AN EFFECTIVE "SINGLE SOLUTION" STAIN

DAVID LEVOWITZ AND MAURICE WEBER

New Jersey Dairy Laboratories, New Brunswick, New Jersey
(Received for publication March 9, 1956)

Editorial Note: A modification of Newman's No. 2 Stain has been developed at the New Jersey Dairy Laboratories. This discription of its preparation and use is given encourage interested laboratories to test it and to report their experiences with it.

A. CONSTITUENTS:

1. Ethyl Alcohol, 95%[a] .....520.0 bl.
2. Tetrachlorethane, technical[b] .....440.0 ml.
3. Acetic Acid, glacial ..... 40.0 ml.
4. Methylene Blue (chloride), Certified 6.0 gm.

[a]Or Formula 3A Denatured (95% ethyl + 5% methyl)
[b]Acetylene Tetrachloride

B. STAIN PREPARATION:

1. Pass the dry dye through a fine sieve or strainer, to insure against caking; then add it slowly to the alcohol and tetrachlorethane in a 2-L. flask. Swirl to dissolve, let stand 12-24 hours at 40-45° F. Then add acetic acid.
2. Filter through paper which retains "fine" precipitates (Whatman No. 42 or equivalent). package in bottles equipped with plastic closures.

C. SLIDE PREPARATION:

1. Apply Bon Ami (powder or cake) lightly, using a moist cloth or sponge pad, to the surfaces of new slides or thoroughly washed old slides. After the Bon Ami has dried thoroughly, wipe it off with a clean, dry (oil free) chamois or cheesecloth.
2. Flame each slide and store in a dispensing cabinet which prevents free access of air. If such a cabinet is not used, flame the slide and let it cool to room temperature immediately before applying samples. (This routine insures slides' surface being properly receptive to smears).
3. Prepare a convenient slide-drying cabinet (a large index card file box is satisfactory) by (a) perforating ends to permit air circulation; (b) mounting a glass shelf level, under the top, and (c) installing a small bulb (15 or 25 watt) to keep the glass at not over 125°F. (Check with Tempilstik; do not use area which warms above 125°F.)

D. SLIDE STAINING:

1. Dry smeared slides rapidly (on the shelf in the cabinet); cool to room temperature, then immerse in the stain for 2 minutes. Drain off excess stain by resting slides on edges on absorbent paper. Dry thoroughly – preferably by forced air from blower or fan.
2. Rinse dried, stained slides in three changes of tap water at 100-110°F., then dry them thoroughly and rapidly.

E. ADVANTAGES OF THIS STAIN AND METHOD:

Whether the sample is a raw or pasteurized (unhomogenized or homogenized) milk or cream—

1. The stain is retained lightly and very uniformly by only the milk protein. There is no "mottling" or "network effect" on the background which is tinted enough to reduce eyestrain and fatigue and to make focussing easier, but not enough to keep any cellular constituents from standing out in bold relief.
2. The outlines of almost completely plasmolyzed microorganisms retain the stain sufficiently to be distinguished readily; cells exhibiting lesser degrees of plasmolysis are stained more heavily. Unplasmolyzed organisms are colored strongly.
3. The cytoplasmic portions of body cells are distinctly darker than the background with cell outlines well defined. Nucleoplasmic areas are stained deeply but individually differentiated (with no "mossy" effect), so that primary leucocyte classification into myelocytes, young and mature polymorphonuclears, etc., is accomplished directly without employing differential stains on special smears.
4. Smears remain fixed even if rinsing is energetic—will not slough off. Smears continue to be firm, when treated with fat-solvent to remove immersion oil. Neither crystals nor dye particles, which might obscure the preparation and prevent its proper examination, will ever form.

ORIGIN OF THIS SYSTEM:

1. Newman's procedure, when followed, results very frequently in (a) loss of smears at rinsing; (b) obliteration of large smear areas by crystal formation and dye precipitation; (c) such deeply colored backgrounds that only heavily stained cellular units can be seen; and (d) mottled backgrounds whose darker areas cannot be examined at all.
2. Newman's stain, as published, was not suitable for our use. However, the principle of defatting, fixing and staining by one solution, in which decolorizer was also incorporated, to

(Continued on Page 127)

contacted by the dairy products. In other words, it was recommended that where the dairy processing plant is responsible for the sanitation of the dairy products surfaces, the 3-A Sanitary Standards Group would undertake the preparation of the standard; and that where the food handling establishment is responsible for the sanitation of the surfaces contacted by dairy products, the standards would be developed by the National Sanitation Foundation's Joint-Committee on Food Equipment Standards.

It was further recommended that closer liaison should be maintained between the two organizations by interchange of tentative drafts of standards during the formative period of their preparation, where it is evident that there is definite interest of both organizations in the details of the sanitary standards under consideration. Where necessary to achieve an adequate understanding, a conference of representatives of coresponding committees would be called.

There was complete agreement that both organizations have a common objective in the preparation of effective sanitary standards for equipment used in the food service and dairy fields. Past accomplishments by both serve to point to the necessity of further coverage of such additional items of equipment as have known public health significance.

The presentation of the 1954 Annual Report of this Committee included an "off-the-record" report of progress toward the formation of an organization to promote and police the use of the 3-A Symbol. It is now possible formally to announce the formation of "The 3-A Sanitary Standards Symbol Administrative Council," consisting of the following members:

Representing the Technical Committee of D.I.S.A.

Paul K. Girton, Girton Manufacturing Co. Millville, Pa.

George W. Putnam, Creamery Package Mfg. Co., Chicago, Ill.

Representing the Sanitary Standards Subcommittee of D.I.C.

William A. Dean, Jr., Bowman Dairy Co., Chicago, Ill.

A. E. Nessler, Kraft Foods Co., Chicago, Ill.

Representing the Committee on Sanitary Procedure of this Association.

Paul Corash and *C. A. Abele* (3-year terms)

Mark D. Howlett, Jr., and *K. G. Weckel* (2-year terms)

The Association representatives on the Council were appointed by President Parkin.

The organization meeting of the Council was held in conjunction with the joint meeting on 3-A Sanitary Standards at Bethesda, Md. The following officers were elected:

CHAIRMAN—*William A. Dean, Jr.*

VICE-CHAIRMAN—*A. E. Nessler*

SECY.-TREAS.—*C. A. Abele*

Attendance of Committee members at this initial meeting of the council (one member from Los Angeles) was made possible by a loan from the Association Treasury. Letterheads and envelopes, application forms for authorization to use the 3-A Symbol, and authorization certificates have been printed, also at the expense of this Association. The 3-A Symbol Council is now indebted to this Association to the amount of $491.86, plus the express for shipment of the stationery and forms. Repayment will be made from the initial receipts of the Council. The fees to be charged for use of the 3-A Symbol have not yet been fixed by the Council, but will probably be decided upon at a meeting of the Council to be held next week.

The mechanics for processing applications for authorization have not been fully crystallized. It is possible to state, however, that the plans of the Council with respect to this procedure have been materially advanced by the availablility of checklists for Storage Tanks, Stainless Steel Automotive Transportation Tanks, and Homogenizers and High-Pressure Pumps, drafted by the Committee on Sanitary Standards for Dairy Industry Equipment, of the New York State Association of Milk Sanitarians. These check-lists of mandatory provisions of 3-A Sanitary Standards were drafted for use by sanitarians in determining compliance of individual pieces of equipment, none of which is now identified by the 3-A Symbol.

It is anticipated that, after formal review — possibly some modification — by the Task Committees which presented the original drafts of the respective sanitary standards, such checklists, filled out by a responsible officer of the applicant concerned, will become a feature of the formal applications for authorization to use the symbol. The New York State Association Committee has indicated its intent to prepare similar checklists for other types of equipment for which 3-A Sanitary Standards have been adopted. This activity indicates a high degree of interest in sanitary standards, which is reflected in assistance to individual sanitarians, and to the 3-A Symbol council, as well.

H. E. BREMER
E. B. BUCHANAN
PAUL CORASH
JOHN CULP
DAVID H. EVANS
MILTON R. FISHER
H. CLIFFORD GOSLEE
MARK D. HOWLETT, JR.
WILBUR KEMPA
BEN LUCE
C. K. LUCHTERLAND
JAMES A. MEANY
SAM D. NOLES
IVAN E. PARKIN
C. W. WEBER
DICK B. WHITEHEAD
H. L. THOMASSON
Ex.Officio
C. A. ABELE, *Chairman*

AN EFFECTIVE "SINGLE SOLUTION" STAIN" CONTINUED FROM PAGE 121

result in a constant stain itensity in background and cells was intriguing. We therefore balanced the ingredients to the point we desired.

3. Our criterion was the light, uniform background, and the differentiation of cellular structure that is attainable by employing very dilute stains. This is particularly necessary when heat-treated dairy products are examined, where all stages of plasmolysis may be anticipated. We have used the system detailed above for twenty years. (We test an average of 100 raw and 150 unhomogenized and homogenized milks and creams daily).
4. Undoubtedly, the origin of many of the conflicting statements in the literature regarding the utilization of the microscopic method on pasteurized products, is due to the use by some investigators of uncritical stains which yielded smears so dense that partially plasmolyzed and even unplasmalyzed cells were frequently missed.
5. The three stains recommended for official use by the current 10th Edition of Standard Methods recover cellular populations of a magnitude equivalent to that obtained by this modified stain on raw milk and creams. They recover much lower levels, however, on heat-treated products—especially those which have been homogenized.