A Research Note

RAPID DIAGNOSIS FOR STREPTOCOCCUS AGALACTIAE AND STREPTOCOCCUS UBERIS

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ABSTRACT

The TKT-ferric citrate medium lends itself well for use in a rapid screening method for Streptococcus agalactiae and Streptococcus uberis isolation and identification from milk samples in that: (a) nonprofessional technicians can rapidly identify positive colonies as S. agalactiae or S. uberis on initial isolation; (b) additional confirmatory tests (CAMP, etc.) are usually unnecessary, thereby reducing labor and media costs; and (c) the medium is selective for streptococci and inhibitory to contaminants thereby increasing the number of isolations.

In eradication procedures for the Streptococcus agalactiae type of mastitis, it is important that a diagnosis be made as soon as possible. The infected animal can then be treated shortly thereafter, thus limiting the chance of spreading the disease to other animals in the herd. As some Streptococcus uberis cultures will give a CAMP (1) reaction, it is necessary to differentiate them from S. agalactiae. The labor, time, and material needed to accomplish this diagnosis was too cumbersome, and hence another procedure, described in this report, was devised.

MATERIALS AND METHODS

Milk samples (5-60 ml) were obtained in "whirl-pak" bags from approximately 3,000 cows in herds on the Wisconsin Department of Agriculture Albion Mastitis Control Project. The samples were composite cow samples, aseptically drawn, immediately placed on ice, and delivered to the laboratory the same day.

Two comparisons were made using three media: blood agar (BAP), Bacto Difco Lab. blood agar base plus 2% citrated ovine blood; an inhibitory medium (TKT) (2) and then ferric citrate added to the inhibitory medium (TKT-FC) (3). Approximately 1,000 samples were used in each comparison in the following manner:

(a) BAP vs. TKT-FC. Duplicate samples were streaked on BAP and TKT-FC plates divided to accommodate six cow samples. All plates were incubated 24 hr at 37 C.

(b) TKT vs. TKT-FC. Duplicate samples were also streaked here using the same procedure as in comparison study (a).

Positive reactions (clear zones of hemolysis surrounding Streptococcus colonies with or without darkening of the medium and colonies) were recorded from the TKT-FC. All TKT-FC plates were reincubated and held another 24 hr for detection of slow-growers or delayed reactions. All suspect Streptococcus colonies were picked from the blood agar and TKT and tested for CAMP and aesculin-splitting reactions using a medium containing aesculin and ferric citrate plus blood agar.

The media were compared for accuracy and efficiency in the rapid detection of S. agalactiae and CAMP positive S. uberis. Special consideration was given to the time-saving aspects of each when culturing milk samples in the laboratory.

RESULTS AND DISCUSSION

Correlations were based on duplicate results whether positive or negative on the plates being compared, while non-correlations were recorded whenever results differed in isolation results. The differences are tabulated below.

In both studies, the TKT-FC showed an advantage in detecting S. agalactiae colonies where medium

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<thead>
<tr>
<th>TABLE 1. COMPARISON OF FOUR MEDIA FOR ISOLATION OF STREPTOCOCCI FROM MILK.</th>
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<tr>
<td><strong>Study (a)—BAP vs. TKT-FC</strong></td>
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<tr>
<td><strong>Total tests</strong></td>
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<td>1,087</td>
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| **Study (b)—TKT-FC vs. TKT**                                    |
| **Total tests** | **Correlation** | **TKT** | **S. agalactiae** | **S. uberis** | **TKT-FC** | **TKT** | **S. agalactiae** | **S. uberis** | **TKT-FC** |
| 986            | Negative       | 831     | 96 (9.7%)        | 14           | 17        | 14      | 7             |
|                | Positive       | 112     | 2 (0.2%)         | 6            | 4         | 6       | 2             |

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was not in agreement.

The BAP in study (a) required that 680 colonies be checked for CAMP and aesculin-splitting reactions. It meant a 24-hr delay of diagnosis. Recognition of Streptococcus colonies on BAP requires a more experienced technician; more colonies must be selected and tested per sample and other contaminating organisms frequently overgrow the staphylococci and streptococci on this noninhibitory medium.

Since the TKT-FC is selective as well as inhibitory, showing the CAMP and aesculin-splitting reactions immediately, it naturally saves time and increases positive isolations making it most valuable for use in the culturing of milk samples submitted to the laboratory.

The TKT does not show the aesculin-splitting reaction; 135 CAMP tests were necessarily made on aesculin BAP's to differentiate S. agalactiae and S. uberis isolates. Thus, incorporation of ferric citrate in the TKT medium would seem beneficial.

**NEW MILK STORAGE STANDARD IS ADOPTED**

A new sanitary standard that will make it possible to safely store unlimited volumes of cold, raw milk on the farm prior to transporting it to market has been developed by the 3-A Sanitary Standards Committees, a voluntary group of industry, government and regulatory representatives.

The new standard sets guidelines for the cleanliness of large tanks with greatly increased storage capacities to assure proper protection of pre-cooled stored milk. "This standard is highly significant in an era of increasingly large dairy herds, in which large milk volumes must be effectively handled prior to tank loading," said Donald H. Williams, secretary of the committee, who is technical director of Dairy and Food Industries Supply Association.

Until the new code was approved at the 3-A’s spring meeting May 23-25, 1972, at Louisville, Ky., existing 3-A standards had applied only to smaller, conventional farm tanks suitable for combined cooling and storage.

The new guideline is expected to facilitate the already rapidly changing operations in milk marketing and the trend to farm storage tanks with capacities in excess of 2,000 gallons.

In other action, the 3-A committees completed an amendment to the basic standard for fittings by setting criteria for fittings and components to be used in systems for aseptic processing. The amendment is important in applications requiring that sterilization of the equipment be achieved as well as maintained during entire processing operations. The amended standard will also provide the basis for aseptic applications of pumps and other equipment used in high temperature environments.

Other tentative standards which were reviewed and passed on to appropriate action groups for further study and revision include drafts on scraped surface heat exchangers, uninsulated storage tanks, and revisions in published 3-A standards for instruments, pumps, and fillers and sealers of single service containers.

The meeting marked the beginning of the 27th year of operation of the joint 3-A Sanitary Standards Committees. About 65 health officials, equipment manufacturers and dairy processors attended.

The 3-A program for dairy equipment is the result of cooperation among three groups. (1) dairy processors, the users of dairy equipment; (2) dairy industrial suppliers and equipers, the manufacturers and sellers of dairy equipment; and (3) public health officials and sanitarians, the regulatory officials under whose jurisdiction the equipment is installed and used.

Voluntarily supported by the national trade associations in the dairy processing industry, the program has resulted in the adoption of a total of 32 standards and practices for dairy industrial equipment. Equipment complying with the standards may carry the 3-A Symbol, provided its manufacturer received authorization to do so from the 3-A Symbol Council.

In general, 3-A Standards and practices are acceptable in public health jurisdictions in nearly every town, city and state in the U.S. The 3-A Sanitary Standards and Practices are cited in the recommended Grade "A" Pasteurized Milk Ordinance of the U. S. Public Health Service.