a. Milk: Immediately after a sample is mixed, pipet 17.6 ml (18g) of milk (using a calibrated Babcock milk pipet) into a clean 8% milk test bottle. (The bottom of the milk meniscus should be adjusted to the line on the suction tube of the pipet). Allow the pipet to drain for at least 30 seconds and then blow out the milk remaining in the pipet tip. Remove the pipet from the milk test bottle.

   b. Cream: weigh 9g ± 0.03g or 18g ± 0.03g (depending on bottle) into 9g or 18g cream bottle.

c. Skim milk, buttermilk and whey: weigh 9g ± 0.01g into a skim test bottle. (Multiply result by 2)

d. Chocolate milk: Weigh 18g ± 0.01g of well-mixed sample into a Babcock bottle. For chocolate milk containing more than 1% fat, use 0 – 8% bottle. For chocolate milk containing less than 1% fat weigh 9g ± 0.1g into a skim test bottle. (For skim test bottle, multiply result by 2)

e. Half and Half: Weigh 9g ± 0.01g into a milk bottle (0 – 8%). Add 9mL DI water (temperature?). Proceed from this point with the "milk" determination in A1 below. (Multiply result by 2.)

2. Cheese: weigh 9g ± 0.01g grated cheese into a 9g Paley or cream bottle. Pipet about 10mL of hot (60°C) LG water in to the Paley bottle. Mix to suspend the cheese thoroughly before adding acid. Cool to 21° ± 1°C.

3. Yogurt, Ice Cream, Sour Cream and like products: weigh 9g ± 0.03g into a 9g bottle (0 – 10% or 0 – 20%, depending on fat level). For fat free version of yogurt, ice cream, sour cream and cottage cheese, it can be very difficult to obtain an accurate fat test via Babcock; Mojonnier method is preferred for the fat free samples, if it is available.

C. Tempering sample and acid

After all samples have been pipetted or weighed into test bottles, temper the samples plus bottles to 21° ± 1°C (68°-72°F) before adding 21° ± 0.1°C sulfuric acid in step D below. Have extra samples included in the set to use as a temperature check at this stage and for the measurement of the product-acid reaction temperature. (Refer to detailed instructions under "measurement of product-acid reaction temperature and determination of amount of sulfuric acid to use", in section C1.)

D. Procedure

1. Acid Addition

a. Milk: Using an Akrofil or other suitable acid dispenser, add approximately 17.5ml of 1.825 ± 0.005 sp. gr. sulfuric acid at 20°- 22°C (68°-72°F) to the Babcock bottle while rotating the bottle between your fingers to wash all traces of milk from the inside of the bottle neck. Enough sulfuric acid must be added to produce a milk-acid reaction temperature of 108 ± 2°C (224°-228°F) with a digital thermometer. Temperature control samples must be run first and the amount of sulfuric acid added should be increased or decreased slightly to obtain a milk-acid reaction temperature in this range.) (Refer to detailed description of reaction temperature measurement in section C1. Immediately swirl the milk plus acid and place in a mechanical shaker and shake until all traces of curd disappear (approximately 1minute). Keep adding bottles to the shaker until all bottles in the set have had acid added to them. Shake for 1 minute after the last bottle has been added to the shaker. The temperature of the milk-acid mixture in the first bottle (or any bottle in the set) should not be less than 50.5°C (122.9°F) when it is placed in the centrifuge.

b. Cream: Add the amount of 1.825 ± 0.005 sp. gr. acid determined in 3A (approximately 9mL for 9g bottle and 16mL for 18g bottle) in one delivery that washes all traces of cream into bulb and cleanly layers acid under cream. Immediately shake bottle by hand rotation until all traces of curd disappear. Place bottle in shaker set at medium speed. Add acid to all test samples, and then shake the full set 1 additional minute. Add 6mL distilled water at 47° to 52°C to all bottles and then shake full set 1 additional minute.

c. Cheese: Add the amount of 1.825 ± 0.005 sp. gr. acid determined in 3A (approximately 15 mL, added portion wise, 8, 4 and 3mL), swirling by hand after each addition. Place bottle with sample + water + acid on shaker. Shake the full set for 1 minute after the last sample is placed on shaker.

d. Skim milk, lowfat milk, buttermilk and whey: Add the amount of 1.73 sp. gr. acid determined in 3A (approximately 7 to 9mL). Mix the sample-acid mixture thoroughly and place on shaker. Shake for 1 minute after the last bottle with sample + acid is added to the shaker.

e. Chocolate milk, ice cream, frozen desserts, yogurt and ice cream: Add 2mL of NH_4OH and rotate the bottle slowly to thoroughly mix the reagent with the sample. Add 3mL of normal butyl alcohol

and mix thoroughly by swirling the bottle. Add the amount of 1.73 sp. gr. sulfuric acid determined in 3A (approximately 17.5mL portion wise, 8, 5 and 4mL), rotating the bottle to wash traces of the milk product from the neck. Shake bottles on shaker until all traces of curd disappear (approximately 1 minute after placing last bottle on shaker.)

f. Cottage Cheese: Add 2mL of NH_4OH through the side opening of the Paley bottle and mix thoroughly. Add 3mL of normal butyl alcohol, again through the side opening. Tightly insert the rubber stopper and mix the samples and reagents thoroughly by rotating the bottle. Shake on a mechanical shaker for 3 minutes. Add the amount of 1.73 sp. gr. acid determined in 3A (approximately 9mL) and mix the contents thoroughly for an additional 3 minutes on the shaker. Add a small amount of hot (51°C) LG water to the bottle through the side opening to dislodge any fat adhering to the stopper area.

2. Centrifugation

a. For all sample types, after the samples have been mixed thoroughly, place them in a heated centrifuge (46° - 51°C). Centrifuge for 5 minutes after the proper speed has been reached. (Refer to table on page 6 for proper centrifuge speeds).

b. At the end of 5 minute whirl, gently stop the centrifuge and add enough 50 - 52°C (122°- 125.6° F) distilled water to bring the contents of each bottle to a point just below the base of the neck of the bottle.

c. Centrifuge at the proper speed for two minutes.

d. At the end of the two minute whirl, gently stop the centrifuge and add 50°- 52°C (122°- 125.6°F) distilled water to each bottle until the fat column approaches the upper graduation of the neck of the bottle.

e. Centrifuge at the proper speed for one minute.

f. At the end of the one minute whirl, allow the centrifuge to come to a complete stop and then carefully remove each bottle from the centrifuge and place them in a 48 ±1°C (116.6 -120.2°F)water bath. The water should be deep enough to cover the entire fat column.

Temper the bottles at 48 ±1°C (116.6° -120.2°F) for at least 5 minutes before reading the test results.

3. Reading Fat Test

a. Remove the fat test bottles one at a time from the water bath. The fat columns should be translucent, golden yellow or amber color, free of any charred particles, curdy matter or visible impurity. The liquid under the fat column should be clear.

b. For cream, half and half, cheese and chocolate milk products, carefully add 2 drops of room temperature Glymol, allowing the liquid to flow down inside neck of Babcock bottle to top of fat column.

b. Wipe excess water from the neck of the bottle. With the aid of a reading light and magnification, quickly (excessive time delay will result in cooling and contraction of the fat column) measure the fat column with dividers or calipers. The fat column should be measured from the lowest point on the lower meniscus to the highest point of the upper meniscus. (If Glymol is used, read from lower meniscus to sharp line of demarcation between fat and Glymol). Read and report your result to the nearest 0.01% for skim milk, buttermilk and whey, 0.05% for milk and chocolate milk, 0.1% for cottage cheese, 0.25% for cream, cheese, ice cream and frozen desserts. For lower fat products and other products not detailed here, read to the smallest percentage possible for the type of bottle used. For 18g test bottles where 9g sample is called for, multiply reading on bottle by 2 to determine final % fat.

Maximum recommended difference between duplicates 0.1% fat
At 3.6% fat:

Sr = 0.029% RSDR = 1.014% SR* = 0.037% r value = 0.081%
RSDr = 0.742% R value* = 0.104%

*using regression equations for milk containing 3.6% fat.

$$SR = (0.0080 \times 3.6\%) + 0.0080$$
$$R\ value = (0.0227 \times 3.6\%) + 0.0226$$

The method of reading the percentage of fat is shown in Figure 1. The reading is made from the extreme bottom of the lower meniscus, at point D, to the extreme top of the upper meniscus, at point A. Do not set dividers at B or at C. The points of the dividers are placed at the upper and lower limits. Then being careful not to change the spread, lower the dividers until one point is at the 0 mark; the other point will indicate on the scale the correct percentage for the sample tested.

Do not use Glymol on milk tests.

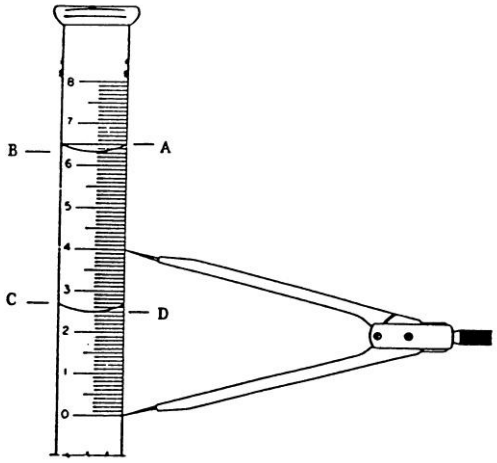

Figure 1. Butterfat Test of Milk

A perfect test for butterfat appears as follows: A. Translucent, golden yellow or amber.
B. Extremities of fat column sharply defined.
C. Fat column free from foreign substances such as curd and/or charring.
D. Liquid beneath fat column clear.
E. Fat column within graduated scale on test bottle.

Reject and retest all tests not meeting the above standards.

GERBER METHOD

(As outlined in the Standard Methods for the Examination of Dairy Products)

This method is applicable to raw, pasteurized, homogenized, and composite (preserved), milks, excluding chocolate-flavored milks.

A. Milk

1. Apparatus and reagents:

a. Standard Gerber milk test bottle, 8% - Clear, transparent, colorless, resistant borosilicate glass, well annealed and free from defects; neck, body, flat tube, and bulb on a straight median axis, joined smoothly to permit free flow of liquid; wall thickness adequate to provide sufficient strength but not less than 0.9 mm at any point (Refer to Fig.19:1); and of the following dimensions:

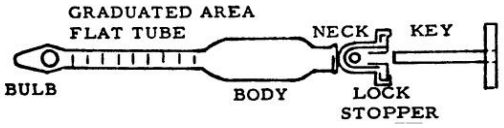

Figure 19:1. Gerber milk test bottle, lock stopper and key.

Total length…….190 + 2.5 mm Neck and body…….cylindrical Neck length……...15 + 1.0 mm Neck, ID……...11.5 + 0.5 mm diam. Neck rim, optionally reinforced - total thickness of external rim…not more than 2.5 mm diam. Body, OD……....not more than 25.0 mm diam. Body capacity……...21.5 + 0.4 ml Flat tube, uniform in cross-section beyond each terminal graduation……..not less than 3.0 mm Flat tube, external width…not less than 10.0 mm Graduation lines, permanent, cleanly etched, perpendicular to flat tube axis, filled with pigment contrasting sharply with color of fat; width of lines……0.10 to 0.20 mm Bulb, tapered, and with matte surface on side above graduations for sample identification, OD……not more than 15.0 mm Graduated volume in the flat tube at 20 °C (68°F) is 1.000 ml (13.546 g Hg). It extends not less than 70 mm on the flat tube and is divided into 80 equal parts, each equivalent in volume to0.0125 ml. Error of total calibrated length is not to exceed + 1/2 of the smallest graduation.

Graduation lines are uniformly centered on the flat tube face, with the 0.1% lines not less than 3 mm long, the 0.5% lines extending 1 mm on each side beyond the 0.1% lines, and the 1.0% lines extending not less than 1 mm on each side beyond the 0.5% lines, or perhaps extending across the entire flat face.

Each whole-per cent graduation is identified by etched and pigmented numbers running serially from 0 to 8, at the right and just above the whole- per cent lines, with the zero measurement nearest the body. Capacity of the bulb at 20 °C (68 °F) to the 8% line is not less than 1.5 ml. Bottles are identified "Milk," with the name or symbol of the manufacturer or distributor permanently inscribed on the body and a State symbol applied after recalibration where required to attest conformance with state specifications.

b. Bottle support rack - To hold bottles in a secure, vertical position. Stainless steel (Series 300) racks consisting of two perforated shelves and a solid bottom, supported by solid sides, provide convenience and durability. Optionally use racks with stainless steel "rapid clamp-on" covers to lock bottles securely when using shaking machines.

c. Milk test pipet - To contain 11 ml. Clear, transparent, colorless glass tubing, free from defects and well annealed; bulb, suction and delivery tubes on a straight median axis; bulb cylindrical and tapered to tube junctions to permit free flow of liquid; top of suction tube smooth and perpendicular to the tube axis; and delivery tube wall straight above the tapered tip, with dimensions as follows:

Wall thickness…….not less than 1.0 mm
Total length…….340 + 20 mm
Delivery tube length, including tapered tip……..105 + 5 mm
Tapered tip, length……7 + 1.5 mm
Delivery tube, OD……..6.5 + 0.5 mm
Bulb, OD……..16.0 + 1.0 mm
Suction tube, to graduation not……less than 110 mm
Suction tube, OD……6.5 + 0.5 mm
Graduation to bulb…….40 + 15 mm
Graduation line. 0.1-0.2 mm wide, is cleanly the end of the suction tube, the line filled with adherent dark pigment. The pipet may be filled to the mark with water at 20 °C (68 °F) to discharge within 5-8 seconds, TC 11.07 + 0.03 ml at 20 °C (68 °F); and is identified "TC 11.07ml at 20 °C (68 °F)," with the name or symbol of the manufacturer or distributor permanently inscribed on the bulb and a State symbol applied after recalibration where required, to attest conformance with state specifications.

d. Sulfuric acid (H_2SO4) - Concentrated commercial (technical) grade, clean, colorless, free from fat, sp. gr. 1.820-1.825 at 15.5 °C (60 °F), corresponding to 90-91% sulfuric acid by weight, stored in tightly closed containers.

e. Isoamyl alcohol for Gerber test - Clean, clear, colorless or almost colorless. CP primary isobutyl carbonyl, free from water, acid, fat and furfural; sp. gr. to be 0.814-0.816 at 15.5°C (60 °F); more than 95% distills at an atmospheric pressure of 760 mm Hg within a 2°C range between 128° and 132°C (294°-302°F) (it evaporates, leaving no residue); store in tightly closed containers, in amber bottles, or in darkness.

Test each batch of isoamyl alcohol identified by the manufacturer as suitable for Gerber milkfat tests by transferring 10 ml of sulfuric acid, 11 ml of water, and 1 ml of the isoamyl alcohol to a test bottle. Shake and centrifuge bottles as required in the test for fat in milk. If oily particles are recovered, discard the batch. Use four numbered bottles, a single pipet, and a single sample of milk for tests in the same bottles, both with new and in-use isoamyl alcohol. Determinations should agree, with less than 0.05% variation.

f. Reagent dispensers - For sulfuric acid, use a measure to deliver 10 ml. For isoamyl alcohol, use a measure to deliver 1ml. Optionally use a tilt measure, burette, or short cylinder for dispensing 10-ml quantities; optionally use a tilt measure, semiautomatic syringe, or other suitable apparatus to dispense1-ml portions; use of pipets to measure sulfuric acid and isoamyl alcohol is unsafe. Dispensers that operate by adjustable positive displacement (syringes), when properly used, combine uniform delivery speed with no loss of time for drainage. Burets and automatic devices of various types are available.

g. Lock stopper and key for bottle - The lock stopper is of rubber or synthetic resilient, reagent-resisting material, of standardized dimensions, molded to provide a seat for the ball or plug, and a channel for the key; rim and channel are reinforced by metal, plastic, or other firm binding or insert. The key is of no corrodible material suitable for inserting into the stopper.

h. Centrifuge for bottles - Standard style, with disc and trunnion head to rotate at 1.100 + 100 rpm; or an angle head to rotate at 1.350 + 100 rpm. Trunnion-head models are supplied with heaters to maintain internal temperature at approximately 60°C (140°F). Use a centrifuge that attains full rotating speed within 2 min of operation. If not equipped with permanent speed indicators, check the operating speed of all Gerber centrifuges under full load at monthly intervals, or more frequently if necessary. Bottles should receive an acceleration of 350 + 50 times the acceleration of gravity.

Optionally use Babcock centrifuges for 9-in. cm) bottles, equipped with heaters and cup adaptors for Gerber bottles, and operate at 1.100+ 100 rpm.

i. Water bath - Depth to permit vertical immersion of bottles to the terminal bulb, equipped to provide intermittent or constant agitation and to control temperature at 60°-62.8°C (145°F).

j. Shaking machine (optional) - To standardize test conditions and to save time. Available models may be readily modified to hold Gerber racks.

k. Desk reader (optional) - Improvised to expedite reading of fat columns. Models with a shielded tubular electric bulb standardize background illumination for the bottles.

l. Washing device (optional) - Consisting of a rack and perforated cover (Item b), expedites washing. After reading, return bottles to the rack, remove the stoppers, lock the rack cover; empty, wash, rinse and drain bottles as a unit.

2. Procedure:

Measure 10 ml of sulfuric acid + 0.2 ml at 15.5°-21.1°C (60°-70°F) into a test bottle. Temper milk sample to not more than 23.9°C (75°F). Using an11-ml pipet accurately measure 11 ml. into the test tube with the top of the meniscus resting on the graduation line. . Allow the milk to drain into the bottle - slowly, at first, to prevent reaction with the acid - then permit the pipet to empty and touch off any part of a drop at the tip. Add 1 ml of isoamyl alcohol and insert the lock stopper securely. With stoppered end up, grasp the test bottle at the graduated column and shake it until the curd is completely digested. Holding the bottle (CAUTION - HOT) at stopper and neck, invert it at least four times to mix the acid remaining in the bulb with the rest of the contents.
Machine- shaking of filled test bottles, secured in racks equipped with quick-locking covers provides safe, rapid, uniform treatment and optimal temperatures for centrifuging.

Before contents of the bottles have cooled below 60°C (140°F), place them in a centrifuge and counterbalance the load as needed. Close and lock the cover before starting the centrifuge. After the required speed is attained, centrifuge for 4 minutes. Stop the centrifuge and temper the bottles in a water bath at 60°- 62°C (140°-144°F) for at least 5 minute, leaving only the bulbs exposed. Holding a test bottle vertically, remove it from the water bath and fit the reading key into the lock stopper. Gently push the straight line at the bottom of the fat column upward so that it coincides with the nearest whole-per cent graduation mark. Promptly read the scale at the bottom of the meniscus and the top of the fat column to the nearest 0.10% graduation. Subtract the lower reading from the upper reading. The difference is the fat content in each sample. Experienced operators can accurately estimate fat tests to the nearest 0.05%.

Repeat tests where fat columns are imperfect. As fat cools in the column, the slope of the meniscus increases, tending to lower the per cent fat reading. Soft, glareless background lighting and a desk reader stand, which is readily adjusted to bring the column to tester eye level, expedite accurate readings.

Proper fat columns contain no light or dark particles or zones. They are pale to strong yellow in color, depending on the season of the year and on feed given the animals.

B. **CREAM**

1. Apparatus and reagents:

a. Standard Gerber cream test bottle, 50% - 5-g capacity, construction and dimension a except that the external width of the flat tube is not less than 13 mm.

Graduated volume at 20°C (68°F) in the flat tube is 2.839 ml (38.457 g Hg). It extends not less than 70 mm on the flat tube and is divided into 100 equal parts, each equivalent in volume to 0.02839 ml. Error of total calibrated length is not to exceed + 1/2 of the smallest graduation. Graduation lines are uniformly centered on the flat tube face, with the 0.5% lines not less than 3 mm long, the 1.0% lines extending 1 mm on each side beyond the 0.5% lines, and the 5.0% lines extending not less than 1 mm equally on each side beyond the 1% lines, or perhaps extending across the entire flat face.

Each successive 5% graduation is identified by etched and pigmented numbers running serially from 0 to 50, at the right and just above the per cent lines, with the zero measurement nearest the body. Capacity of the body, at 20°C (68°F), from junction with the neck to the 0% line is

19.0 + 0.4 ml; capacity of the bulb, at 20°C (68°F), to the 50% line is not less than 2.0 ml. Bottles are identified "Cream, 5 g ", with the name or symbol of the manufacturer or distributor permanently inscribed on the bulb and a State symbol applied after recalibration where required to attest conformance with State specifications.

b. Gerber cream test bottle, 25% - Graduated volume at 20°C (68°F) in the flat tube is 1.4195 ml (19.2285 g Hg). Graduation lines are uniformly centered on the flat tube face, with the 0.2% lines not less than 3 mm long, the 1.0% lines extending 1.5 mm on each side beyond the 0.2% line, and the 2.0% lines (and the 25% line) extending not less than 1 mm on each side beyond the 1.0% line, or they may extend across the entire flat face. Even-numbered per cent graduations should be numbered, in addition to the 0% and 25% lines. Capacity of the body, at20°C (68°F) from junction with the neck to the0% line is 20.5 + 0.4 ml.

c. Gerber cream test bottle, 15% - Graduated volume at 20°C (68°F) in the flat tube is 0.8517 ml (11.537 g Hg). Graduation lines are uniformly centered on the flat tube face, with the 0.2% lines not less than 3 mm long, the 1.0% lines extending 1.5 mm on each side beyond the 0.2% line, and the 2.0% lines (and the 15% line) extending not less than 1 mm on each side beyond the 1.0% line, or they may extend across the entire flat surface. Even-numbered per cent graduations should be numbered, in addition to the 0% and 15% lines. Capacity of the body, at 20°C (68°F), from junction with the neck to the 0% line is 21.0 + 0.4 ml.

d. Balance for weighing cream - Accurate, having a sensitivity reciprocal not greater than 0.03 g, equipped with a convenient taring or counterbalancing device, and with a pan bottle support on the balance to provide firm vertical support for the bottles.

e. Weight, 5-g - One-piece, accuracy 5.000 + 0.005 g.

f. Transfer device for sample - A pipet of not less than 5-ml capacity, or a syringe with a 10- to 14-gauge cannula, will be satisfactory.

Other apparatus and reagents - (see pages 13 and 14)

2. Procedure:

Measure 10 + 0.2 ml of sulfuric acid at 15.5°-21°C (60°-70°F) into a cream test bottle. Place the bottle in the support on the balance and tare, weigh 5.00 g of prepared cream sample into the bottle. Remove the bottle from the balance and add 5 ml of water at 15.5°-21°C (60°-70°F) and1 ml of isoamyl alcohol. Insert the lock stopper securely and proceed as in Read the scale to the nearest 0.5% graduation mark and record results.

C. CHOCOLATE MILK

This method is applicable to sweetened, flavored milk and drinks.

1. Apparatus and reagents:

a. Same as for milk (see page 12 - 15) and for cream (see pages 15-16), also adding:

b. Sulfuric acid, diluted - Slowly add 94 parts by volume of cold sulfuric acid to 6 parts by volume of cold water.

c. Weight - Special one-piece 11.125 + 0.005-g weight, or an equivalent combination of weights, kept clean and free from corrosion.

2. Procedure:

Measure 10 + 0.2 ml of diluted H_2SO_4 (see above) at 15.5°-21.1°C into a milk test bottle. Pipet the test charge(see page 14) If viscous, tare and place the 11.125-g weight on the balance pan, and weigh 11.125 g of properly prepared sample into the bottle. Remove the bottle from the balance and add 2 ml of isoamyl alcohol. Insert the lock stopper securely and proceed as page 14. Read the scale to the nearest 0.1% graduation mark and record results.

FAT DETERMINATION IN RAW MILK BY MODIFIED MOJONNIER METHOD

A. Apparatus

1. **Flask** - a Mojonnier style ether extraction flask with a volume of 21 to 23ml in the lower bulb plus neck at the bottom of the flask. The flask should have a smooth and round opening at the top that will seal when closed with a cork.

2. **Weighing dishes** - metal; 8.5 to 9.5cm diameter and 4.5 to 5.5cm tall or 250ml glass beakers.

3. **Calibration weight**s - class S, standard calibration weights to verify balance accuracy within weight range to be used for weighing flasks plus samples and weighing dishes plus fat.

4. **Analytical balance** - readability to nearest 0.0001g. Accuracy on verification within 0.0002g, checked periodically and whenever the balance is moved or cleaned. A record should be kept of balance calibration checks.

   The analytical balance should be checked using a set of Class S standard reference weights. The weights should be handled carefully with forceps and should be equilibrated to the ambient temperature of the balance before the calibration check. First, the balance should be given a broad spectrum check across the entire weight range. Then the balance should be checked using a combinations of weights that mimic the approximate weights of an empty fat extraction flask, the flask plus sample weight, empty fat pan, and fat pan with residue. This second check at the approximate weights being used is most important when using old style mechanical balances or multiple range electronic balances. Check the balance's ability to maintain a zero with the holder for the extraction flask on the balance. This check should be done on a routine basis and the results dated and recorded in a balance log book. Any time a balance is cleaned, moved, or serviced it should be checked for accuracy.

5. **Desiccator** - room temperature for cooling weighing dishes after preliminary and final drying. Should contain coarse desiccant (mesh size 6-16) that contains a minimum of fine particles and that changes color when moisture is adsorbed.

6. **Tongs** - for handling weighing dishes.

7. **Hot plate (steam bath or other heating device)** - for evaporation of ether ≤100°C (212°F).

8. **Corks** - high quality natural cork stoppers for flasks. Soaking corks in distilled water for several hours will give a better seal.

9. **Vacuum or forced air oven** - vacuum oven capable of maintaining a temperature of 70°-75°C (158°- 167°F) at greater than 20 inches of vacuum or a forced air oven capable of maintaining a temperature of 100 ± 1°C (212 ± 1.8°F).

10. **Water bath for tempering milk samples prior to weighing** - provided with thermometer and device to maintain temperature of 38 ± 1°C (100.4 ± 1.8°F).

B. Reagents

1. **Ethyl ether** - ACS grade, peroxide free, should leave no residue on evaporation.

2. **Petroleum ether** - ACS grade, boiling range 30°- 60°C (86°- 140°F), should leave no residue on evaporation.

3. **Ammonium hydroxide (NH_4OH)** - concentrated, ACS grade, sp. gr. 0.9.

4. **Ethyl alcohol** - 95%, no residue on evaporation.

5. **Distilled water (DI)** - free of oil and mineral residue.

6. **Phenolphthalein indicator** - 0.5% (W/V) in ethanol.

CAUTION: Both ethyl and petroleum ethers are highly flammable and explosive, and the utmost care must be used in handling. Store in a cool place and do not permit smoking, open flames, or electrical equipment, which causes sparks where these reagents are handled. If refrigerated storage is used, be sure that the refrigeration system is explosion proof. Purification of these ethers by redistillation is of doubtful value, extremely dangerous even in experienced hands, and is not recommended.

Ether fumes are heavier than air, so special considerations are necessary for proper vapor removal during the evaporation steps in ether extraction procedures.

Ethyl ether will form explosive peroxides on exposure to sunlight and oxygen. Opened bottles of ethyl ether (even those with an oxidation inhibitor such as BHT) should not be kept more than 3 months because of peroxide formation due to contact with oxygen. When peroxide containing ether is distilled or evaporated the peroxide residues may explode causing injury and fires. (Reference - Prudent Practices for Handling Hazardous Chemicals in Laboratories, National Research Council, National Academy Press, 2101 Constitution Ave. N.W., Washington, D.C. 20418).

C. Procedure

1. Sample preparation –The test portion size should not exceed 10g and, for full fat products, the test portion weight should be chosen to yield 0.3 – 0.6g of extracted fat.. When sample size is less than 10g, after recording weight of sample add enough 60°C DI water to bring the total sample plus water weight to approximately 10g. For acidic products it may be necessary to increase the amount of NH_4OH added from 1.5mL to 3.0mL.

   a. **Milk:** Prepare by tempering milk to 38°C ± 1°C (100°F) and weigh about10g of milk (to the nearest 0.0001g) directly into a clean and dry sample flask. Check balance zero between samples.

   b. **Cream:** Prepare by tempering cream to 38°C ± 1°C (100°F) and weigh approximately 1g 40% cream, 2g 20% cream, 3g half and half into clean and dry sample flask.

   c. **Egg Nog, Buttermilk and Kefir:** Prepare by tempering sample to 38°C ± 1°C (100°F) and weigh 4 – 5g (to the nearest 0.0001g) directly into a clean and dry sample flask. Check balance zero between samples. For buttermilk, add 3 mL of NH_4OH instead of 1.5ml.

   d. **Condensed skim milk, buttermilk and whey:** Prepare by tempering condensed to 38°C ± 1°C (100°F). Weigh about a 5g sample, except in the case of condensed buttermilk where 3g should be used.

   e. **Sweetened condensed milk:** Temper unopened can in water bath at 30° - 35°C until warm. Open, transfer to beaker large enough to permit stirring thoroughly, scrape out all milk adhering to interior of can, and mix until whole mass is homogeneous. Weigh 100g thoroughly mixed test sample into 500mL volumetric flask, dilute to volume with DI water,

and mix thoroughly. Transfer a portion of the thoroughly mixed diluted sample into a sample vial and temper to 38°C ± 1°C (100°F). Weigh out 10g of the diluted sample. (Weight of original sample, flask and water needs to be recorded to apply dilution factor to final fat result).

f. Evaporated Milk (Unsweetened): Temper unopened can in water bat at 60°C. Remove and vigorously shake can every 15 minutes. After 2 hours, remove can and let cool to room temperature. Remove entire lid and thoroughly mix by stirring contents in can with spoon or spatula. Weigh 40g thoroughly mixed test sample into 100mL volumetric flask, dilute to volume with DI water and mix thoroughly. Transfer a portion of the thoroughly mixed diluted sample into a sample vial and temper to 38°C ± 1°C (100°F). Weigh out 10g of the diluted sample. (Weight of original sample, flask and water needs to be recorded to apply dilution factor to final fat result).

g. Cottage Cheese: Blend thoroughly a full retail carton of cottage cheese or a representative sample of larger containers until the product is uniform and pourable. The final temperature of the mixed sample should not be more than 25°C. Temper sample to 21°C and weigh4 – 5g into Mojonnier flask. Record weight. Add sufficient 60°C DI water to bring total weight to 10g. Shake vigorously to dissolve sample. Add 3mL NH_4OH and again shake vigorously. If sample has not completely dissolved, refrigerate to aid dissolution.

h. Ice Cream: Cut frozen blocks of product into ca ½ pint pieces. Select 2 or 3 pieces at random, place in the cup of a high-speed blender, and close tightly. Let soften at room temperature and mix (2 minutes for plain products and ≤7 minutes for those containing nuts or hard candy chips). Do not let the temperature exceed 12°C (54°F) at any time during the softening and mixing steps. If fat separation or "churning" occurs, discard the sample and repeat, using a shorter mixing time. Immediately pour mixture into a wide-mouth jar and cap tightly. If allowed to stand, shake vigorously before removing test portion. Weigh 4 – 5 g thoroughly mixed sample into Mojonnier flask. Record weight. Add 5 – 6mL 60°C DI water and mix. Add 2mL NH_4OH, mix thoroughly, and heat in a water bath 20 minutes at 60°C with occasional shaking. Cool.

i. Powders: Weigh 1g thoroughly mixed powdered sample into Mojonnier flask (0.6g for whey protein concentrate) and record weight. Add 10mL Ethyl alcohol. Mix thoroughly. Add 1.5mL NH_4OH. Add 9g 60°C DI water and shake vigorously. It can be very difficult to get the powders into solution. It may be necessary to heat in a water bath at 60°C with occasional shaking to get the powder into solution. Note that the order of addition of the first two reagents is reversed for powders, to aid in getting the powders into solution.

j. Yogurt: Weigh 4 – 5g 21°C well mixed sample into Mojonnier flask. Record weight. Add 5–6mL DI water. Mix thoroughly.

2. Weighing dish preparation - Number the clean weighing dishes and predry under the same conditions that will be used for the final drying after fat extraction. Be sure that all surfaces where weighing dishes will be placed (i.e. hot plate, desiccator, etc.) are clean and free of particulates. At the end of oven drying, place the pans in a room temperature desiccator and cool to room temperature. Weigh the dishes to the nearest 0.0001g and record weights. (Weighing should be done on same day as fat extraction). Check balance zero after weighing each pan. Once pans have been weighed, protect them from contamination with extraneous matter.

3. Fat extraction

   a. First Extraction: Add 1.5 ml ammonium hydroxide and mix thoroughly. If NH_4OH added in sample preparation step, do not add additional here. The ammonium hydroxide neutralizes any acid present and dissolves casein. Add 3 drops of phenolphthalein indicator. The indicator will help sharpen the visual appearance of the interface between the ether and aqueous layers during the extraction. Add 10ml ethyl alcohol, cork, and shake for 15 seconds. For the first extraction, add 25ml ethyl ether, stopper with cork, and shake flask very vigorously 1 minute. Add 25ml petroleum ether, stopper with cork, and repeat vigorous shaking for 1 minute. Centrifuge the flasks at about 600 rpm for at least 30 seconds to obtain a clean separation of aqueous (bright pink) and ether phases.(A centrifuge is highly recommended, but if a centrifuge is not available, allow the stoppered flask to stand for at least 30 minutes until the

ether layer is clear and distinctly separated from the aqueous layer.) Decant ether solution into suitable weighing dish prepared as described on page 17. When decanting the ether solution into the dishes, be careful not to pour over any suspended solids or aqueous phase into the weighing dish. Ether can be evaporated at ≤100°C (212°F) from the dishes while conducting the second extraction. (Avoid any spattering by lowering temperature) Prior to the second extraction, if the interface between the ether layer and the aqueous phase is below the neck of the flask, add DI water slowly down the inside surface of the flask (avoid disturbing the separation layer) to bring the interface to nearly the top of the neck of the flask).

b. For the second extraction, add 5 ml of ethyl alcohol, stopper with cork, and shake vigorously for 15 seconds. Next, add 15 ml ethyl ether, stopper with cork, and shake flask vigorously 1 minute. Add 15ml petroleum ether, stopper with cork, and repeat vigorous shaking for 1 minute. Centrifuge the flasks at approximately 600 rpm for at least 30 seconds to obtain a clean separation of aqueous (bright pink) and ether phases. Decant ether solution for the second extraction into the same weighing dish as for the first extraction.

c. For the third extraction, omit the addition of ethyl alcohol, and repeat the procedure used for the second extraction.

4. Evaporate solvents completely on hot plate at ≤100°C (212°F) (avoid spattering). Dry the extracted fat plus weighing dish to constant weight in a forced oven at 100 ± 1°C (212 ± 1.8°F) (30 minutes or more) or vacuum oven at 70°-75°C (158°- 167°F) under pressure ≥50.8 cm (20 in.) Hg (7 minutes or more). Remove weighing dishes from oven and place in a desiccator to cool to room temperature. Record the weight of each weighing dish plus fat.

   A pair of reagent blanks should be run each day that tests are conducted. To run a reagent blank, replace the milk sample with 10ml of distilled water and run the test as normal. Record the weight of any dry residue collected and use it in the calculation. Reagent blank should be less than 0.0020g of residue. If the reagent blanks for a set of samples are negative, use the negative number in the calculation. A negative blank usually indicates that the pans were not completely dry when you started or that the balance calibration shifted between the weighing of the empty pans and the pans plus fat. Cause of negative blanks should be identified and corrected.

Calculation

$$\text{Percent Fat} = \frac{[(\text{wt. pan + fat}) - \text{wt. pan})] - (\text{aver wt. blank residue})}{\text{(wt. of milk)}} \times 100$$

Maximum recommended difference between duplicates <0.03% fat

At 3.6% fat:
Sr = 0.015% RSDR = 0.512% SR = 0.020% r value = 0.044% RSDr = 0.396% R value = 0.056%

APPENDIX

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