Repeatability and Standardization in Cattle Blood Typing

Abstract. Thirteen laboratories engaged in cattle blood typing have participated in tests to compare their blood-typing reagents. Over 75,000 individual tests of cattle erythrocytes were made. When the laboratories tested duplicate samples without knowing they were duplicates, the results were in agreement 99 percent of the time. Tests on samples from the same 199 animals with 56 different reagents from two or more of the laboratories showed agreement 89 percent of the time. The agreement was unexpectedly good, since the primary purpose of the tests was to allow comparison of independently developed reagents and since the sources of error were numerous.

The last two decades have witnessed great progress in the study of blood groups in cattle (see *1* for a review). New discoveries intensified the problems of standardization and nomenclature for cattle blood-group workers around the world. In March 1956, a cooperative program of comparative testing was initiated by the Dairy Cattle Research Branch of the U.S. Department of Agriculture to aid workers in this field. Blood samples from 40 cattle were sent to the participating laboratories for concurrent testing. Thus each laboratory had the opportunity to compare its findings with those of others testing blood from the same animals. Six trials have been completed, and over 75,000 individual tests on the red blood cells of cattle of various breeds were made. Laboratories (*2*) in Canada, Denmark, Finland, Holland, Norway, South Africa, Sweden, the United States (California, Ohio, Wisconsin, and Wyoming), and West Germany (Göttingen and Munich) participated. Laboratories in Belgium and France will join the program soon.

Primarily, the program facilitates comparison of blood-typing reagents and genetic studies. It has also provided data on the repeatability of cattle blood-typing tests. This report is a summary of the results of the analysis of repeatability. A more detailed account will be published elsewhere (*3*).

Two aspects of repeatability were examined: (i) the agreement within laboratories when they unknowingly tested samples of blood from the same animal twice; and (ii) the agreement among laboratories when they tested the same cells with the same reagents (reagents produced independently and presumed to detect the same antigenic factors). Some samples were repeated within trials and others between trials. Samples from 41 animals were repeated during the course of the six trials. There was an opportunity for agreement each time a laboratory tested repeat samples with the same reagent. Table 1 summarizes the results of the repeat sample tests with laboratories designated by letter codes. The high repeatability of 99 percent is striking. It is obvious, however, that laboratory G experienced a great deal of difficulty, and over-all means are shown with and without the inclusion of that laboratory's results. To estimate agreement among laboratories, reports of reactions in the blood-typing tests were studied within reagents and trials. For example, in a particular trial, if the participants used a reagent which was supposed to detect the same blood factor, then the results with that reagent were compared to determine the number of samples of the 40 tested on which they agreed. The cells either had the blood factor in question (+ reaction) or did not (− reaction). An agreement was recorded when all laboratories testing a particular sample reported the same result. Results were compiled for 56 different reagents which had been used in one or more trials by some or all of the laboratories.

The over-all percentage of agreement among laboratories was 82.1. When the results of laboratory G were excluded, this figure became 89.0 percent, indicating that G was responsible for much of the disagreement. The analysis was carried further and results were calculated without including those from whatever laboratory caused the most disagreement in each trial-reagent group. The term "trial-reagent group" refers to the results reported by all the laboratories in which a certain reagent was used in a particular trial. With this procedure the over-all mean was 94.6 percent agreement. These results indicate that one laboratory was usually responsible for most of the disagreement in each group. This is not unexpected since the purpose of the trials was to facilitate comparison of independently developed reagents.

These results are a tribute to the accomplishments of the workers in the relatively new field of cattle blood grouping. The high degree of repeatability is especially noteworthy when the numerous sources of error are considered. The program requires a great deal of transcription of results, and some transcription errors have occurred. Although some of these were discovered by the laboratories after reports were submitted, no corrections for them have been made. Occasionally the blood arrived at a laboratory in poor condition due to delay en route. This was particularly true in two instances, but all the results were included since there was no objective way to pick and choose among them. There is no doubt, however, that the tests were not as accurate as they would have been if some of the samples had not deteriorated. Also included are the technical errors inherent in any complex test, such as the hemolytic test, which involves thousands of tubes to which red blood cells, reagents, and complement are added a

Table 1. Summary of results of tests on repeat samples* by laboratory.

Laboratory	Between trial repeats†				Within trial repeats‡				All repeats
	No. of animals	No. of possible agreements§	Actual No. of agreements	Percent of agreements	No. of animals	No. of possible agreements	Actual No. of agreements	Percent of agreements	Percent agreement (weighted mean)‖
A	26	1398	1388	99.3	15	768	768	100.0	99.5
B	0	0	0		3	54	52	96.3	
C	7	168	167	99.4	6	156	156	100.0	99.7
D	13	480	473	98.5	12	486	484	99.6	99.1
E	13	494	483	97.8	12	468	468	100.0	98.9
F	13	538	522	97.0	12	528	526	99.6	98.3
G	11	273	213	78.0	9	249	219	88.0	82.8
H	5	296	292	98.6	9	549	549	100.0	99.5
I	5	181	168	92.8	9	405	403	99.5	97.4
J	0	0	0		3	108	107	99.1	
K	0	0	0		3	111	111	100.0	
Unweighted means¶									
Over-all				95.2				98.4	96.9
Without G				97.6				99.4	98.9
Weighted means ‖									
Over-all				96.7				99.0	97.9
Without G				98.2				99.8	99.0

* Repeats: duplicate samples sent to the laboratories without their knowledge. †Samples from the same animal included in two different trials. ‡ Samples from the same animal included twice in the same trial. § Opportunity for agreement occurred whenever a sample was tested twice with the same reagent. (Reagents considered by a laboratory to be experimental were not included.) ‖ Over-all number of agreements as a percentage of over-all number of possible agreements. ¶ Mean of laboratory means.

drop at a time. Still another source of error—and the main reason why these comparison tests are so useful to any one of the blood-group workers—is the limited choice of animals and especially breeds, which is available to most of the workers for production and standardization of reagents.

These results indicate the accuracy with which blood types are regularly determined in cattle when the tests are made by experienced persons. They also indicate that these trials are helpful in solving the problems of standardization. Comparison of new reagents, developed independently in different laboratories, often indicates that some are detecting the same antigenic factor and leads to an agreement on nomenclature. The trials will be continued on an annual basis.

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References and Notes

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2. Thanks are due to the following blood-group workers and their associates who have participated in this program and contributed to its success: J. Bouw, Holland; M. Braend, Norway; R. J. Humble, Canada; E. J. Lazear, Ohio; Miss C. Lindstrom, Finland; D. R. Osterhoff, South Africa; J. Moustgaard and A. Neimann-Sørensen, Denmark; J. Rendel, Sweden; A. Meyn and D. Schmid, Munich; W. H. Stone and M. R. Irwin, Wisconsin; C. Stormont and W. J. Miller, California; C. P. Stroble, Wyoming; E. Mitscherlich and A. Tolle, Göttingen, Germany.
3. C. A. Kiddy and N. W. Hooven, Jr., *U.S. Dept. Agr. ARS*, in press.

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Some Characteristics of a Thermophilic Blue-Green Alga

Abstract. An alga identified as *Synechococcus lividus* has an exponential growth rate of nine doublings per day at 52°C with illumination of 1500 foot-candles. It uses nitrate or urea as a nitrogen source and does not use acetate or glucose. It seems a promising organism for atmospheric regeneration in sealed cabins.

Photosynthesis by algae is one of the most promising methods for atmospheric regeneration in sealed space cabins. In order for such a system to be practical, the algae used must be capable of achieving high growth and photosynthetic rates. We wish to present some data on a thermophilic blue-green alga that appears to have value in this respect.

The organism was originally obtained by the USAF School of Aviation Medicine in a collection of mixed specimens from the hot springs in Yellowstone National Park. It was received in our laboratory in a mixed culture of algae and bacteria and was isolated in unialgal culture by serial transfer in liquid cultures maintained at a temperature between 50° and 55°C. The cells are about 1.4 μ in diameter and 4 to 9 μ long, the most common length being about 6 μ. They are straight or slightly curved. Some occur in pairs joined at the ends. Polar granules are occasionally observed. From the description given by Copeland (see *1*) this species has been tentatively identified as *Synechococcus lividus*.

A nitrate medium described by Gafford and Craft (*2*) gives good growth and was used for all experiments reported here. Best growth occurs when the *p*H of the medium is adjusted to about 7.5. We have recently found that a urea medium (*3*) recommended for the culture of the thermophilic strain of *Chlorella pyrenoidosa* will also provide maximum growth at *p*H 7. There is no growth in the dark when acetate or glucose is provided as the carbon source.

Growth was measured by determining the optical density of the suspension at 500 mμ with a Beckmann model DU spectrophotometer, using 1-cm cells. Agreement between optical density and packed cell volume was best when the suspension was diluted to keep the measured optical density below 0.25. Thus the measurements were all made in much the same density range. The measured density was multiplied by the dilution factor to express the cell concentration of the culture.

Cultures of 100 ml were grown in test tubes suspended in thermostatted water baths. The bath containers were glass jars 12 in. in diameter, each supported over a grid of ten 15-watt fluorescent lamps. The bottoms of the tubes were about 12 cm above the lamps. Carbon dioxide was provided by bubbling 2 percent carbon dioxide through small polyethylene tubes. We have estimated the effective light intensity, by measurements with a Weston model 614 footcandle meter, to be about 400 ft-ca. Other cultures were grown in the same tubes at higher light intensity. These tubes were placed in a narrow water bath sandwiched between two vertical panels, each of which held eight 107-watt Powergroove fluorescent lamps. Here the effective light intensity is about 1500 ft-ca.

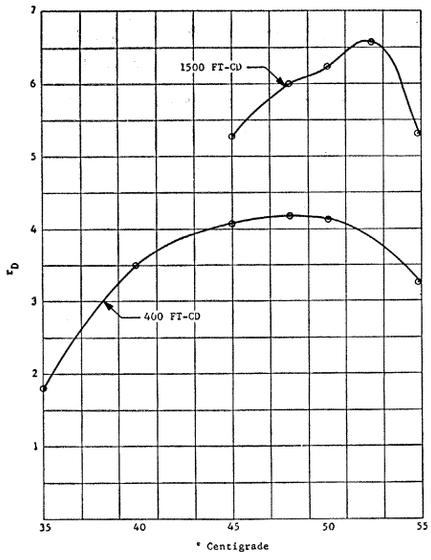

Fig. 1. Growth rate of *S. lividus* in doublings per day (r_D) with respect to temperature at 400 and 1500 ft-ca illumination.

Figure 1 shows the growth rate at various temperatures for both light intensities. The general shape of the curves is rather similar to those shown for *Chlorella pyrenoidosa* TX 71105 (*4*). With the higher intensity light the cells did not tolerate temperatures below 40°C as well as with less light. The optimum temperature is higher with more light. Growth occurs even at 60°C, but at a lower rate.

The curves of Fig. 1 were determined by measuring growth over periods of 21 to 23 hours. Since it appeared that the cultures were light-limited at the end of that time, we measured density at shorter intervals. Exponential growth rates determined in this manner fell in the range of 6 to 7 doublings per day at 400 ft-ca and 8 to 9 doublings per day at 1500 ft-ca for periods of 7 hours or more. The shift to linear growth occurred after 12 to 14 hours. At this time the calculated cell density is 0.4 and 1.8 ml of cells per liter at low and high intensity light. Thereafter growth continued at about 0.09 and 0.3 ml of cells per liter per hour. As expected, the linear growth rates are in approximately the same ratio as the light intensities.

We find this alga of particular inter-