UPDATING ABNORMAL MILK TESTS

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Abstract

The U. S. Public Health Service and the National Conference on Interstate Milk Shipments have agreed to require that producers meet standards for the abnormal milk program. Effective July 1, 1970, all raw milk shall contain less than 1,500,000 leucocytes per milliliter. The regulations of various states now require that qualified personnel in approved laboratories make total bacterial plate counts on all raw supplies at least once a month. A proposed modified direct microscopic somatic cell count can effectively be used as a rapid screening test by the same personnel, eliminating the need for other screening tests. This procedure also utilizes low power "spotting" to detect clustered leucocytes with enmeshed bacteria to establish the presence of bacterial mastitis. When these clusters are found, the need for counting leucocytes is eliminated. Photographs of clustered leucocytes with enmeshed bacteria under low and oil immersion are shown. Typical epithelial cells are photographed. The proposed procedure is advocated because it is accurate, not time-consuming, and can establish the presence of bacterial mastitis.

Public Health Service and National Conference on Interstate Milk Shipment Regulations

The National Conference on Interstate Milk Shipments (NCIMS) has decided to limit considerations of abnormal milk to only mastitis and not to include other contaminants such as pesticides, radioactive materials, etc. The NCIMS and the National Mastitis Council have agreed that mastitis is present if the somatic cell count of a milk is 1,500,000 or more per milliliter. The recorded indicator tests for this determination are detailed in U. S. Public Health Service Publication No. 1306. Laboratory examinations or screening procedures of the milk for the presence of unwholesome, altered mammary secretions—whether of an inflammatory, infectious, physiological, or environmental origin, should be made at the same frequency as specified for bacterial tests in milk. When a herd milk contains 1,500,000 leucocytes per milliliter, the following procedures should be followed:

(a) A warning letter shall be sent to the producer notifying him of the high leucocyte count. The letter shall also list the principal causes of excess leucocyte counts. (b) Following the second consecutive test indicating a raw milk count of 1,500,000 or more leucocytes per milliliter, an inspection shall be made by an official sanitary or a person designated by him. (c) A third herd milk sample shall be taken. If this sample also indicates a leucocyte count of 1,500,000 or more per milliliter, the milk regulatory authority shall, if he deems it necessary, require the producer to: (aa) have milking equipment analyzed by a milking equipment servicereman, and (bb) have individual animals examined by a veterinarian. Cows producing abnormal milk shall be milked separately and the milk shall be withheld from the milk supply.

Effective July 1, 1970, milk supplies containing 1,500,000 or more leucocytes per milliliter on three out of five of the last tests shall have their permit suspended and/or court action shall be taken in accordance with the recommendations of the USPHS Grade A Pasteurized Milk Ordinance, "provided that leucocyte counts of 1,500,000 or more per milliliter shall not have been officially recorded nor penalty applied unless corroborated by the direct microscopic leucocyte count or the equivalent as published by the U.S.P.H.S. and subjected to the State Laboratory Certification Program."

Presently 47 of the States utilize the NCIMS Program. There are slight variations in regulations of the States: for example, New Jersey lists as unsatisfactory a leucocyte count of 2,000,000 per milliliter or more. For milks produced in New York state, the only acceptable test is the Whiteside; for milks produced out of the State for import there, other screening tests are accepted, with qualifications.

Figure 1. Low-power photomicrograph (100X) of stained milk smear showing appearance of leucocyte cluster in center of field. In observation through microscope, individual leucocytes in cluster are better delineated as field is taken in and out of focus.

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MASTITIS

There is widespread disagreement as to the definition of "mastitis." The dictionary definition and, in fact, the nation-wide dairy-industry-accepted definition is "inflammation of the mammary gland . . . whether of an inflammatory, infectious, physiological, or environmental origin." It seems to me that regulatory enforcement agencies should be concerned, from a public health hazard standpoint, only with bacterial mastitis. Certainly, the ingestion of somatic cells has not and will not make anyone ill. The existing regulations of the various states nevertheless detail screening tests which use only the leucocyte count to determine if milk is "abnormal." It is my expectation that these regulations will be changed, but in the meantime we must live with them.

The screening test of choice should be one which will most accurately indicate the presence of bacterial infection. In many, many cases the mere presence of high somatic cell counts does not establish the presence of bacterial mastitis. The California Mastitis Test, the Catalase Test, the Modified Whiteside

**Figure 2.** Same cluster as in Fig. 1 under oil immersion objective (970X). Only some bacterial cells can be seen photographically; many more can be seen under microscope when it is focused to different depths.

**Figure 3.** Low-power magnification similar to Fig. 1.

**Figure 4.** Oil immersion objective magnification of cluster in Fig. 3.

**Figure 5.** Oil immersion objective magnification of stained epithelial cells. Note that most cells are part-moon shaped.

Test, and the Wisconsin Mastitis Test all indirectly estimate the somatic cell count. There has been a tremendous diversity of opinion as to the accuracy of these tests and much of the published work has established their unreliability.

DIRECT MICROSCOPIC SOMATIC CELL COUNT

The direct microscopic somatic cell count in milk reported by the Subcommittee on Screening Tests of the National Mastitis Council, in the November, 1968 Journal of Milk and Food Technology, details quite comprehensively the procedure for determining the nucleated somatic cell count. It is unfortunate that nucleated epithelial cells (Fig. 5) are also included in the count. The presence of these cells very frequently is not associated with bacterial infections. The direct microscopic somatic cell count test as detailed is quite elaborate and consumes a considerable amount of time per test, but this report does establish the microscopic count to be reproducible and reliable.

Let's go back to the regulations which will go into effect July 1, 1970: "Penalties will be applied only if
the leucocyte count has been corroborated by the direct microscopic leucocyte count."

A modified direct microscopic somatic cell count can effectively be used as a rapid screening test. Ten fields spaced out over the length of a strip are adequate to establish that the leucocyte count is significantly below or above 1,500,000 per milliliter. In these instances there is no point in making full strip counts. If the field count yields a figure close to the 1,500,000 cutoff, then full strip counts should be made. This modification is rapid for the majority of the milks encountered and we now have a definite, accurate procedure for determining the leucocyte level on which action must be taken. On this basis, there is no logic for the continued use of the indirect screening tests in connection with the official Abnormal Milk Program.

Although better qualified personnel are needed to perform the direct microscopic somatic cell count than any of the indirect screening tests, (and I might say that there has been much controversy as to whether technicians are properly reading the indirect screening tests) the regulations of the various states now require that qualified personnel in approved laboratories make total bacterial plate counts on all of the raw supplies at least once a month. I know from experience that the same qualified technicians can quickly learn how to make microscopic leucocyte counts and thereby eliminate the use of these controversial indirect screening tests.

**Low-Power "Spotting"**

For over 30 years at our laboratory we have automatically made microscopic smears for type analysis of all milks subjected to plate counts. It is important for the field man to know the source of the bacterial count found, whether it be from utensils, from udder infections, or whether it is complicated by inadequate cooling. These same smears are available for leucocyte counting. One thing that we have always done, and which I propose be included as an integral part of the microscopic method for determining abnormal milk, is to examine these smears for positive evidence of the presence of bacterial infection. When bacterial mastitis exists there is a clustering of leucocytes with bacteria enmeshed in these clusters. These clusters can be readily spotted under low-power magnification (see Fig. 1 and 3). Oil immersion observation of this grouping then will confirm the actual mastitic condition (see Fig. 2 and 4). There are only about five low-power strips in an entire square centimeter smear. The length of time required to examine this area for evidence of clustered leucocytes is minimal. Keep in mind that if you do locate clustered leucocytes with enmeshed bacteria, there is no need to make a leucocyte count because you have already established the presence of bacterial infection. If it is the intent, and I think it should be, of the Abnormal Milk Program to locate and then eradicate the presence of bacterial infections in the milk supply, the combination of leucocyte counts and low-power examination for detecting mastitic clusters is essential. Routine examination has disclosed a large percentage of herd milks showing the presence of infection, even though the leucocyte count was significantly below 1,500,000 per milliliter.