A COMPARISON OF SIX METHODS OF PREPARING AND USING THE METHYLENE BLUE STAIN FOR BACTERIAL COUNTS BY THE DIRECT MICROSCOPIC METHOD*

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A cooperative study, involving 12 federal, state, local, and private laboratories throughout the country, was conducted to evaluate six methods of preparing and using the methylene blue stain for the direct microscopic count of bacteria in milk. Three of the six methods were found superior, yielding significantly higher bacteria counts, at the same time providing greater ease in counting. These three methods are: Levine and Black's acid-and-water-free stain; North's aniline oil stain; and Anderson's polychrome stain.

In 1948 Levine and Black[1, 2] proposed a new method for preparing and using the methylene blue stain (methylene blue chloride, certified) when making bacterial counts by the direct microscopic method. By comparison with counts made with conventional methods[3] of preparation and use of the stain, the counts by the new procedure were appreciably higher. Also in 1948 Anderson, Moehring, and Gunderson[4] proposed a different method of preparing and using the stain and by similar comparisons demonstrated that the counts by this method were appreciably higher also. Levine and Black[5] reported additional comparisons in 1949. Following collaborative studies on stains in 1951, Olson and Black[6] showed that some of the newer methods of preparing and applying methylene blue stains were superior to certain modifications of the time-honored method. Later Levine[7] presented an explanation of staining phenomena as it applied to the direct microscopic method. Because of these reported failures[1, 2, 4, 5, 6, 7] to get as high counts by staining procedures prescribed in the eighth and ninth editions of *Standard Methods for the Examination of Dairy Products*[3] as were obtained by the more recently proposed methods, the APHA* Committee organized a plan for comparing counts on stained milk and cream films in order to discover the one or more most satisfactory among staining procedures.

STAINS IDENTIFIED

The chief criterion for selecting a satisfactory stain was that it give the highest count with maximal uniformity when used by different operators. Recognizing certain assumptions, the study was organized so as to give at least 95 percent assurance that the best of the 6 stains would show up best if it were at least 5 percent superior (capable of giving 5 percent higher counts) to the next best stain. The study required obtaining counts on replicately prepared films from 25 raw milks, 25 pasteurized milks, 4 raw creams, and 4 pasteurized creams.

The comparison included the following stains:

Stain A A slight modification[3] (23 percent alcoholic base) of the one used in 1911 by Breed[8]

Stain B The Newman-Lampert Type 2 stain[9]

Stain C North's aniline oil stain[10]

Stain D Levine and Black's acid-and-water-free stain[1, 2, 5]

Stain E Borman's stain[11] (experimental, described below)

Stain F Anderson's polychrome stain[4]

The method for preparing the modified Stain A is described in paragraph 2.46, lines 4-7, of the ninth edition of *Standard Methods*. Because of confirmed observations that the carbolated methylene blue stain,[1, 2, 4] also described in the same paragraph, was appreciably inferior to Stain A, the former was omitted from the study. In the absence of documental reference to Borman's stain, directions for its preparation and use follow:

To 970 ml of water, add 30 ml (3.5% aqueous) methylene blue chloride, certified, 8 g Na Cl, and 5 ml (2% aqueous) merthiolate. Store stock supply of prepared stain in cleaned, tightly closed container. Submerge slides with fixed, dried films in xylene for 1 minute. Drain and dry slides, optionally using forced air current. Submerge slides for 2 minutes in solution consisting of 1000 ml ethylene glycol monomethyl ether and 3 ml pyruvic acid, EK No. 498. Drain and dry slides completely, optionally using forced air current. Submerge in staining solution for exactly 1 minute. Remove and wash in gentle stream of tap water, stand on edge and dry, preferably using a forced air current.

For cream films proceed as for milk films, using 2 successive submersions in xylene with drying following each. Apply 2 successive submersions in conditioning solution with drying following each stain for exactly 1 minute.

The description and application of the Modified Polychrome Methylene Blue Stain used by Anderson in the current investigation follows. To prepare chloroform-alcohol, defatting-fixing reagent, completely dissolve 0.2 g gelatin, Eastman Kodak No. 5247, in 20 ml H_2O by heating gently at not over 70°C. Cool to 25°C. Slowly add gelatin solution with intermittent

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TABLE 1.—ASSEMBLED REPORT FOR FILMS ON RAW MILK No. 19.
Each Count Is the Total of Clumps Seen in 60 Fields, 30 for Each of Two Films.

Participant	Microscope factor	Stain A Count	A Rank	B Count	B Rank	C Count	C Rank	D Count	D Rank	E Count	E Rank	F Count	F Rank
1	300,000	11	1½	12	3	14	5	13	4	11	1½	19	6
2	600,000	7	1	13	2½	15	4	416	6	13	2½	18	5
3	600,000	8	1	13	2	25	4	29	5	14	3	30	6
4	300,000	23	1	42	6	28	3	30	4	36	5	25	2
5	300,000	10	1	11	2	26	6	16	4	14	3	18	5
6	600,000	3	1	8	4½	8	4½	10	6	6	2½	6	2½
7 D*	300,000	7	1	24	2½	29	4	42	6	24	2½	30	5
8 C	500,000	7	1	8	2	23	5	11	3	15	4	24	6
9 A	300,000	2	1	4	2	16	5	13	4	19	6	6	3
10 E	300,000	2	1	6	2	13	5	10	4	7	3	15	6
11 F	300,000	11	1	18	2	44	5	36	3	38	4	47	6
Totals for participants, 1-6, non-stain sponsoring													
Counts		62	1	99	3	116	4½	514	6	94	2	116	4½
Ranks		6½	1	20	3	26½	4½	29	6	17½	2	26½	4½
Totals for participants, 7-11, stain sponsors													
Counts		29	1	60	2	125	6	124	5	103	3	122	4
Ranks		5	1	10½	2	24	5	20	4	19½	3	26	6

Counts = Number of bacterial clumps, without adjustment for microscope factor.
*Numbers identify participant; letters identify stain used.

shaking to flask holding 330 ml methyl alcohol, absolute, refined, Mereck, Baker, or Mallinckrodt (other brands tested were unsatisfactory). Add gelatin-methyl alcohol mixture to 650 ml chloroform in liter flask. Filter through rapid filter paper into stock reagent container fitted with tight closure. (Stock solution ordinarily is stable for at least 6 mo.) Replace solutions in current use monthly or after treating 75 slides or at shorter intervals if necessary. Optionally replenish reagent in current use with fresh stock reagent or filter used reagent to remove fat and other accumulations before returning filtrate to *clean* container.

Completely dissolve 1 g certified methylene blue chloride in 500 ml H_2O in liter flask. Add 6 ml (1% aqueous) H_2SO_4 and mix. Slowly add 20 ml (5% aqueous) potassium dichromate and mix. Add 375 ml H_2O and mix. Autoclave mixture at 121°C (15 lbs steam pressure) for 30 min. Cool to about 20°C and filter. Wash precipitate with 900 ml H_2O into graduated liter flask. Add 2 gm Na_2HPO_4, anhydrous. Heat to 95-98°C for 15 min, cool to 20°C, add 50 ml methyl alcohol, absolute, Merck, and make up volume with H_2O. Mix and filter into stock reagent container fitted with tight closure. Replace stains in current use monthly or after treating 125 slides or at shorter intervals if necessary. Optionally replenish staining solution in current use with fresh stock reagent or filter, if precipitates or other accumulations collect, before returning filtrate to *clean* container.

To treat film, submerge slides in defatting-fixing reagent for 2 min. Drain and air dry (without aid of heat or fans) thoroughly for 3 min. Submerge in staining solution for 18-20 sec. Rinse in container of non-flowing, cold tap water for 2-3 sec. Stand slides on end on blotting or bibulous paper, drain and air dry films before examining microscopically.

Submerge cream films in defatting reagent for 2 min. Drain and air-dry films. Repeat above defatting operation *once* before proceeding as directed above for staining milk films.

The identities of (stain) sponsors and of (counting) participants follow in accompanying tabulation:

Lab. No.	*Participants, but Non-stain Sponsors*
1	Nicholas D. Duffett Department of Public Welfare Division of Health St. Louis, Missouri
2	Frost Claiborne Department of Health State of West Virginia Charleston, West Virginia
3	Betty Cunningham Beatrice Foods Company Decatur, Illinois
4	David Levowitz New Jersey Dairy Laboratories New Brunswick, N. J.
5	Harold J. Barnum Department of Health and Charity City and County of Denver Denver, Colorado
6	J. C. McCaffrey Department of Public Health Division of Laboratories Chicago, Illinois

Lab. No.	*Stain**	*Participants and also Stain Sponsors*
7	D	Benjamin S. Levine Milk and Food Sanitation Laboratory U. S. Public Health Service Cincinnati, Ohio
8	C	M. T. Bartram Federal Security Agency Food and Drug Administration Washington, D. C.
9	A	Charles Livak

*For complete identity, see previous description.

		Penn Dairies, Inc. York, Pennsylvania
10	E	Earle K. Borman Bureau of Laboratories State Department of Health Hartford, Conn.
11	F	C. W. Anderson Department of Public Health Rockford, Illinois
12**	B	R. W. Newman Division of Animal Industry Department of Agriculture Sacramento, Calif.

Although not responsible for originating the modified Stain A, Charles Livak, Laboratory Director at Penn Dairies, Inc., York, Pennsylvania, used it in this study and is referred to herein as its sponsor. Films of each milk and cream were prepared by B. Eugene Pellet and Richard Eglinton under the direction of E. K. Borman, in the Laboratories of Connecticut State Department of Health, Hartford.

Procedure

Exclusive of extra slides for replacement of lost or damaged ones, a total of 58 (milks and creams) x 6 (stains) x 12 (participants) x 2 (films in duplicate per slide), or 8,352 films, were prepared for counting. This was accomplished by taking 25 pairs of milks (1 raw, 1 pasteurized) and 4 pairs of creams, making a total of 29 pairs. For *each* pair, 98 slides with 4 films on each (2 raw, 2 pasteurized) were prepared. Of each batch of 98 slides, 14 slides were sent to *each* of the 6 stain sponsoring laboratories for defatting and staining, and 14 slides were retained in reserve at Hartford. After defatting and staining 14 slides for *each* of the 29 pairs, each sponsoring laboratory shipped them in groups as completed to J. C. McCaffrey, Illinois Department of Public Health, Chicago. There coding on the stained slides was completed and the slides from the 6 sponsors were randomly regrouped before shipment to the 12 laboratories for counting. One stained slide from each set of 14 was sent to each of 12 laboratories and 2 were kept in reserve at Chicago.

Sponsoring laboratories were directed to use 4 staining solutions, each prepared from stain (powder) with a different lot identity. Batches of prepared stain could be replaced as needed, but 4 different solutions were maintained. A different solution was used for staining approximately ¼ of each set of 14 slides so as to reduce the possibility of finding that any one stain was either unusually good or unusually poor.

To avoid possible systematic biases resulting from orderly selection of slides, special care at each stage was taken to randomize the assignment of slides to stains, their reassignment to participating laboratories, and the order of counting slides. On each film 15 vertical and 15 horizontal fields were counted by starting about midway at one edge of each film. Fields were selected by passing over the first 0.4 – 0.5 mm. and then counting each successive third field across the film, assuming that diameter of field is 0.206 mm.

A completed report form for each slide counted was sent by the participating laboratories to the writers where records were assembled for statistical analysis. Preparation of slides was begun in June 1951 and counting completed between December 1951 and May 1952. One laboratory failed to submit completed reports.

Statistical Considerations

The original intent to use for reference the counts by Stain A was abandoned when it was found that the counts by different participants on the same sample varied so widely that mean counts partially lost their significance in comparisons. Contributing to irregularities in laboratories, 3 different microscope factors were used (table 1) instead of a fixed factor of 300,000. Still more important, the counts reported were much more erratic than had been anticipated. Initially when erratic results were discovered, the participant was asked to recount the film, but recounts never differed appreciably from original counts. Erratic counts were not confined to any particular milk, stain, or participating laboratory. Unusual counts could not be omitted from the comparison because there was no consistent way to identify them or to distinguish between them and many less erratic ones which were not completely out of line. Ac-

**No counts reported.

TABLE 2.—TOTAL BACTERIAL COUNTS FOR 58 MILKS AND CREAMS, BY PARTICIPANT AND STAIN.
Each Figure Represents a Total Count from 3,480 Fields.

Participant	Microscope Factor	Stain A	B	C	D	E	F	Total
1	300,000	8,461	8,561	10,356	11,111	10,730	9,912	59,131
2	600,000	4,597	5,250	6,180	6,331	5,498	5,999	33,855
3	600,000	6,262	6,863	7,353	7,378	6,705	7,761	42,322
4	300,000	9,888	10,106	12,748	12,439	11,832	12,405	69,418
5	300,000	8,263	9,228	11,127	11,090	10,891	11,688	62,287
6	600,000	3,952	4,041	5,135	4,827	4,606	4,878	27,439
7 D	300,000	8,470	9,789	11,993	14,223	11,314	12,258	68,047
8 C	500,000	6,295	7,066	8,999	8,465	8,613	8,556	47,994
9 A	300,000	5,526	6,417	8,175	8,247	7,213	7,828	43,406
10 E	300,000	8,803	8,820	11,147	11,441	12,355	11,772	64,338
11 F	300,000	9,930	10,630	13,103	12,926	12,548	15,699	74,836
Total for non-sponsoring participants, 1-6		41,423	44,049	52,899	53,176	50,262	52,643	294,452
Total for sponsoring participants, 7-11		39,024	42,722	53,417	55,302	52,043	56,113	298,621

cordingly the approach for statistical analysis was changed so as to circumspectly include the erratic counts.

This change required the simple ranking of counts in order of magnitude (lowest on each sample, 1; next higher, 2; etc.) and substituting in the statistical analysis the ranking numbers for actual counts, (table 1). Thus no more weight was given to an erratically high count than to a count only 1 higher than the next lower count. In case of identical values, the rank was split equally.

The resulting analysis was actually simpler than would otherwise have been required. Fortunately, the difference between counts obtained with certain stains was greater than anticipated, thereby making possible unmistakable distinctions. Table 1 illustrates the application to results on raw milk No. 19. Although by no means typical, this milk shows (1) the completely erratic count of 416 for participant 2 with Stain D, (2) the greater-than-anticipated variation for the remaining counts, and (3) an extreme example of the generally low counts on films treated with Stain A.

In a few cases extremely high counts were obtained where objects were counted which could not be unmistakably identified as bacterial clumps. Unfortunately the participants were not instructed to count only clumps which could be positively identified. It seems inappropriate to attempt herein to explain individual differences in counts possibly attributable to use in laboratories of different microscopic factors, to different ages of films before counting, to possible break-up of clumps, to presence of direct accidental contamination of films, to lack of familiarity with the stain, to staining failures of Stain A, etc.

STATISTICAL ANALYSIS

Totals of counts: Table 2 shows a summary total count for the 58 milks and creams for each participant with each stain. Noteworthily, each of the 11 participants obtained lowest totals with Stain A, and 10 of the 11 obtained next to lowest totals with Stain B. Typically, Stain E gave the third lowest total, being rated third by 7 of the 11 participants. Three participants obtained their highest totals with Stain C, 4 with Stain D, 1 with Stain E, and 3 with Stain F.

TABLE 3.—AVERAGE RANK OF BACTERIAL COUNT FOR 58 MILKS AND CREAMS BY PARTICIPANT AND STAIN.

	Stain					
Participant	A	B	C	D	E	F
1	2.05	2.58	4.08	4.34	3.85	4.10
2	2.12	2.66	4.35	4.34	3.46	4.07
3	2.32	2.99	3.75	4.43	2.96	4.55
4	2.11	2.61	4.22	4.52	3.53	4.02
5	1.82	2.75	4.22	4.38	3.16	4.68
6	2.40	2.92	4.44	3.97	3.28	4.00
7 D	2.06	2.84	3.89	4.78	3.48	3.95
8 C	1.63	3.18	4.60	4.04	3.56	3.98
9 A	1.70	2.54	4.64	4.69	3.45	3.98
10 E	1.83	2.20	4.09	4.38	4.10	4.41
11 F	1.52	2.22	4.03	4.15	3.55	5.53
Average for non-sponsoring participants, 1-6	2.14	2.75	4.18	4.33	3.37	4.24
Average for sponsoring participants, 7-11	1.75	2.60	4.25	4.41	3.63	4.37

Of the 5 reporting sponsoring participants, 4 reported highest totals with the particular stain sponsored, suggesting that familiarity with a stain often leads to higher counts. The exception to this was Stain A, where similarity was insufficient to compensate for its markedly low staining properties.

Total counts obtained with Stains C, D and F agreed closely with each other. If the average total for these 3 stains is assumed to represent 100 percent, counts by Stain A would be 75 percent, Stain B, 80 percent, and Stain E, 95 percent.

Ranks of count: A summary of average rank for the 58 milks and creams for each participant with each stain is shown in table 3. These correspond to averages of the ranks illustrated in table 1. Judging from the ranks, the relations between the stains are much like those indicated in table 2. Each one of the 11 participants obtained the lowest average rank with Stain A, and 10 of the 11 obtained next to the lowest average rank with Stain B. Stain E received the third lowest average rank by 9 participants, being rated second lowest by 1 participant and fourth lowest by its sponsor. Stains C, D and F have ranks very close to each other and among themselves account for all of the highest individual participant average ranks. Three participants obtained their highest average ranks with Stain C, 4 with Stain D, and 4 with Stain F.

Statistical Significance: To the extent that 11 participants could be considered a random sample of laboratories, the results found would be sufficient to establish statistical significance. The general systematic placing of Stain A lowest; Stain B second lowest; and Stain E third lowest is in itself significant. A procedure more sensitive to stain differences would be to consider the 58 milks and creams to be a random sample of all milks and creams.

With this viewpoint, it is necessary only to derive from the data some kind of score or rating value for each of the 6 stains from each of the 58 milks and creams, and then to determine if the average score over the 58 differs significantly from stain to stain. Two ranking scores have been considered and applied separately to sponsoring and non-sponsoring participants. The first score is the rank of each of the 6 stains in the order of the sum of the separate counts the participants obtain for each stain for the particular milk or cream. The second score assigns ranks to the stains for a particular milk or cream on the basis of the sum of the separate ranks each participant obtained for each stain. Both types of scores are illustrated for a single milk in the lower section of table 1. The average of such scores for all 58 milks and creams are in the first section of table 4.

For each of the 4 sets of averages shown in the first section of table 4, the set of differences between the 6 stains is highly significant. It is then proper to

TABLE 4.—AVERAGE SCORE FOR ALL 58 MILKS AND CREAMS, AND FOR RAW AND PASTEURIZED MILKS AND CREAMS SEPARATELY, BY STAIN.

Scores based on	Stain A	B	C	D	E	F
Averages for All 58 Milks and Creams						
Sums of counts						
non-sponsoring participants	1.52	2.28	4.58	4.71	3.27	4.66
sponsoring participants	1.24	2.30	4.41	4.83	3.46	4.77
Sums of ranks						
non-sponsoring participants	1.55	2.33	4.59	4.73	3.23	4.57
sponsoring participants	1.24	2.20	4.52	4.76	3.62	4.66
Averages for 29 Raw Milks and Creams Only						
Sums of counts						
non-sponsoring participants	1.48	2.40	4.64	4.76	3.26	4.47
sponsoring participants	1.24	2.31	4.41	4.55	3.76	4.72
Sums of ranks						
non-sponsoring participants	1.57	2.38	4.60	4.90	3.34	4.21
sponsoring participants	1.21	2.14	4.59	4.45	4.00	4.57
Averages for 29 Pasteurized Milks and Creams Only						
Sums of counts						
non-sponsoring participants	1.55	2.16	4.52	4.66	3.28	4.84
sponsoring participants	1.24	2.29	4.40	5.10	3.16	4.81
Sums of ranks						
non-sponsoring participants	1.53	2.28	4.57	4.57	3.12	4.93
sponsoring participants	1.28	2.26	4.45	5.02	3.24	4.76

select the highest average and assert that it corresponds to the best of the stains. Each of the successively lower averages can then be compared with the highest average until a significant difference is obtained. This provides a demarcation between the possibly best and the presumably inferior stains. In all 4 sets of averages the possibly best stains consisted of C, D and F. In addition, in each case Stain E was significantly superior to Stain B, which in turn was significantly superior to Stain A.

In comparing any 2 stains with each other, the procedure was to total the number of milks and creams among the 58 with a higher score. A significant departure from a 29-29 split (a minor modification was necessary in the case of equal distribution) would indicate a significant difference between the stains.

It was not anticipated that the findings above would be altered appreciably if the sample was considered to consist of 29 pairs of milks and creams rather than 58 milks and creams. Since pairs instead of individual milks and creams were used for each set of slides, treating the sample as 29 pairs may be more appropriate.

The 58 milks and creams may also be separated into 29 raw milks and creams and 29 pasteurized milks and creams, and a similar analysis performed separately on these 2 groups. The average scores for these 2 separate groups are shown in the lower 2 sections of table 4. The results for the 6 stains are substantially the same for both groups. Apparently, then, the stains which have been selected as superior are superior for both raw and pasteurized products.

SUPPLEMENTAL INFORMATION

In addition to bacterial counts on the report forms, participants were required to record their judgment about certain factors on each slide, generally relating to the ease of counting. This supplemental information served two purposes: first, a basis for rejecting a stain, based on film appearance alone, as difficult to work with even though it gave higher counts, and second, a reinforcement of the selection of a stain as superior, if the relative ease of counting supported such a choice. Conceivably, a stain could give high counts by virtue of a tendency to cause bacterial clumps to dissociate into smaller clumps or separate bacteria. If, as is true in the present study, the stains giving high counts were also easy to work with, the presumption would be that the high counts were attributable to such ease. Therefore, the case for dissociation is weakened.

As implied above, the supplemental information furnished results which were very much in line with the bacterial count data. On each of the 4 supplemental factors considered, Stain A was poorest by far, Stain B very poor, and Stain E a little inferior to the remaining Stains C, D and F. The results for Stains C, D and F were all roughly comparable. Table 5 presents a summary tabulation of the data on supplemental information.

Care should be taken in interpreting the results of this supplemental information. If an operator has difficulty in making counts on a particular slide, he is very likely to downgrade the stain used on each of the factors considered instead of only the factors which caused the difficulty. The existence of this halo effect has been recognized in other judgment problems, i.e., taste testing. The halo effect makes more difficult the exact pinpointing of the weaknesses of the item being judged. In the present problem it may exist between the different factors considered in the supplemental information but not between these factors and the bacterial counts.

TABLE 5.—DISTRIBUTION OF RESPONSES TO SUPPLEMENTAL INFORMATION SECTION; TOTALS FOR ALL PARTICIPANTS.

Factor considered		Stain A	B	C	D	E	F
Eyestrain and fatigue	Much	383	179	114	57	42	46
	Average	205	362	249	287	383	299
	Little	41	92	264	288	208	286
	No answer	9	5	11	6	5	7
Stained bacteria and protein contrast	Poor	285	100	23	30	44	27
	Fair	216	216	120	141	174	115
	Good	79	217	242	256	300	294
	Excellent	35	86	237	198	109	185
	No answer	23	19	16	13	11	17
Uniformity of background, absence of "cloud roll" effects	Poor	298	219	66	29	36	39
	Fair	238	240	127	119	190	171
	Good	72	159	205	275	277	315
	Excellent	22	15	213	202	128	104
	No answer	8	5	27	13	7	9
Refocusing of microscope z	Much	357	212	75	69	67	49
	Average	185	281	240	250	306	288
	Little	32	78	258	258	200	234
	No answer	64	67	65	61	65	67

SUMMARY AND CONCLUSIONS

A comparison of bacterial clump counts by the direct microscopic method discloses that Levine and Black's Acid-and-Water-Free Stain, North's Aniline Oil Stain, and Anderson's Polychrome Stain are equally satisfactory and appreciably superior to the stains prescribed in the ninth edition of *Standard Methods for the Examination of Dairy Products.* Borman's experimental stain proved slightly but significantly inferior to the stains identified above. The Newman-Lampert Type 2 Stain was superior to the other two conventional stains described in the ninth edition of Standard Methods, but all three of these stains were distinctively inferior to the three above mentioned appreciably superior stains.

For reasons given above, it was concluded that the superior stains should be recognized as soon as practicable by the American Public Health Association for official use for making bacterial counts in milk and cream by the direct microscopic method.

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STATE REVISES SANITATION RULES FOR MEAT MARKETS

New regulations governing sanitary conditions in Oregon's retail meat markets have been adopted and are now being enforced, announces the state department of agriculture.

They come partly as an outgrowth of the expanded inspection and sanitation program the department got under way a few months ago when it brought Chester B. Liechty into the headquarters to direct work under the meat dealer's law. And they come partly as a result of needs voiced at the recent consumer conference on meats held by Oregon State College.

Under the new regulations, the department will insist that all fresh meats sold from self-service counters or display cabinets be wrapped if the cold box is open.

It is also required that all cut portions of bacon, hams, picnics or other smoked or prepared meats be covered with transparent paper or a glass container. This applies whether the product is cut in half or lesser size.

The department will make an effort to bring about a reasonable cleanliness of aprons and other clothing worn by meat handlers, and will encourage in every way it can a careful check on the health of meat handlers, says M. E. Knickerbocker, chief of the department's division of animal industry.

He says that 3,000 retail meat establishments—those with grade B state licenses—are now operating in Oregon, with 600 of these in the city of Portland. In Portland, the sanitation program is handled by the city inspection service; otherwise, the state department of agriculture is covering the field as thoroughly as it can with its limited personnel.