This manual contains instructions and information relating to sampling, testing, and grading of milk and cream, in accordance with Colorado Revised Statutes 25-1-107(o)(IV) and 25-5.5-107 (2). The

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SECTION I: LICENSE INFORMATION

The Colorado Department of Public Health and Environment, Consumer Protection Division (CDPHE), must be assured that the holder of a sampler and/or tester's license is capable of properly sampling, testing, and/or grading milk or cream and is qualified to handle dairy products in the best possible manner.

LICENSE REQUIREMENTS

Any person who samples and/or tests milk, cream, or other dairy products for the purpose of determining the value of such products when bought and sold or who instructs another person for such purpose is required to have first made proper application to CDPHE. Applicants should be prepared to answer questions based on material contained in this manual. The applicant will be required to pass an examination given by CDPHE and based on material contained in this manual.

VALID LICENSE

The license fee of three dollars ($3.00) must accompany the application. A sampler and/or tester license is valid from the date of issuance until the following June 30 (one fiscal year or any part of that fiscal year) unless canceled by CDPHE. The license must be posted at the place of business at all times.

TRANSFER OF LICENSE

If a sampler and/or tester transfers from one plant to another, a new license will be issued at no fee. The sampler and/or tester or the employer must notify CDPHE of the change of location. If CDPHE is not notified, the license will be automatically canceled.

RENEWAL OF LICENSE

Applications for renewal of license will be mailed from CDPHE during June of each year and samplers and/or testers are requested to fill out the renewal and return it by mail with the necessary license fee. Plants are required to notify CDPHE if any of the samplers and/or testers do not receive a renewal. Plants must also notify CDPHE when new samplers and/or testers are hired.

EXAMINATION

Applicants are required to submit their applications to CDPHE. The person conducting the examination will contact the company to arrange for the written examination either at the place of business or at CDPHE. Laboratory supervisors applying for a tester's license will be subject to a practical performance test prior to licensure, conducted at the place of business by a representative of CDPHE. Samplers are required to pass a practical performance test prior to licensure, and every two years thereafter.

FAILURE OF EXAMINATION
If the applicant fails to make a passing grade of 70 or above on the first examination, the applicant will be notified by CDPHE, and must contact CDPHE within 30 days of this notification to schedule a second examination.

**PENALTIES**

A license is subject to cancellation by CDPHE at any time that CDPHE finds that the person holding the license is incompetent or guilty of violating any of the provisions of the applicable statutes. The burden of the accuracy and correctness of the testing or sampling lies upon the licensee and it is not necessary to prove intent to defraud. A person found making false results of milkfat contained in milk or cream is subject to a fine of not less than $10.00 nor more than $200.00 and sixty (60) days in jail for each offense.

**SECTION II: PLANT SAMPLING**

The test of any milk sample is only as accurate as the sample delivered to the laboratory. This fact makes the collection of samples one of the most important steps in the milk sanitation and laboratory control programs. Following standard sampling procedures can eliminate contaminated samples and can provide the milk producer, processor, and regulatory agencies with valid laboratory results.

The following procedures and requirements are for industry and regulatory samplers who obtain samples at receiving stations, milk plants or dairy plants. These samples are used for fat, chemical, bacteriological and temperature determinations. Samples obtained by either certified industry or regulatory personnel can be used for enforcement of the standards set forth in Section 7, *Standards for Milk and Milk Products, 1999 Colorado Grade A Pasteurized Fluid Milk and Milk Products Regulations*. Samples may include individual producer shipments, commingled shipments, bulk shipments and processed milk products and dairy products.

**MILK SAMPLERS**

Milk samplers are persons licensed by CDPHE who are qualified and trained to sample milk or cream for the purpose of payment. This includes bulk milk haulers who collect milk from dairy farms. Bulk milk hauler sampling procedures and requirements are outlined in the *2000 Colorado Milk Haulers Manual* (which can be obtained from CDPHE).

Samplers who are evaluated and licensed by CDPHE may obtain samples from dairy farms and plants. These samples include individual producer samples; commingled raw samples of milk or cream; and samples of bulk shipments of milk, cream or other milk products intended for processing in the plant.

**A. EQUIPMENT**

1. **THERMOMETER**
a. **Accuracy** - The sampler’s thermometer (dial type or electronic) shall be checked initially and every six months against a standard thermometer and adjusted, if necessary, to an accuracy of $\pm 1^\circ F/1^\circ C$.

b. The date checked and the person’s initials who checked the thermometer shall be taped to the case or a suitable record shall be kept containing this required information.

2. **AGITATION**

Odor-free, pressurized, filtered air, or electrically driven stirring equipment should be used for the agitation of shipments of milk or cream.

3. **SAMPLE TRANSFER INSTRUMENTS**

a. All sampling instruments must be clean and sanitized or sterilized.

b. Various sampling instruments include metal dippers and seamless metal tubes or individually wrapped, sterile single service tubes of at least 10 milliliter (ml) capacity, or other approved means for removing samples aseptically.

c. Samples may be obtained from a sanitized sampling cock.

4. **SAMPLING INSTRUMENT CARE**

Sampling instruments must be clean and maintained in good repair.

5. **SAMPLE CONTAINERS**

a. Clean and properly sanitized or sterilized containers are required for sampling of non-packaged products.

b. The containers shall be properly stored and handled to keep the container free from contamination.
6. **SAMPLE STORAGE CASE**

An approved case is required when samples are transported or shipped to the laboratory for analysis.

a. The case shall be clean, of rigid construction, and have a tight cover to protect samples from contamination. The case shall maintain samples at 32°F - 40°F (0°C - 4.4°C).

b. Ample space is required in the case for ice/refrigerant. Racks, if needed, should be sized to accommodate the largest sample container.

7. **CLEANING AND SANITIZING OF EQUIPMENT**

a. All sampling instruments shall be maintained in a clean and dry condition prior to sanitization.

b. Sampling instruments such as stirrers, sampling tubes (non-single service) or dippers shall be sanitized between samples as follows:
   
   1. Adequately rinse all milk or cream residue from the instrument.
   
   2. Submerge the instrument in hot water, at least 180°F (82°C), for at least one minute;  
      
      or submerge the instrument in an approximately 200 parts per million (ppm) hypochlorite (chlorine) solution or another bactericidally equivalent halogen for at least one minute.

   3. Alternatively, dry heat or steam heat may be used as specified in Standard Methods.

   4. The strength of sanitizing solutions, if used, shall be determined with an applicable test kit.

B. **SAMPLING PROCEDURES**

1. **METHODS USED TO OBTAIN A UNIFORM MIXTURE FOR SAMPLING**

   Samples should be thoroughly mixed before sampling to permit the withdrawal of a representative sample. Withdraw the sample immediately after thorough mixing by:

   a. suitable mechanical agitation. Any cream adhering to the sides of the container is to be uniformly incorporated into the milk prior to sampling;
b. pouring from one vessel to another at least three times; or

c. stirring with an ordinary stirring rod.

2. GENERAL SAMPLING PROCEDURES - Raw and Processed Samples

Samples shall be stored under refrigeration in suitable racks or containers. Any samples for regulatory purposes shall be delivered to the certified laboratory within 36 hours after collection.

a. Wash and dry hands prior to sampling and rewash as necessary during the sampling procedure.

b. Determine and record the sample temperatures at all sampling locations with an approved thermometer.

c. Obtain a temperature control sample at the first sampling location and label the container with the following information:

1. Temperature Control (TC)
2. Date of collection
3. Time of collection
4. Temperature of the product collected
5. Collection site (producer, milk plant, tank or silo number, milk plant truck number and route number)
6. The identity of the sample collector by initials or number.

d. Legibly identify sample containers at the collection point and enter all required information on the collection reports.

e. Handle sample containers and closures in an aseptic manner. Do not use any container that may be contaminated.

f. When sampling an open vessel such as the manhole on a milk tank truck, or an open port on a storage vat or constant level tank, do not hold the sample container over the product when transferring the sample into the container.

g. Protect sampling instruments from contamination before and during sampling.
h. Fill the sample container to the 3/4 level of the container capacity.

i. Immediately place all samples into the sample case.

3. **RAW MILK, CREAM OR OTHER PRODUCT(S) FOR PASTEURIZATION**

   **Milk Tank Trucks and Plant Storage Tanks** *(see Section II, item B-2 for applicable sampling procedures).*

   a. When sampling cream, thoroughly mix before sampling to permit proper withdrawal of a sample representative of the entire volume and uniform in fat content. Take the sample immediately after thorough mixing so that an accurate test can be made as to the fat content of the cream. Since cream is quite viscous, it ordinarily requires prolonged mixing to bring about a uniform mixture for sampling. Insure that the entire contents of the container are thoroughly mixed including the portion usually adhering to the sides and shoulders of the container or tank. Freshly separated sweet cream may, however, be sampled in the same manner as that prescribed for milk.

   b. Do not sample frozen or partly frozen milk or cream, and milk containing butter granules or flakes due to partial churning of the milkfat.

   c. In the case of raw milk or other fluid products such as condensed skim milk, milk tank trucks and silos or other plant storage, agitate tanks for at least 20 minutes prior to sampling. Some storage tanks may specify agitation times on the information plate. See instructions for cream sampling in section (a) above.

   d. Collect samples aseptically from:

      1. tank openings such as manholes or ports;

      2. pipelines using a syringe extending through the sampling device into the product;

      3. the constant level tank. When commingled milk, as an example, is sampled at the constant level tank, the milk must represent the entire source;

      4. the sanitized sampling-cock. If the sample is to be used for regulatory purposes, no other sample can be withdrawn prior to the regulatory sampling. Samplers will need to know the plant routine as to what time the sample must be obtained.

   e. If using a sanitized sampler dipper, rinse the dipper at least two times in the product before transferring the product to the sample container.
f. Extend the sample dipper or tube 6-8 inches into the product in order to obtain a representative sample.

g. Rinse the dipper or other multi-use tubes of all product residue after each use and place the instrument in a sanitizing solution.

4. SANITIZE THE SAMPLING-COCK IN THE FOLLOWING MANNER:

a. Prepare the proper strength sanitizer of approximately 200 ppm chlorine and place in a plastic container such as an 18 ounce whirl-pak bag.

b. Place the container of sanitizer over the entire exterior of the sampling-cock, sanitizing the hole where the product is released.

c. Hold the bag tightly around the sampling-cock beyond the sample hole and gently massage the sanitizer container for two minutes.

d. Collect at least two liters of product from a properly agitated silo or tank (B3c) into a container prior to obtaining the sample from the sampling-cock. Avoid contamination from overhead condensation or from hands when pushing against the tension of the sampling-cock springs. Flow of the product shall be started before the sample container is placed under the sampling-cock and continued until the container is removed.

5. PROCESSED PACKAGED PRODUCT SAMPLES (see Section II, item B-2 for applicable procedures).

a. Collect a temperature control, the size of which must be at least one-half the size of the largest sample being collected. Insert a thermometer into the temperature control container to determine product temperature. This container can be used as the temperature control submitted to the laboratory.

b. Collect regulatory samples while they are still in the possession of the processor. Possession includes all processor delivery vehicles and distribution centers.

c. Randomly select representative samples of all plant processed products.

d. Obtain samples of product from unopened dispensers by using a sanitized utensil, such as scissors or a knife, to cut the tube. The sample shall be collected without sanitizing or flushing the spigot opening.

6. SAMPLING PRODUCT CONTAINERS AND CLOSURES (see Section II, item B-2 for applicable procedures).
a. Collect at least four randomly selected, representative multi-use and/or single-service containers used for packaging on each machine used to fabricate containers.

1. Do not touch the lip or the interior of the bottles or containers.
2. Do not allow product or water to drip into the empty containers.
3. Use sterilized caps, aseptically applied to the containers, or containers sealed or capped with line equipment. Do not touch inside surfaces of the caps.
4. Deliver these containers to the laboratory without refrigeration.
5. Protect the containers from damage or crushing during transport to the laboratory.

7. **SAMPLE STORAGE AND TRANSPORTATION**

   a. Use ice or other refrigerant maintained slightly above the product level in the sample container to keep samples at 32°F-40°F (0°C - 4.4°C). Do not freeze, except those products normally frozen.

   b. Protect all samples against contaminants, including ice water. Do not bury the tops of containers in ice.

   c. Promptly submit all samples, sample collection reports and data to the laboratory. Raw milk, cream, and processed packaged products (except high acidity milk products such as cottage cheese) shall be delivered to the laboratory within 36 hours after collection. High acidity products shall be delivered to the laboratory within 24 hours after processing.

   d. When shipping samples via a common carrier, use a tamper proof shipping case with the top labeled "This Side Up."

**NOTE:** Questions contained in the sampler examination and the evaluation of sampling techniques for certification will be based on information contained in sections I and II.

**SECTION III: FAT TESTING**

**MILK TESTERS**
Milk testers are persons licensed by CDPHE who are qualified and trained to test milk, cream and/or other dairy products for the purpose of payment.

It is unlawful for any person engaged in buying, selling, testing, handling, or determining the value of milk, cream or processed products by the use of an approved test, to give any false reading of the test. It is unlawful to manipulate the test in any way so as to give a higher or lower percent of milkfat than the milk, cream, or processed products actually contain. It is unlawful to cause any inaccuracy in reading the percent of milkfat by securing from any quantity of milk, cream or processed products to be tested, an inaccurate sample for the test. It is unlawful for any person to use any instrument or glassware in such test which is not clean. Unclean glassware is declared to be inaccurate.

The Colorado Department of Public Health and Environment has approved the following tests to be used for the determination of fat in milk and cream.

1. **Babcock Method.** Testing procedures shall be in accordance with Standard Methods for the Examination of Dairy Products or Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). If procedures vary from these two methods, it is the responsibility of the milk plant to prove that the technique used is equivalent, and this information must be submitted to CDPHE.

2. **Roese-Gottlieb Method.** The Roese-Gottlieb ether extraction and the Mojonnier modification of this procedure are the methods of choice because of their inherent analytical capacity for total milkfat.

4. **Infrared technology such as the Dairy Lab or similar equipment.** Refer to page 20.

**MILK AND CREAM TESTING PROCEDURES - BABCOCK METHOD**

**Apparatus**

A. **Standard Babcock Milk-test Bottle.** An 8%, 18 gram, milk-test bottle with a total height of 160-170 millimeters (6.3-6.7 inches). The bottom of the bottle is flat, and the axis of the neck is vertical when the bottle stands on a level surface. The quantity of milk for the bottle is 18 grams.

1. **Bulb.** The capacity of the bulb to the junction with the neck must be ≥45 milliliters. The shape of the bulb may be either cylindrical or conical. If cylindrical, the outside diameter of base must be between 34 and 36 millimeters. If conical, the outside diameter of the base must be between 31 and 33 millimeters, with a maximum diameter between 35 and 37 millimeters.

2. **Neck.** The neck must be cylindrical and of uniform diameter from ≥5 millimeters below the lowest graduation mark to ≥5 millimeters above the highest mark. The top of the neck is flared to a diameter of ≥10 millimeters. The graduated portion of the neck has a length ≥75 millimeters and is graduated in whole, half, and tenth percents, respectively, from 0.0 to 8.0%. Graduations may be etched, with black or dark pigment annealed to the graduation, or may be un-etched black or dark lines permanently annealed to the glass. The graduation line widths must be ≤0.2 millimeters. The tenth-percent graduations are ≥3 millimeters long and the half-percent graduations are ≥4 millimeters long and project 1 millimeter to the left. The whole percent graduations extend at least halfway around the neck to the right, but no more than three quarters of the way around, and project ≥2 millimeters to the left of the tenth-percent graduations. Each whole percent graduation is numbered, with the number placed to the left of the scale. A vertical line may be etched and annealed with black or dark pigment. Alternatively, a vertical line may be an un-etched black or dark line permanently annealed to the glass located 1 millimeter to the right of the tenth-percent graduation marks and extending ≥1 millimeter above the 8% line and ≥1 millimeter below the 0% line. The zero line must be etched, annealed with black or dark pigment and be ≤0.2 millimeters wide. The capacity of the neck for each whole percent on the scale is 0.200 milliliters. The maximum error of the total graduation or any part thereof must not exceed 0.008 milliliters (.04% fat).

Each bottle must be constructed to withstand the stress to which it will be subjected in the centrifuge.
3. **Testing.** The accuracy of each bottle shall be determined. The testing laboratory should receive certification from the bottle supplier indicating that this glassware has been calibrated.

B. **Pipette.** A standard milk pipette conforms to the following specifications:

<table>
<thead>
<tr>
<th>SPECIFICATION</th>
<th>Mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>≤ 330</td>
</tr>
<tr>
<td>Outside diameter of suction tube</td>
<td>6-8</td>
</tr>
<tr>
<td>Length of suction tube</td>
<td>130</td>
</tr>
<tr>
<td>Outside diameter of delivery tube [must fit into bottle(a)]</td>
<td>4.5–5.0</td>
</tr>
<tr>
<td>Length of delivery tube</td>
<td>100–120</td>
</tr>
<tr>
<td>Distance of graduation mark of bulb</td>
<td>15–45</td>
</tr>
</tbody>
</table>

The nozzle should be parallel with the axis of the pipette, but slightly constricted so as to discharge in five to eight seconds when filled with water.

The pipette must have a graduation, marked to contain 17.6 milliliters of water at 68.0°F (20°C) when the bottom of the meniscus coincides with the mark on the suction tube.

The maximum error in graduation shall be ≤ 0.05 milliliters.

**Testing the pipette.** Place the tip of the pipette against a firm rubber surface, clamping the pipette in a vertical position, and filling the pipette to the graduation mark with water at 68.0°F (20°C) using a buret (Class A - graduations ≤ 0.05 milliliters).

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

D. **Centrifuge.** A standard centrifuge, however driven, must be constructed throughout and mounted as to be capable, when filled to capacity, of rotating at necessary speed with minimum vibration and without liability of causing injury or accident. It must be heated, electrically or otherwise, to a temperature of 131°F-140°F (55°C-60°C) during centrifugation. It must be provided with a speed indicator, permanently attached if possible. The proper rate of rotation may be determined by reference to the table on the following page. The rotation speed with a full centrifuge should be checked periodically with a tachometer. The diameter of wheel(s) defined as the distance between the inside bottoms of opposite cups measured through the center of rotation of the centrifuge wheel while the cups are hori-
zontally extended.

<table>
<thead>
<tr>
<th>DIAMETER OF WHEEL, INCHES</th>
<th>rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1074</td>
</tr>
<tr>
<td>12</td>
<td>980</td>
</tr>
<tr>
<td>14</td>
<td>909</td>
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<td>16</td>
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</tr>
<tr>
<td>22</td>
<td>724</td>
</tr>
<tr>
<td>24</td>
<td>693</td>
</tr>
</tbody>
</table>

E. **Dividers or calipers.** Used to measure the fat column.

F. **Water Bath for Test Bottles.** The bath should be provided with a thermometer and a device to maintain the temperature of the fat column at 135.5°F ± 1°F (57.5°C ± 1°C).

G. **Water bath for tempering milk samples prior to pipetting.** The bath should be provided with a thermometer and a device to maintain the temperature of the milk at 100.0°F ± 1°F (37.8°C ± 1°C).

H. **Water bath for addition of water after first and second centrifugation.** The bath should be provided with a thermometer, a device to maintain the temperature of the soft water at 140°F ± 1°F (60°C ± 1°C), and a device to deliver water into the Babcock bottles.

I. **Bottle shaker.** The shaker should operate at variable speed, matched to the maximum capacity of the centrifuge.

J. **Digital and/or mercury thermometer for measurement of milk-acid reaction temperature.** This thermometer should read to the nearest degree in the range of 212°F - 248°F (100°C - 120°C). The length of the probe should be such that its tip is approximately one centimeter above the bottom of the bottle when fully inserted. For a digital thermometer, use an acid-resistant probe with a small diameter, ≤ .5 millimeters, to ensure a rapid response time.

K. **Reading light.** Used as background when measuring fat columns. The light should be diffused (soft white color) and provide illumination from angles above and below the level of the fat column. A magnification device must be used to aid reading.
DETERMINATION

A. Sample preparation and temperature adjustment. Use a pipette to transfer 17.6 ± 0.05 milliliters of prepared sample at 100.0°F ± 1°F (37.8°C ± 1°C) to the milk-test bottle. Blow out the milk remaining in the pipette tip about 30 seconds after free outflow ceases. Adjust the milk in the test bottles to 69.8°F ± 1°F (21°C ± 1°C). Adjust the sulfuric acid (specific gravity 1.82 to 1.83) at 68°F to 69.8°F ± 1°F (20°C to 21°C ± 1°C). Pipette some additional milk samples for use as temperature control samples.

B. Measurement of milk-acid reaction temperature and determination of the amount of sulfuric acid to use. Prior to testing a group of samples, determine the correct amount of sulfuric acid to be used by measuring the milk-acid reaction temperature. Start by adding 17.5 milliliters of 69.8°F ± 1°F (21°C ± 1°C) sulfuric acid to a bottle containing 18 grams of milk of the same temperature. Add the 17.5 milliliters of acid in one delivery that washes all traces of milk into the bulb and cleanly layers the acid under the milk. Fully insert the thermometer probe down the bottle neck and immediately shake by hand rotation until all traces of curd disappear. The reaction temperature should be 215.0°F (101.6°C) to 226.4°F (108.0°C). Adjust the amount of 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 batches of acid. Ammonia and/or butyl alcohol may be added to some samples, to make the sample more basic and to aid in fat extraction, respectively.

C. Testing milk samples. Add the appropriate amount of sulfuric acid [as previously determined in (B)] by delivering the acid in one addition that washes all traces of milk into the bulb and cleanly layers the acid under the milk. Immediately shake by hand rotation [as in (B)] until all traces of curd disappear. Place the bottle in a mechanical shaker set at medium speed. Continue to add acid to all the samples to be tested. After acid has been added to all samples, shake the full set one additional minute. The temperature of the milk plus the acid in the first bottle should not be less than 140°F (60°C) at the time the bottles are transferred to the centrifuge. Place the bottles in a heated centrifuge, counterbalance, and after proper speed is reached, centrifuge for five minutes. Add soft water at 140°F ± 1°F (60°C ± 1°C) [AOAC: 124°F ± 2°F/(51°C ± 1°C)] until the bulb of the bottle is filled. Centrifuge for two minutes. Add soft water at 140°F ± 1°F (60°C ± 1°C) until the top of the fat column approaches the 7% mark of bottle calibration. Centrifuge one minute longer at about 135°F-140°F (57°C-60°C) [AOAC: 119.5°F ± 4.5°F (48.5°C ± 2.5°C)]. Transfer the bottle to a warm water bath kept at 135.5°F ± 1°F (57.5°C ± 1°C) [AOAC: 118.5°F ± 2°F (48°C)], immersing the bottle in the bath to a level slightly above the top of the fat in the column. Leave the bottle in the bath until the column is in equilibrium and the lower fat surface assumes final convex form, 5 five minutes. Remove one bottle from the bath, wipe it, and with the aid of a reading light and magnification (see Apparatus, Section K) use dividers or calipers to quickly measure the fat column before it begins to cool and contract. Place the caliper points in the vertical line on the neck of the bottle with one point at the lowest surface of the lower meniscus and the other point at the top of the upper meniscus. Without
changing the distance between the two points on the calipers, move the calipers down the bottle neck until the lower point rests in the etched horizontal graduation mark at 0%. Place the upper point of the calipers against the bottle graduation and read the test in percent (%) by weight to nearest 0.05%. Repeat for each bottle.

At the time of measurement, the fat column should be translucent, golden-yellow or amber, and free of visible suspended particles. Reject all tests in which the fat column is milky or shows presence of curd or charred matter, or in which the meniscus is indistinct or distorted. Repeat the test, adjusting the volume of sulfuric acid added to obtain proper color and milk-acid reaction temperature.

After reading the test, replace the bottle in the water bath in order to maintain the proper level of the water. Glymol is not used in reading milk tests.

The maximum recommended difference between duplicates is 0.1% fat.

**SUMMARY OF THE STEPS IN MILK TESTING**

1. Have the temperature of the samples at approximately 100°F ± 1°F (37.8°C ± 1°C).
2. Transfer 17.6 ml ±0.05 ml of the prepared sample with a pipette to a milk test bottle.
3. Adjust the milk in the test bottle to 69.8°F ± 1°F (22°C ± 1°C).
4. Add the appropriate amount of sulfuric acid in one addition that washes all traces of milk into the bulb and cleanly layers the acid under the milk. The amount of acid ranges from 14 to 19 milliliters, with an average of 17.5 milliliters. The time for acid addition should not exceed 20 seconds.
5. Immediately shake the sample by hand rotation until all traces of curd disappear.
6. The reaction temperature should be 215.0°F (101.6°C) to 226.4°F (108.0°C ±1°C).
7. Place the bottle in a mechanical shaker set at medium speed and shake for at least five minutes.
8. The temperature of the milk plus the acid in the first bottle should not be less than 140.0°F (60.0°C) at the time the bottles are transferred to the centrifuge.
9. Centrifuge at the proper speed for five minutes in a centrifuge heated to 135°F-140.0°F (57°C - 60.0°C) [AOAC: **119.5°F ± 4.5°F (48.5°C ± 2.5°C)**].
10. Add soft water at 140.0°F ± 1°F (60.0°C ± 1°C) [AOAC: **124°F ± 2°F**] until the bulb of the bottle is filled.
11. Centrifuge for two minutes.

12. Add soft water at 140.0°F ± 1°F (60.0°C ± 1°C) [AOAC: 51°C ± 1°C (124°F ± 2°F)] until the top of the fat column approaches the 7% mark of calibration.

13. Centrifuge for one minute.

14. Transfer the bottle to a water bath maintained at 135.5°F-140°F ± 1°F (57°C-60°C ± 1°C) [AOAC: 118.5°F ± 2°F (48°C)], immersing it to a level slightly above the top of the fat column for five minutes.

15. Remove the bottle from the water bath, wipe it, and with aid of reading light and magnification, use dividers or calipers to quickly measure the fat column. Repeat the measurement for each bottle.

16. Record the test, as read, on the test sheet.

17. Clean the testing equipment immediately after use.

**CREAM TESTING PROCEDURES - BABCOCK METHOD**

Cream is weighed into the test bottle instead of measured because cream varies in weight according to the fat content. Cream containing a high fat content is lighter in weight than cream containing a lower percent of fat. Additionally, thick cream adheres to the side of the pipette which prevents delivery of the full measured volume into the test bottle, and cream often contains variable amounts of air and other gases which can cause weight variations for a given volume.

**TEST BOTTLES**

The 50 or 55%, 9 gram, sealed 165 ml (6.5 inch) cream test bottle is approved by CDPHE. The total graduation shall be 50% or 55%. The graduation shall represent 5%, 1%, and 0.5%. The total height of the bottle shall be 165 ml. (6.5 inches). The capacity of the bulb up to the junction of the neck shall not be less than 45 cubic centimeters.
TEST SCALES

An electronic scale may be used if it is sensitive to at least ten milligrams and is currently certified by the Colorado Department of Agriculture, Weights and Measures. The cream scale should be set on a level, firm foundation, free from vibration, and shall be protected from drafts. The one bottle torsion balance is also approved by CDPHE, if it is sensitive to at least ten milligrams. Contact CDPHE to receive additional information on the requirements for the one bottle torsion balance.

THERMOMETER

In order that proper temperatures may be observed, an thermometer accurate to ± 1°F/± 1°C is required.

PREPARING CREAM SAMPLE FOR TESTING

The preparation of cream for testing is similar to that for milk. Cream samples should be heated in a water bath until the cream has reached a temperature of approximately 100°F (37.8°C). Temperatures above 100°F may cause the fat to "oil off." Bottle racks should be used in the water bath to keep the bottles in a fixed, vertical position. The bottles should be kept sealed with a stopper or lid while heating to avoid evaporation and to prevent accidental dilution by water. Mix the sample thoroughly by pouring from one container to another three or four times, and then pipette the sample immediately for weighing.

WEIGHING THE CREAM

While the cream sample is warming, mark the test bottle with the same number given the corresponding sample. If using an electronic scale, tare the scale with the cream test bottle on the scale. Weigh nine grams (9g) of cream. No cream should be allowed to get on the outside of the bottle or on the scale while weighing. If too much cream is added to the test bottle, the excess may be removed with a clean wire or glass rod. Do not touch or remove the bottle from the scales until the weighing is complete.

ADDING WATER

Pipette nine (9) milliliters of soft water with a clean pipette. Mix the water and cream thoroughly by swirling the bottle.

ADDING ACID

Adjust the temperature of the cream in the test bottle to 71.6°F ± 1°F (22°C ± 1°C). Hold the bottle at a slight angle and add about 17.5 milliliters of sulfuric acid [specific gravity 1.82 - 1.83 at 68°F (20°C)] portion wise (approximately 8, 5, 4 milliliters). Hand shake between the portions, rotating the bottle slowly during each addition to rinse down any cream that may cling to the neck. The time for complete acid addition should not exceed twenty (20) seconds. Immediately swirl until all lumps completely disappear. Shake on a mechanical shaker for at least five minutes.
CENTRIFUGING

Place bottle in a heated centrifuge 135°F-140°F (57°C-60°C), counter balance, and turn on the centrifuge. Centrifuge for five minutes after the proper speed has been obtained. Stop the centrifuge and add hot soft water 140°F (60°C) [AOAC: 119.5°F±4.5°F (48.5°C±2.5°C)] until the bulb of the bottle is filled to within .6 centimeters of the neck. Centrifuge the sample for two (2) minutes. Stop the centrifuge and add hot soft water at 140°F (60°C) until the liquid column approaches the top graduation of the scale. Centrifuge the sample for one minute.

HOT WATER BATH

Transfer the bottles immediately to a water bath with the temperature controlled at 135°F-140°F (57°C –60°C) and leave the bottles at this temperature for a period of five minutes. The water should be at a level which surrounds the fat column in the necks of all bottles. The water bath must always be at the specified temperature for which the standardized bottles are calibrated and to obtain the correct percent of fat. This assures the proper expansion of the fat globules in the fat column.

READING THE FAT COLUMN

Immediately before removing the bottle from the water bath, add a few drops of glymol by allowing it to flow down the inside of the neck, rather than to fall directly into the fat. Remove one bottle at a time from the water bath, and wipe the bottle before reading. With the aid of sharp pointed dividers or calipers, measure the fat column by placing one point of the dividers at the bottom of the lower surface of the column and the other point at the top of the column, which is the division line between the fat and glymol. The dividers are then carefully lowered until the lower point of the dividers rests on the zero graduation around the neck of the bottle, reading the graduation mark on which the upper point of the dividers is placed. It is suggested that this operation be repeated to be sure the dividers have not slipped. All readings should be read to the nearest one-half percent indicated on the scale on the test bottle. When the reading is completed, it should be recorded opposite the patron’s name, assuring that this number corresponds with the number on the test bottle.

To obtain an accurate reading, hold the test bottle perpendicular and at eye level. If the test has been properly run, the fat column will be translucent, of golden-yellow to amber color and free from suspended particles. If the fat column is milky or shows presence of curd or of charred material, the test should be rerun. After reading each test, replace the bottles back in the water bath to maintain the proper water level for the remainder of the test bottles.
SUMMARY OF CREAM TESTING STEPS

1. Have the temperature of the samples at approximately 100°F ± 1°F (37.8°C ± 1°C).
2. Tare the scale with the cream test bottle on the scale.
3. Mix each sample thoroughly by pouring from one container to another three or four times.
4. Weigh nine (9) grams (±.01 g) of cream.
5. Pipette nine (9) milliliters of soft water into the cream test bottle with the cream.
6. Cool the weighed sample to 71.6°F ± 1°F (22°C ± 1°C).
7. Add 17.5 milliliters of acid at 68°F (20°C) in portions of approximately 8, 5, and 4 milliliters. Manually swirl between portions, not exceeding twenty (20) seconds for the acid addition.
8. Hand shake between portions.
9. Shake on a mechanical shaker for at least five minutes.
10. Centrifuge at the proper speed for five minutes.
11. Add hot soft water at 140°F (60°C) \([\text{AOAC: } 124°F ± 2°F]\) until the bulb of the bottle is filled to within 0.6 centimeters of the neck.
12. Centrifuge for two minutes.
13. Add hot soft water at 140°F (60°C) \([\text{AOAC: } 51°C ± 1°C (124°F ± 2°F)]\) until the liquid column approaches the top graduation of the scale.
14. Centrifuge for one minute.
15. Place the bottle in a hot water bath, maintained at 135°F-140°F (57°C-60°C) \([\text{AOAC: } 118.5°F ± 2°F (48°C)]\), for a minimum of five minutes with the water level above the top of the fat column.
16. Carefully add a few drops of colored gylmol or (red or blue) reading oil.
17. Remove the bottle from the water bath and read the test immediately.
18. Record the test, as read, on the test sheet.
19. Clean all testing equipment immediately after use.

GENERAL INFORMATION - BABCOCK METHOD

ABNORMAL TESTS

Abnormal tests are frequently due to the condition or use of the acid. The amount of acid used to obtain a perfect milk or cream fat test depends upon the strength and temperature of the acid being used. Consequently, it is difficult to specify the exact amount of acid to use in performing the test. The temperature of the milk or cream at the time the acid is added is also an important factor.

1. Dark or charred fat columns are usually caused by:
   a. The use of too much acid or acid that is too strong.
   b. Using milk or cream that is too warm.
   c. Using acid for which the temperature is too high.
   d. Violent mixing of the acid with the milk or cream.
   e. Allowing the bottles, after the acid is added, to stand too long before mixing.
   f. Mixing of the acid and the milk or cream interrupted before complete digestion.

2. Curd, light colored, cloudy or milky fat columns are usually caused by:
   a. An insufficient amount of acid or acid that is too weak.
   b. Acid that is too cold when added to the milk or cream.
   c. Milk or cream that is too cold when the acid is added.
   d. Mixing of the milk or cream with the acid is not continued long enough to dissolve the protein.

3. Foam or air bubbles on top of the fat column are usually caused by adding hard water to the test. Carbonates, which are present in hard water, liberate carbon dioxide in the presence of sulfuric acid, thereby causing the foam to form.
   a. A few drops of sulfuric acid added to the water before it is heated will usually correct the condition.
b. Distilled water is recommended for use.

LABORATORY/TEST ROOM RECORDS

All plants purchasing milk and/or cream on a milkfat basis shall maintain easily accessible written or electronic records of the tests for a period of at least ninety (90) days. Licensees are responsible for the accuracy and completeness of the test records. If electronic files are maintained, the files shall be secured by password and access to the files must be limited to authorized personnel.

In cases where there are two or more persons doing the testing, each person shall initial the test sheets to designate the work performed.

PROCEDURES FOR ELECTRONIC METHODS FOR BUTTERFAT TESTING

All licensed testers using electronic equipment such as the Dairy Lab or Milko-Tester (an electronic transistorized apparatus utilizing light transmission for determining the fat contents of raw milk on which payment is based) shall follow explicitly the current instructions issued by the manufacturer for calibration, operation, maintenance and cleaning of the equipment. In no case shall the method of calibration deviate from the current instructions of the Association of Official Analytical Chemists (AOAC).

The following shall govern the operation of electronic methods for butterfat testing, including light transmission and infrared:

a. The latest edition of the manufacturer's operation manual shall be available at all times in conjunction with the instrument.

b. The electronic equipment shall be operated according to the manufacturer's instructions. This includes cleaning, maintenance and calibration.

c. When electronic methods are used, supplemental Babcock or Roese-Gottlieb/Mojonnier equipment must be provided. If this equipment is not provided at the plant, it must be readily accessible at another location specified by the plant, available during all hours that butterfat testing is run.

d. It is the responsibility of the licensed tester to determine that the electronic equipment is operating correctly. If at any time the tester believes the results obtained are biased, all further testing must be done by another method approved by CDPHE until the bias has been eliminated.

e. Samples used for calibration shall be prepared in the same manner as samples upon which producer payments are to be based. They shall be retained for the same time
and under the same conditions as for official samples used for basis of payment.

f. When any sample differs in milkfat content by greater than 2.0 percentage points from the sample preceding it through the instrument, there shall be an immediate retest and the second test shall be the one recorded. On all retesting of samples, the retest shall be the test used.

g. All calibration results and check tests for initial calibrations and all tests for checking the accuracy of calibration shall be recorded in a permanent record book or electronically.

h. "Official Test" means milk sampled and tested for its milkfat content by means of the Babcock test or any equally accurate and efficient test approved by CDPHE.

i. Any instrument shall read to the second decimal place and shall report findings to the second decimal place.

NOTE: Questions contained in the examination and evaluation of fat testing techniques for certification are based on the information contained in Section III above.