

A COMPARISON OF DETERGENT TESTS FOR BUTTERFAT IN MILK WITH OFFICIAL METHODS

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SUMMARY

A collaborative comparison of the Schain and Dairy Products Section (DPS) tests with the official methods, the Babcock and Mojonnier tests, was performed by a group of experienced research workers. The volumetric tests were performed in duplicate by eight men; the Mojonnier test in quadruplicate by one man. Twelve cows, comprising four breeds with three cows of each breed, were used. Morning and evening milk was analyzed one day of the first, third, and fifth week.

The Babcock test has a closer correlation with the Mojonnier test than does either the Schain or the DPS test. The Schain test correlation is .9889, the DPS .9886, and the Babcock .9963, whereas a perfect correlation is 1.000.

The three volumetric tests averaged slightly higher than the Mojonnier: the Schain test 0.11% butterfat, the DPS 0.06%, and the Babcock 0.09%.

The standard deviation between testers was 0.15%, 0.18%, and 0.10% for the Schain, DPS, and Babcock tests, respectively. Therefore, the tester is a significant variable in these tests.

The butterfat content of the milk from these individual cows varied considerably, up and down, over the 5-wk. period. Differences of 1% fat were found in five out of the 12 cows, using the average of the morning and evening samples. This variation is far greater than that attributable to the analytical procedures or to the individual testers.

The dairy industry in the United States has relied on gravimetric analysis (Roese-Gottlieb or Mojonnier) and volumetric analysis (Babcock) for butterfat in milk for many years. Through continued research and collaborative study, these methods have been developed into highly accurate standard procedures. They are official methods of the Association of Official Agricultural Chemists and are incorporated in the laws of many states.

Alternative fat tests have been studied previously (2, 8, 10, 11, 17, 18), but the development of tests that employ synthetic detergents was stimulated by the work of Schain (15, 16), published in 1949 and 1950. Later work by Sager

Received for publication October 1, 1957.

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and coworkers (12, 13, 14) resulted in the Dairy Products Section (DPS) detergent test. Both of these tests avoid the use of strong sulfuric acid, a definite liability of the Babcock test. The DPS test requires more equipment and is more complicated to perform than the Schain test, but avoids the use of the empirical nomogram required to calculate the results of the latter test.

A survey of the published results of these detergent tests (3-7, 12-16)² revealed a need for a careful comparison of them with the official methods. The experiment reported herein was, therefore, designed and carried out cooperatively by the EURDD, Animal Husbandry Research Branch, District of Columbia Health Department, and the Dairy Husbandry Department of the University of Maryland. The contribution of the University of Maryland must be especially acknowledged for, besides providing the analytical work of the four testers, the samples of milk, and the calibration of glassware, it performed other essential parts of the experiment. The design of the experiment was developed by the Biometrical Services, ARS, in cooperation with AMS. The Biometrical Services analyzed the data.

EXPERIMENTAL DESIGN

The experiment was designed to compare the accuracy of the Schain, the Dairy Products Section (DPS), and the Babcock tests with the Mojonnier (Roese-Gottlieb) ether extraction method for determination of butterfat; and to determine the factors affecting the precision of these methods in the hands of competent analysts.

In order that an accurate, statistical comparison of these methods would be made, it was decided to test 12 cows, comprising four breeds, with three cows of each breed. The three within each breed, i.e., three Guernsey, three Holstein, three Ayrshire, and three Jersey, were chosen as far as possible to be high, medium, and low fat producers of the respective breed. Morning and evening milk of each cow being tested was analyzed for butterfat content.

As required by the design, nine testers were assigned to perform the analyses. One tester analyzed every sample for butterfat content by means of the Mojonnier method. These determinations were performed in quadruplicate by Tester A. The results of these tests were considered to be the actual per cent of butterfat in the sample being tested. All results of the other methods of butterfat determination studied were compared with these Mojonnier results.

The eight other testers, B, C, D, E, F, G, H, and I, analyzed every sample by the methods of Schain, Dairy Products Section, and Babcock butterfat tests, except for one set not analyzed because of illness and the unavoidable loss of a few samples. The tests were run in duplicate; the results were recorded as individual readings for each test performed, rather than as averages of the duplicates.

The experiment was conducted over a 6-wk. period consisting of 3 wk. of

² The valuable study of E. O. Herreid and collaborators (*J. Assoc. Offic. Agricultural Chemists*, 40: 499. 1957.) was reported after the completion of the experimental work reported herein. The results of the two studies are in general agreement but, because of their differing purposes, can not be closely correlated.

testing and 3 wk. of assembling the data. The 1st, 3rd, and 5th wk. were the actual testing weeks. The experimental program for testing was as follows: On the first day of the first testing week (Tuesday, October 3, 1955), the Guernsey breed was tested, i. e., the morning (*m*) and evening (*e*) milks of the high, medium, and low test cows of the Guernsey breed were analyzed by Tester A, who used the Mojonnier method of testing (see Methods of Analysis). The other eight testers, B, C, D, E, F, G, H, and I, tested aliquots by the Schain, DPS, and Babcock tests. On the second day of the first testing week, the same procedure was carried out with the Holstein breed; on the third day with the Ayrshire breed, and on the fourth day of testing the Jersey breed was tested in a similar manner. In the 2nd and 3rd wk. of testing the same plan was followed.

PROCEDURE FOLLOWED IN CARRYING OUT THE STUDY

A. *Equipment and materials.* The equipment was carefully selected and checked, since emphasis was placed on accuracy. The glassware was standardized by the Dairy Inspection Service of the State of Maryland. All glassware which did not meet the requirements of the State was discarded. It was also ascertained that the speed of weighing might also be a factor in the accuracy of the tests to be performed. Therefore, Mojonnier dishes were purchased which were tared to within 3 mg. of each other. Thus, only a limited amount of weight-adjusting was necessary for each aliquot of each sample, which was accomplished on a rapid indicating balance.

The chemicals and reagents used in the testing study were as follows: The sulfuric acid used in making the fat determinations by the Babcock method had a specific gravity of 1.84; therefore, each tester had to standardize his own acid to a specific gravity of 1.82-1.83, which is the specific gravity of the sulfuric acid designated Babcock acid. The Schain reagent was obtained in one lot of eight individual gallons from Dr. Philip Schain of Staten Island, N. Y. The sodium tetraphosphate (Quadrafos)³ was obtained from the Rumford Chemical Company, Rumford, Rhode Island, and the methyl alcohol (99.6%) from Mojonnier Brothers,³ Chicago, Illinois. The alcohol was standardized by the individual testers to 50%. Triton X-100³ and Quadrafos were obtained in a large container and portions were distributed to the testers, because these chemicals were not available in small lots. All other chemicals were purchased in individual containers. Each tester was supplied with the necessary equipment (except the centrifuge and water baths) and chemicals to carry out the tests. This was done for the purpose of keeping conditions as similar as possible, to minimize sources of error.

B. *Methods of analysis.* A working handbook was prepared for each tester, that contained the method and step-by-step procedure for each test. The directions for the Babcock test were taken directly from official methods (1). The Mojonnier test was run according to directions published by the company (9),

³The mention of commercial products and manufacturers is for the purpose of identification and does not constitute endorsement by the USDA.

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except that three extractions were performed and the final extraction of the fat was made using only petroleum ether and ethyl ether. This modification made the procedure essentially equivalent to the Roesse-Gottlieb method, as described on page 233 of the AOAC methods (1).

Testers were instructed to read their results as accurately as possible to the second decimal place, for the Babcock and for the two other volumetric tests. Directions for the DPS test were taken from the paper of Sager *et al.* (14). The Schain test was performed according to directions described in photostatic copies of the method, as revised February 1955, with minor inked-in changes made by Dr. Schain just prior to the initiation of this study.

C. Manipulation of samples. The evening milk samples were obtained from the University of Maryland dairy barns by one of the cooperating testers. The milk was obtained by machine-milking and placed in individual milk cans. It was immediately taken to the dairy manufacturing building, agitated thoroughly by pouring from one container to another three times, then 9-pt. bottles of each sample were poured. The pint aliquots were stored overnight at 40° F.

The morning milk was handled in an identical fashion, except that it was not stored but was delivered to the cooperating testers as soon as possible, usually by 8 A.M. Both sets of samples were warmed to from 105 to 110° F., mixed thoroughly in the bottles, and then tempered to the standard temperatures of analysis before pipetting the samples.

RESULTS

The data before analysis are not presented, because of their great number. Photostats of the tabulated results can be obtained by writing to the senior author.

Averages of the volumetric analyses by the eight testers, for each sample plotted against the Mojonnier data, are shown (Figure 1). Certain features of the data, such as the fact that most of the samples had fat contents between 3 and 6%, and that the standard deviation for the DPS and Schain tests is greater than for the Babcock test, are apparent by inspection of this figure.

The experimental design called for the collection and analysis of 72 samples of milk; two from each of the 12 cows in each of the three testing weeks. However, three samples were not analyzed, because they were either lost or churned before reaching the tester. In addition, some of the testers were unable to obtain determinations on several of the remaining 69 samples. The loss of determinations, as reported by the eight testers (B through I), with the Schain and DPS tests, was almost five times and three times as great, respectively, as with the Babcock test. Loss of determinations with the Babcock test was negligible.

The four determinations with the Mojonnier (by Tester A) on each of the 69 samples were averaged and compared with the averages of the duplicates for each of the other tests, separately, for each of the eight testers. In addition, the 69 samples were divided into a high and low group, based on the fat content as determined by the Mojonnier method. The high group consisted of 35 samples, with the average Mojonnier reading of the lowest sample being 4.234% fat and

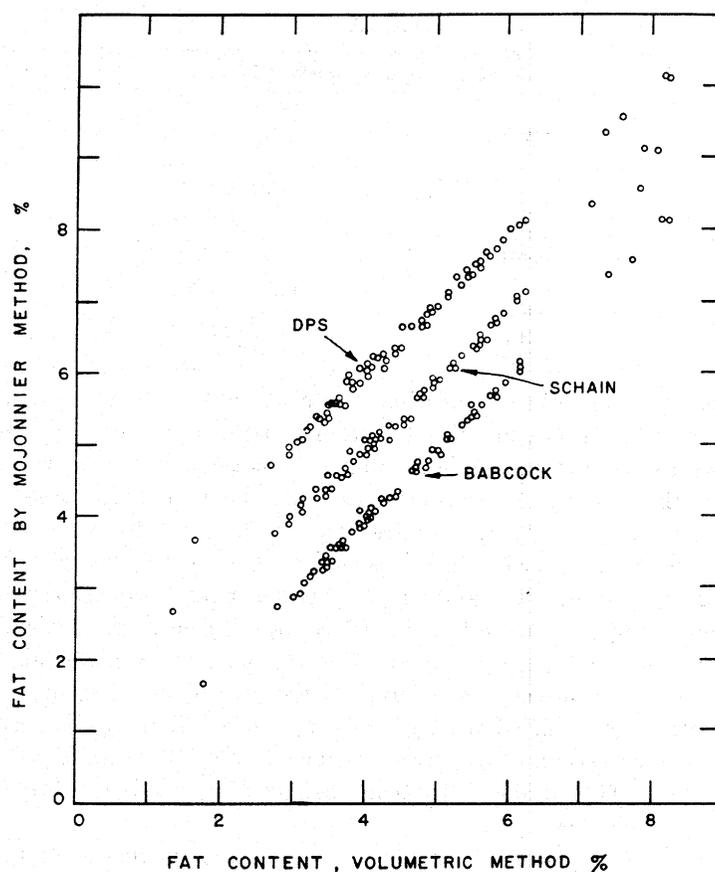


FIG. 1. Average of the volumetric analyses for the eight testers *versus* average of Mojonnier run in quadruplicate by one tester. The ordinate is raised by 1% for the Schain test and by 2% for the DPS test, that is, all points are arbitrarily raised by these amounts to separate the data for the three sets of results.

of the highest sample, 8.118% fat. The low group consisted of the 34 other samples, with the low sample containing 1.658% fat and the high sample, 4.218% fat. The reason for placing the samples into two groups was to determine if the linear regression of the Mojonnier reading on the Schain, DPS, or Babcock tests differed at different fat levels, which would be good evidence of the existence of curvilinearity. A summary of the linear regression and simple product-moment correlation coefficients is given (Table 1).

The difference between the regression in the low group and regression in the high group is 0.175, 0.046, and 0.022 for the Schain, DPS, and Babcock tests, respectively. These results suggest that the true relation between the Mojonnier and Schain tests can best be described with a curved, rather than a straight, line. The relation between the Mojonnier and DPS tests appears to be only slightly curvilinear; whereas, the Mojonnier and Babcock tests are essentially linear in

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TABLE 1
Intratester correlations and regressions of the Mojonnier Test with the Schain, DPS, and Babcock Tests

Groups based on fat content	Schain	DPS	Babcock
		Regressions	
Low	0.851	0.916	1.011
High	1.026	0.962	0.989
All samples	0.964	0.961	0.991
		Correlations	
Low	0.9647	0.9533	0.9815
High	0.9798	0.9774	0.9936
All samples	0.9889	0.9886	0.9963

their relation to each other. Additional samples are needed, especially in the upper and lower fat levels, in order to establish these relations accurately.

It should also be noted (Table 1) that the Babcock test is more closely correlated with the Mojonnier reading than with the two other tests. Although these differences are not great, they are responsible for differences in the standard errors of estimate, which are significant statistically (as can be seen in Table 2). The linear regression and simple product-moment correlation coefficients of the Mojonnier test with the Schain, DPS, and Babcock tests were calculated separately for each of the eight testers. These did not vary materially from one tester to another. However, the average difference of the Mojonnier test and each of the other tests varied widely between testers. These differences and the standard errors of estimate are given (Table 2) for all samples and for each tester separately. The standard deviation between testers is given at the bottom of the table for each test.

Only one tester (C) had a larger standard error of estimate for the Babcock test than for either of the two detergent tests. The standard errors of estimate for all 69 samples on an intratester basis were 0.17, 0.18, and 0.10 for the Schain, DPS, and Babcock tests, respectively.

The variability between testers within samples, as measured by the standard deviations (bottom of Table 2), originates from three possible sources. These are (1) differences that would exist between determinations (using the same test) on the same sample by the same tester, (2) real differences between testers, and (3) the interaction of samples and testers. The importance of these sources of variation in the bias observed between the Mojonnier test and each of the three other tests is more critically evaluated in subsequent analyses.

Duplicate determinations. The general procedure followed by most testers in the conduct of these tests was to repeat the determinations, when the first set of duplicate determinations failed to agree within the limit set in the instructions (0.10% fat).⁴ The discarded determinations were not reported by the

⁴ Although the directions for the Babcock test and the Schain test call for repetition of the test, where duplicates vary by more than 0.10% fat, and where those for the DPS test do also by inference, inspection of the raw data shows that this was not always done. The analysis of variability between duplicates is affected by these facts, but the validity of the major correlations is not affected significantly thereby.

TABLE 2
Average differences from the Mojonnier Test, standard errors of estimate, and standard deviations between testers for the Schain, DPS, and Babcock Tests

		Schain	Test	
			DPS	Babcock
Tester B	D ^a	0.14	0.18	0.20
	SE of E ^b	0.12	0.09	0.07
Tester C	D	0.13	0.10	0.06
	SE of E	0.15	0.14	0.16
Tester D	D	0.08	0.14	0.16
	SE of E	0.13	0.10	0.07
Tester E	D	0.12	0.04	0.08
	SE of E	0.19	0.27	0.11
Tester F	D	0.08	-0.01	0.13
	SE of E	0.15	0.23	0.12
Tester G	D	0.07	-0.03	0.11
	SE of E	0.20	0.17	0.06
Tester H	D	0.15	0.09	0.03
	SE of E	0.13	0.19	0.08
Tester I	D	0.12	-0.05	-0.07
	SE of E	0.21	0.14	0.12
All:	D	0.11	0.06	0.09
	SE of E	0.17	0.18	0.10
	SD ^c	0.15	0.19	0.12

^{a, b, c} D refers to differences from the Mojonnier, SE of E to standard errors of estimate, and SD to standard deviations between testers.

tester. The standard deviation between duplicate determinations was calculated for each tester and for the Schain, DPS, and Babcock tests separately. These standard deviations are shown (Table 3), along with the number of samples that were reported with duplicate determinations in each case.

The variability between duplicate determinations varied between testers. On the average, the greatest difference occurred between duplicate determinations that were reported for the DPS test (standard deviation =0.073), intermediate for the Schain test (standard deviation =0.052), and smallest for the Babcock test (standard deviation =0.046).

Variability among daily means. Because of missing values in some cases, it was necessary to average over the morning and evening samples from the same cow to simplify the analyses of variance. Data collected by the last tester (I) were omitted from these analyses, because he made no determinations on 12

TABLE 3
Standard deviations between duplicate determinations and number of samples in each case

	Standard deviations			No. samples		
	Schain	Test DPS	Babcock	Schain	Test DPS	Babcock
Tester B	0.035	0.044	0.027	67	69	69
Tester C	0.035	0.053	0.042	67	68	68
Tester D	0.050	0.040	0.025	65	68	68
Tester E	0.062	0.134	0.032	67	68	68
Tester F	0.044	0.041	0.090	57	57	68
Tester G	0.033	0.036	0.034	61	67	68
Tester H	0.077	0.106	0.024	67	68	68
Tester I	0.062	0.060	0.055	58	58	58
Intratester	0.052	0.073	0.046	509	523	535

samples. Both a morning and an evening sample were collected and tested for each cow, except in four instances, where one of these two samples either was not collected or had no readings by most testers. The table of daily means, set up from the original data, was a tester \times test \times cow \times week table. The corresponding daily averages of the Mojonnier test were then subtracted from each of these values. This gave a total of 756 mean differences (biases) from the Mojonnier. These biases were analyzed by the analysis of variance for each breed and for each test separately. The mean squares of the analyses of variance for each test are given (Table 4) and the separate analyses of variance from each of the four breeds are shown (Table 5).

It should be noted (Table 4) that the error variance ($M \times W \times C : B$) is three times and five times larger, for the Schain test and DPS test, respectively,

TABLE 4
Analysis of variance of biases for each test, mean squares only^a

	d.f.	Test		
		Schain	DPS	Babeock
Total	251	0.0368	0.0393	0.0172
Breeds	3	0.0452	0.1760	0.0174
Men (M)	6	0.0376	0.2019	0.1352 ^b
Weeks (W)	2	0.1621	0.0356	0.0540
B \times M	18	0.0204	0.0392	0.0074
B \times W	6	0.1309	0.0232	0.0621
M \times W	12	0.0559 ^b	0.0798 ^b	0.0246 ^b
B \times M \times W	36	0.0246 ^b	0.0450 ^b	0.0078 ^b
Cows (C:B)	8	0.2840	0.1303	0.1042
M \times C:B	48	0.0085	0.0147	0.0024
W \times C:B	16	0.1382 ^b	0.0851 ^b	0.0630 ^b
M \times W \times C:B	96	0.0098	0.0160	0.0031

^a Appropriate error terms were determined by assuming that all effects except breeds were random.

^b Significant at the 0.01 level.

TABLE 5
Analyses of variance of biases for each breed, mean squares only^a

	d.f.	Breed			
		Guernseys	Holsteins	Ayrshires	Jerseys
Total	188	0.0646	0.0323	0.0118	0.0147
Men (M)	6	0.0604	0.0467	0.0396	0.0809
Tests (T)	2	0.0681	0.1367	0.0349	0.0050
Weeks (W)	2	0.5938	0.0082	0.0505	0.0386
Cows (C)	2	1.5131	0.1107	0.0682	0.0281
M \times T	12	0.0774 ^b	0.0631	0.0077	0.0260
M \times W	12	0.0605 ^c	0.0363 ^b	0.0171	0.0308 ^c
M \times C	12	0.0137	0.0097	0.0040	0.0048
T \times W	4	0.0474	0.0076	0.0211	0.0284
T \times C	4	0.0579	0.0880 ^b	0.0077	0.0232
W \times C	4	0.9208 ^c	0.0343 ^b	0.0848 ^c	0.0139
M \times T \times W	24	0.0292 ^c	0.0493 ^b	0.0142 ^c	0.0301 ^c
M \times T \times C	24	0.0070	0.0218	0.0040	0.0025
M \times W \times C	24	0.0093	0.0122	0.0080 ^b	0.0053 ^b
T \times W \times C	8	0.0190 ^c	0.0144	0.0048	0.0077 ^b
M \times T \times W \times C	48	0.0054	0.0279	0.0043	0.0028

^a Appropriate error terms were determined by assuming that all effects except tests were random.

^b Significant at the 0.05 level.

^c Significant at the 0.01 level.

than is the error variance for the Babcock test. These differences in error variances are significant statistically, and clearly show that sources of variation other than those considered in this study were considerably more important in the Schain and DPS test than in the Babcock test. Perhaps this can be explained by the fact that the conditions and manipulations of the Babcock test have been more highly standardized, through long study and use, than have those of the newer tests.

The week \times cow interaction within breeds ($W \times C:B$) is highly significant for all three tests (Table 4). This means that the bias in estimating the average fat content of two samples (in most cases—morning and evening) on the same cow varied from week to week, even when the same test was used. The week \times cow interaction, when averaged over all tests, but separately for each breed (Table 5), is significant for the Holstein data and highly significant for the Guernsey and Ayrshire data. Changes in the average bias from week to week for data from the same cow are shown (Table 6). This interaction is more noticeable in the Guernsey breed because of one low sample collected on Cow No. 1 the first week, which was greatly underestimated on the average by the Schain test.

Analyses of variance similar to those given in Tables 4 and 5 were calculated for the daily means without regard to the Mojonnier readings. The week \times cow interaction was highly significant in all of these analyses. Therefore, because the fat content of samples from these cows varied differentially by weeks and because an association was found between the amount of bias and the fat content

TABLE 6
Subclass means of biases

Breed	Cow	Weeks			Schain	Test		Means	
		1	2	3		DPS	Babcock		
Guernsey	1	-0.500	0.061	0.104	-0.141	-0.117	-0.077	-0.112	
	2	0.209	0.186	0.138	0.267	0.114	0.152	0.178	
	3	0.151	0.140	0.096	0.144	0.086	0.157	0.129	
			-0.047	0.129	0.113	0.090	0.028	0.077	0.065
	1	0.017	0.087	-0.010	-0.015	-0.009	0.118	0.131	
	2	0.029	0.033	0.024	0.114	-0.074	0.047	0.029	
Holstein	3	0.087	0.080	0.141	0.102	0.091	0.115	0.103	
		0.045	0.067	0.052	0.067	0.003	0.093	0.054	
	1	0.095	-0.005	0.061	0.118	0.051	0.082	0.084	
Ayrshire	2	0.118	0.088	0.061	0.098	0.071	0.099	0.089	
	3	0.190	0.151	0.088	0.178	0.135	0.116	0.143	
		0.134	0.078	0.104	0.131	0.085	0.099	0.105	
Jersey	1	0.065	0.155	0.115	0.097	0.123	0.115	0.112	
	2	0.120	0.144	0.140	0.165	0.117	0.122	0.135	
	3	0.094	0.123	0.060	0.046	0.115	0.116	0.092	
Means		0.093	0.141	0.105	0.103	0.118	0.118	0.113	
		0.056	0.104	0.093	0.098	0.059	0.097	0.084	
					Tester				
Schain		B	C	D	E	F	G	H	
DPS		0.130	0.108	0.062	0.107	0.065	0.070	0.141	
Babcock		0.164	0.085	0.114	0.018	-0.017	-0.044	0.091	
		0.191	0.047	0.150	0.065	0.114	0.097	0.013	
Week 1		0.098	0.010	0.090	0.052	0.045	0.047	0.052	
Week 2		0.193	0.098	0.098	0.100	0.025	0.088	0.124	
Week 3		0.194	0.133	0.139	0.038	0.092	-0.112	0.069	
Means		0.162	0.080	0.109	0.063	0.054	0.041	0.082	

of the sample in these data, it seems safe to conclude that a considerable amount of the week \times cow interactions would be eliminated, if adjustment were made for the level of fat.

The breed \times men \times week (B \times M \times W) interaction is highly significant for all three tests (Table 4). This means that the average weekly changes in the biases from one breed to another were not evaluated in the same manner by all testers. The Babcock test was considerably less variable from tester to tester, in this respect, than were the two other tests.

The highly significant men \times week (M \times W) interaction for each of the three tests (Table 4) indicates that these tests were not conducted in the same manner by each tester each week. Changes that took place in the test procedures, reagents, etc., were not the same for all testers. The men \times week (M \times W) interaction, when averaged over all tests for each breed separately (Table 5), is significant for the Holstein data and highly significant for the Guernsey and Jersey data.

Experience in collaborative studies of this type has shown repeatedly that analytical tests are more sensitive to subjective effects than it is commonly believed. The significance of the M \times W interaction indicates clearly that the individual operator is from day to day a variable in the performance of these tests, regardless of extensive efforts to standardize procedures, reagents, equipment, etc., between testers.

The greatest differences between testers occurred with the DPS test. However, these differences varied so much from week to week with the DPS test that the average differences between testers are not quite significant at the 0.05 level, when the effects of weeks are considered as random. On the other hand, the average differences between testers are highly significant in the case of the Babcock test, because of less variation in these differences from week to week.

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