A MODIFIED STAIN AND PROCEDU RE FOR THE DIRECT MICROSCOPE METHOD OF COUNTING BACTERIA IN DRY MILK AND OTHER MILK PRODUCTS

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Summary

The preparation of a stain and its method for use in the direct microscopic examination of milk smears is given. Included are some figures arising from a study in which the new stain was compared with the Levowitz-Weber stain. The new method was found to yield higher counts of bacterial clumps and of leucocytes than the standard stain and this was accomplished more quickly and with less fatigue on the part of the observer.

Our experience in this laboratory with the Levowitz-Weber stain recommended by Standard Methods *(1)* for the microscopic examination of dry milk has not been favorable. Organisms which are lightly stained are particularly hard to discern, the background, on occasion, tends to be unevenly stained, in many cases it was difficult to distinguish dirt from bacterial cells, and the conscientious examination of reconstituted milk films was time consuming and inducive of eye strain. These objections led to trials of other stains recommended for the examination of milk films. Staining methods employing contrast stains such as those suggested by Broadhurst and Paley *(2)*, Gray *(3)* and Charlett *(4)* were tried and discarded in favour of the following stain and technique.

Stain as Modified

Preparation

Mix 0.6 g methylene blue (Bacto-Methylene Blue certified) in 52 ml 95% ethanol and 44 ml tetrachloroethane.

Let stand in water bath at 45 C until methylene blue is completely dissolved, agitate occasionally.

Cool.

Add 4 ml glacial acetic acid.

Filter.

Add 2 ml *saturated* alcoholic solution of basic fuchsin (1 g in 15 ml 95% ethanol).

Technique

1. Prepare smears.
2. Dry smears at room temperature or in warming oven at 35-37 C.
3. Immerse slide in acetone for 2 minutes; for reconstituted milk immerse for one minute.
4. Allow acetone to evaporate to dryness.
5. Apply stain for 2 min.
6. Dry stained smears at 35-37 C within 2 min. *It is important not to dry for too long* otherwise smears may crack and excess methylene blue cannot be removed.
7. Wash with gentle agitation in warm water until excess methylene blue is removed.
8. Dry and examine.

Cells will be blue against a pink background.

NOTE: It is very important to use clean slides to prevent lifting of the smears when washed. It is believed that the treatment with acetone increases the permeability of the cells for the stain.

This stain and method when properly prepared and applied invariably gave fields which could be scanned thoroughly, rapidly and with less eye strain and most important with less doubt in the mind of the observer than any we had used formerly.

On the strength of these observations three other laboratories engaged in the examination of dried milk tried the method and reported satisfaction with it, particularly its superiority to the standard stain in ease and rapidity of reading.

The results from a comparative study in which three observers independently examined milk smears in duplicate for each stain from nine different milk samples are depicted in Table 1. In this study the smears were coded so that none of the observers knew their origin. Thirty fields were counted in each smear. Both bacterial clumps and leucocytes were counted and the counts, thus arrived at, per ml of milk were divided by 10^5 for presentation in Table 1.

From these data it will be noted that the counts by the modified stain almost invariably exceeded those obtained with the standard. This increase is further shown by taking the arithmetic mean of all smears read by each observer for both stains as given in Table 2.

While in our hands the use of the modified stain has yielded similar and frequently higher counts than the L.W. stain, it is mainly to be recommended on the basis of the less fatiguing and more rapid examination of milk smears arising from its use.

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TABLE 1. A COMPARISON BETWEEN THE MICROSCOPIC COUNTS YIELDED BY TWO STAINS ON NINE MILK SAMPLES IN DUPLICATE BY THREE OBSERVERS

Sample		Obs. A Bact x 10^5 Mod.	Obs. A Bact x 10^5 L.W.	Obs. A Leuc. x 10^5 Mod.	Obs. A Leuc. x 10^5 L.W.	Obs. B Bact x 10^5 Mod.	Obs. B Bact x 10^5 L.W.	Obs. B Leuc. x 10^5 Mod.	Obs. B Leuc. x 10^5 L.W.	Obs. C Bact x 10^5 Mod.	Obs. C Bact x 10^5 L.W.	Obs. C Leuc. x 10^5 Mod.	Obs. C Leuc. x 10^5 L.W.
	a	2.0	3.5	5.5	7.5	3.3	3.0	5.9	7.7	2.4	2.1	5.4	3.6
1	b	3.5	2.5	6.5	7.0	2.2	3.0	5.5	7.2	4.8	1.2	5.7	6.0
	av.	2.75	2.0	6.05	7.25	2.75	3.0	5.7	7.45	3.6	1.65	5.55	4.8
	a	3.0	2.0	7.5	7.0	4.1	2.1	9.0	8.7	3.3	1.5	7.2	5.7
2	b	3.0	1.1	6.5	4.5	3.9	1.2	6.8	5.4	4.8	1.8	4.8	4.5
	av.	3.0	1.55	7.0	5.75	4.0	1.65	7.9	7.05	4.05	1.65	6.0	5.1
	a	3.0	2.5	9.0	11.0	5.9	9.0	8.6	8.4	4.8	2.7	4.2	6.0
3	b	3.0	2.0	7.0	7.5	5.9	2.7	5.9	9.9	4.5	2.7	5.9	4.8
	av.	3.0	2.25	8.0	9.25	5.9	5.85	7.25	9.15	4.65	2.7	5.05	5.4
	a	4.8	1.1	8.0	7.0	4.6	4.5	11.0	8.4	2.7	1.2	6.6	4.5
4	b	2.5	1.0	8.5	8.0	2.9	3.6	9.9	9.9	2.7	2.1	4.5	3.6
	av.	3.65	1.05	8.25	7.5	3.75	4.05	10.45	9.15	2.7	1.65	5.55	4.05
	a	1.5	2.0	8.5	6.5	1.8	2.4	8.4	7.2	1.8	1.2	6.0	5.4
5	b	1.4	3.5	6.5	6.5	1.5	2.4	8.4	8.4	2.1	0.9	3.9	3.6
	av.	1.45	2.75	7.5	6.5	1.65	2.4	8.4	7.8	1.45	1.05	4.95	4.5
	a	2.0	1.7	14.0	13.0	5.1	1.8	17.0	10.8	4.2	1.5	7.8	6.3
6	b	1.5	1.2	8.5	8.5	3.9	2.3	11.0	9.0	1.5	1.5	6.9	4.8
	av.	1.75	1.45	11.25	10.75	4.5	2.05	14.0	9.9	2.85	1.5	7.35	5.55
	a	1.5	2.0	7.0	5.5	2.4	1.1	8.1	5.4	1.8	0.9	1.8	2.1
7	b	2.0	0.9	3.2	4.5	1.5	1.1	8.6	5.4	1.8	2.1	2.1	1.8
	av.	1.75	1.45	5.1	5.0	1.95	1.1	8.35	5.4	1.8	1.5	1.95	1.95
	a	1.0	1.4	7.0	7.0	3.9	2.7	9.9	5.6	2.1	0.6	4.2	3.3
8	b	1.5	2.0	6.5	8.5	3.3	3.2	8.1	6.8	2.4	0.9	4.2	3.3
	av.	1.25	1.7	6.75	7.75	3.6	2.95	9.0	6.2	2.75	0.75	4.2	3.3
	a	2.0	1.5	14.0	10.0	1.8	1.1	15.0	12.6	4.2	1.2	11.0	9.0
9	b	1.2	1.0	11.0	13.0	0.9	.9	14.0	11.3	1.8	0.3	7.5	6.0
	av.	1.6	1.25	12.5	11.5	1.35	1.0	14.5	11.95	3.0	0.75	9.25	7.5

TABLE 2. ARITHMETIC AVERAGE FOR ALL COUNTS DEPICTED IN TABLE 1 FOR EACH OBSERVER

	Clump Count x 10^5/ml		Leucocytes x 10^5/ml	
	Mod. stain	L. W. stain	Mod. stain	L. W. stain
Obs. A	2.24	1.83	8.20	8.00
Obs. B	3.28	2.67	9.50	8.23
Obs. C	2.98	1.97	5.54	4.68

REFERENCES

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