METHODS FOR USE IN THE BACTERIOLOGICAL EXAMINATION OF DRY MILK AND RELATED POWDERS

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is formulating bacteriological procedures useful in controlling the quality of dairy products. This committee is acting through sub-committees appointed from men in the organization who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The purpose of this preliminary report is to submit an outline of methods for making bacteriological examinations of dry milk and certain related powdered baby foods. These methods will be revised later in accordance with the suggestions received before they are finally adopted by the American Dairy Science Association and included in a general report on bacteriological methods of analyzing dairy products.

METHOD OF SAMPLING

It is very important that all utensils and containers used for sampling milk powder be clean, dry and sterile, and that the work be done as speedily as possible. Milk powder absorbs moisture from the atmosphere very rapidly. Samples should always be taken at points beneath the exposed surface.

If samples are to be taken from a bulk or mass of powder not contained in standard packages it is convenient, with the aid of a sterile tablespoon or wide spatula, to obtain samples of substantially the same size from various points throughout the powder mass. Such samples are to be combined in a suitable sterile container from which a properly mixed composite sample is obtained later.

Sampling of milk powder from standard small package units may be obtained simply by withdrawing the desired amount from the powder avoiding that immediately adjacent to the exterior surfaces. A sterile tablespoon or sterile spatula is very convenient for this purpose.

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If samples are to be obtained from the standard bulk packages such as barrels, drums or boxes containing 25 pounds or more it is desirable to remove the layer of powder adjacent to the barrel head or opening to the depth of about three inches and insert in such a cleared area a sampling tube or device similar to an elongated butter or cheese trier. Such a device may not remove an unbroken column of milk powder but in general a representative sample obtained through the entire depth of the container can be secured by such a device, the efficiency of which is determined largely by its design and the manipulations involved in its use. At least three such "plugs" or columns of powder should be withdrawn from a single container. The number of samples thus obtained from a single lot or consignment will obviously be determined by the requirements to be met.

When it is necessary to prepare a composite sample from the combined portions obtained as above in order that the final sample may be representative of a given quantity of powder, several good sized samples obtained from the various batches should be collected in a container of suitable size in order to permit thorough mixing of the desiccated product in order that the sample to be withdrawn for analysis may be representative of the combined portions. Single or double friction top cans, previously cleaned, and sterilized are recommended for collecting, holding or shipping samples. Variations which may occur in a given quantity of powder can be more readily detected by examining separate samples rather than a composite sample, and, in some cases, this practice is preferable.

PREPARING THE SAMPLE FOR ANALYSIS

If the sample of powder should completely fill the container in which it is collected or received, the entire contents should be transferred to another clean, dry, sterile container of ample size to permit proper mixing. The detailed manipulations for mixing the sample may be left to the discretion of the individual, it being understood that the necessity of uniform mixture is as desirable in this case as in any other wherein a representative sample is desired. It is important during the mixing and handling of the product to prevent extraneous contamination as from a dusty atmosphere, improperly cleaned or unsterilized utensils. The same general precautions as are applied to the preparation of any sample for bacteriological analysis apply also to dry milk.

SUGGESTED METHODS OF ANALYSIS

(a) Standard Agar Plate Method.

The standard agar plate method of the American Public Health Association as used for fluid milk may be used for determining the number of organisms in dry milk.
Preparing Dilutions:

It is believed desirable to follow as closely as practical, methods already recognized as standard procedures. For this reason the method of bringing the volume or weight up to 100, as used in the Standard Methods of Milk Analysis of the American Public Health Association, is preferred to the method of adding the material to be tested to 100 cc. or grams as the case may be.

For the one to ten dilution completely dissolve or mix ten grams of the prepared sample in ninety grams of distilled water. In certain instances it may be more convenient to prepare a one to ten dilution by using forty-five grams of water and five grams of powder. If a one to hundred dilution is desired, this may be prepared from the one to ten dilution or one gram may be used with ninety-nine grams of distilled water. The dilutions may be conveniently prepared by weighing the dry sample directly into a bottle containing the dilution water; or into the empty sterile dilution bottle, the required amount of sterile water being added just prior to dissolving the powder for plating. If the latter procedure is followed glass stoppered bottles will be necessary in order to sterilize the bottles by hot air. In the examination of milk powders or compounded infant foods which do not readily form a complete solution with water, it is desirable to facilitate such solution by bringing the temperature of the dilution water to 110–120° F. before mixing with the sample. The plating should be completed within 15 minutes after addition of the water in order to prevent growth in the dilution bottle which would affect the final results.

If lactic acid milk powder preparations are being examined a dilution may be made by using N/10 LiOH as the diluent in order to aid in dissolving the powder. This same type of diluent may be also used for dilutions for the bacteriological examination of powders containing calcium caseinate, although in this case a one to hundred dilution in distilled water is preferable in order to prevent excessive foaming.

In the case of incomplete solution of the milk powders or certain constituents of compounded preparations, the diluted product should be kept thoroughly agitated during withdrawal of the sample with the pipette in order that a uniform sample of the solution and suspended material may be transferred to the petri plate.

Preparing Plates:

Dilutions should be made which will give not more than 300 nor not less than 30 colonies on the plate. The preparation of the media and pouring of the plates should follow the suggestions outlined in the latest edition of the Standard Methods of Milk Analysis of the American Public Health Association (Fifth Edition, 1928).
Incubation Temperature:

In addition to the incubation temperature of 2 days at 37° C. as recommended in the standard procedure, other incubation periods and temperatures may be used for special purposes, such as 5 days at 25° C., 3 days at 30° C., 2 days at 35° C. or 2 days at 55° C. The later temperature is especially useful for estimating the number of thermophilic bacteria which are often present in large numbers due to certain steps in the manufacturing process.

Counting of Plates—Precautions:

Extreme precaution should be exercised in counting the incubated plates in order to differentiate between the bacterial colonies and suspended constituents of the sample transferred to the plates from the initial dilutions. Insoluble milk powders or compounded products containing insoluble material frequently cause residues in the plates which are readily confused with bacterial colonies. Devices for counting the plates with the aid of artificial illumination and low magnification are strongly recommended. Doubtful objects should be examined with higher magnification to determine whether they are colonies.

(b) Microscopic Colony Count (Frost Method).

In laboratories already equipped and experienced in counting bacteria in milk by this method, it would seem that this method could be readily used in the routine control of dried milk. Directions as outlined in the Standard Methods of the American Public Health Association should be followed.

The original sample should be diluted as suggested in the methods for the agar plate count. Incubate for 12 to 16 hours at 37° C.

(c) Direct Microscopic Count (Breed Method).

The direct microscopic count applied to milk powders has a certain value for indicating the bacterial character of the fluid milk from which the product was made. Where this method is to be used for purposes of information of this kind it should be carried out according to the directions given in the Standard Methods of Milk Analysis of the American Public Health Association.

The direct microscopic count cannot be relied upon as an adequate procedure for determining the bacterial count of milk powders. It may be of supplementary value in conjunction with certain plating procedures listed herein.

(d) Examination for Hemolytic Streptococci.

Standard nutrient agar plates to which has been added 0.5 per cent sodium chloride and 2 to 5 per cent defibrinated blood is suggested for use
in determining the presence of hemolytic streptococci. The blood should be added aseptically after the agar is melted and cooled prior to pouring the plates. The plates should be incubated for 2 days at 37° C. Examination should be made at the end of 24 to 48 hours.

Suspicious hemolytic colonies should be examined by Gram staining to determine the presence of streptococci. Before definite conclusions can be drawn, reference should be made to standard text books on bacteriology dealing with the differentiation and recognition of hemolytic streptococci.

If conditions permit its use, veal infusion medium will give somewhat better results than the standard nutrient agar for the growth of the hemolytic streptococci. This medium is prepared as follows:

Ground lean veal .................................................. 500 grams
Distilled water .................................................. 1,000 cc.

Infuse over night in a cold room. Strain through cheese cloth by pressing. Make up to the original volume. Bring to boiling with frequent stirring and boil until the infusion is clear and the coagulum brown. Strain through cheese cloth and filter through paper. Adjust reaction to pH 6.8 to 7.0. Add 0.5 per cent Difco peptone and 0.5 per cent sodium chloride. The broth should then be autoclaved for 15 minutes at 20 pounds’ pressure. This is a higher pressure than that used for the final sterilization and is used in order to avoid precipitates in the finished product. Egg may be added previous to autoclaving if a glass clear medium is desired. Filter and sterilize at 15 pounds’ pressure for 15 minutes. Before the final sterilization, 1.5 per cent agar is added and the medium distributed in flasks in readiness for plating. Blood (rabbit, horse, sheep, etc.) may be added aseptically just prior to plating and after the agar has been melted and cooled to 50 degrees C.

(e) EXAMINATION FOR THERMOPHILIC BACTERIA.

For the routine determination of the presence of thermophilic organisms plates may be prepared from standard nutrient agar. For investigation or research purposes it is recommended that the following materials be added to each 1,000 cc. of standard agar.

Bacto Trytophane Broth ........................................ 2.5 grams
Bacto Yeast Extract .................................................... 1.0 gram
Dextrose ................................................................. 1.0 gram

pH .................................................. 7.0 ± 0.2

The plates should be incubated for 48 hours at 55° C. At least 15 cc. of medium should be used in each plate to prevent excessive drying during the incubation. Water should be placed in the incubator in order to reduce the drying of the plates to a minimum.
MEDIA FOR SPECIAL PURPOSES OTHER THAN THOSE LISTED ABOVE

(a) Inasmuch as the standard procedure of the American Public Health Association as outlined above (a) may not be conducive to the growth of all organisms in milk powder any more than it is to the growth of all organisms in natural fluid milk, the following medium is suggested for special purposes.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Bacto Tryptophane Broth</td>
<td>2.5 grams</td>
</tr>
<tr>
<td>Bacto Peptone</td>
<td>2.0 grams</td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
<td>1.0 gram</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.0 grams</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 grams</td>
</tr>
<tr>
<td>Water</td>
<td>1,000 cc.</td>
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</tbody>
</table>

Incubation temperature in this case should be for 3 days at 30°C or at other times and temperatures in accordance with the results desired.

(b) Whey agar may also prove satisfactory for obtaining counts required for investigational or interpretative purposes and may be prepared according to methods outlined in Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 396–397, 1930.

EXAMINATION FOR YEASTS AND MOLDS

The determination of yeast and mold counts in dried milk, while not a common practice, is used quite extensively in the butter industry. No doubt a more complete study of dried milk from this viewpoint might give us some valuable information regarding sanitary conditions. (See Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 380–405, 1930.)

METHOD OF REPORTING COUNTS

Results should normally be reported per gram of dry powder. If desired they may be transformed to the fluid basis by dividing by the factor used in reconstitution. This is usually 8 parts of water to 1 gram of powder.

In the interest of uniformity of practice and interpretation of data from various sources, the medium used and the time and temperature of incubation should always be reported in direct conjunction with the numerical results obtained on the one gram basis. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation periods and temperatures are used.

CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are printed for
the general criticism of other members of the American Dairy Science Association and other interested parties.

Separate copies of this report may be secured at cost from the chairman of the Committee on Bacteriological Methods.

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