Estimation of the Growth of the T1 Strain of *Mycoplasma mycoides* in Tryptose Broth by the Measurement of Lactate Dehydrogenase

**II. Application to Vaccine Production**

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The measurement of lactate dehydrogenase activity has been used to evaluate the growth of the T1 vaccine strain of *Mycoplasma mycoides* var. *mycoides* in broth during the production of vaccine. Estimations were made during incubation at 37 C and storage at 4 C. The results were compared with titers of the vaccine obtained by two conventional methods of assessing viability titers: the counting of colonies formed on solid medium and a 50% end point titer found after dilutions were made in broth. The method of measuring lactate dehydrogenase activity allowed the rapid enumeration of organisms during incubation of the culture, but estimations made on vaccine stored at 4 C did not compare well with the two conventional methods of assessing viability. The lactate dehydrogenase activity of the culture has enabled rapid monitoring of the production stages of this vaccine.

The attenuated strain T1 of *Mycoplasma mycoides* var. *mycoides* (4, 6) has been grown in broth and used as a live vaccine throughout East Africa for the control of contagious bovine pleuropneumonia since 1965 (2). More than 7,000,000 doses have been issued up to the present time.

This broth vaccine has only a short shelf-life of 4 to 8 weeks at 4 C (2). This necessitates constant production of small batches of vaccine at this laboratory, which are released as soon as sufficient quality control results are known. These include bacteriological contamination checks and estimations of the titer by a dilution method (2).

The characteristics of the growth curve of this organism, assessed by a plate colony-counting technique, have been described (3).

This paper describes the introduction of lactate dehydrogenase (LDH) estimations to monitor the growth of the T1 organism during vaccine production to provide additional control over the quality of the vaccine.

**MATERIALS AND METHODS**

**Vaccines.** The method of production of T1 vaccine, by using the second broth passage of the T1 strain of *M. mycoides* var. *mycoides* which has undergone 45 egg passages, has been described (2).

**Sampling.** The vaccine culture was grown in 200-ml volumes in 8-oz. (ca. 240 ml) medical flat bottles fitted with cotton-wool plugs. Samples of routine vaccine were taken by aseptically decanting 10 ml of broth culture without mixing the contents by inversion. A sample of vaccine was taken after the seed had been prepared (0 hr). Further samples were taken at 21, 45, and 69 hr (harvest time) after incubation at 37 C, and an additional sample was taken after 4 weeks of storage at 4 C. A 3-ml amount of vaccine was used for the LDH estimation, 3 ml for estimating the number of colony-forming units (CFU), and 4 ml for estimating the titer by a serial dilution method.

The results in Table 1 only were obtained from samples taken from three 8-oz. screw-capped bottles which were filled with 200 ml of the same batch of seeded broth prepared for vaccine production. The contents of these bottles were mixed by inversion before removing the samples of vaccine for the various estimations. Samples were removed after incubation at 37 C for 0, 21, 45, and 69 hr. Samples were also removed after storage at 4 C for 1, 5, 8, 11, 15, 19, 35, 40, and 43 days.

**LDH estimation.** LDH levels were estimated by measuring the rate of oxidation of reduced nicotinamide adenine dinucleotide as previously described (1).

**Viable counts.** (i) On solid medium: the concentration of colony-forming units, in the culture, was estimated as previously described (1) and is expressed as CFU/ml. This is referred to as the CFU titer. (ii) In liquid medium: the method of determining the titer after making 10-fold dilutions of the culture in broth has already been described (2). This method uses the
calculation of Reed and Muench (5) to find the 50% end point of growth. This is referred to as the dilution titer.

RESULTS

LDH is recorded in the tables with "free LDH" (1) values subtracted. The value of 4.60 × 10⁻⁷ milliunits of LDH/organism (1) has been used to convert LDH concentrations to an equivalent concentration of organisms. This is subsequently referred to as the LD₇₄ titer. Mean titers have been recorded together with standard errors in the tables. The dilution titer has been expressed as

### Table 1. Relationship between lactate dehydrogenase (LDH) and viability* during growth and storage of T₁ vaccine

| Treatment of vaccine | LDH (milliunits/ml) | LDH titer (= CFU/ml) | Plate titer (CFU/ml) | LDH: plate titer ratio |
|-----------------------|----------------------|----------------------|----------------------|------------------------|
| Incubation at 37 C    |                      |                      |                      |                        |
| 0 hr                  | 13.8                 | 2.99 × 10⁷           | 3.29 × 10⁷           | 0.91                   |
| 21 hr                 | 260                  | 5.64 × 10⁶           | 5.66 × 10⁶           | 1.00                   |
| 45 hr                 | 1,409                | 3.06 × 10⁵           | 2.06 × 10⁵           | 1.49                   |
| 69 hr                 | 2,541                | 5.51 × 10⁴           | 3.43 × 10⁴           | 1.61                   |
| Storage period at 4 C |                      |                      |                      |                        |
| 1 day                 | 2,285                | 4.96 × 10⁴           | 4.32 × 10⁴           | 1.15                   |
| 5 days                | 2,174                | 4.72 × 10⁴           | 3.18 × 10⁴           | 1.48                   |
| 8 days                | 2,152                | 4.67 × 10⁴           | 3.59 × 10⁴           | 1.30                   |
| 11 days               | 1,926                | 4.17 × 10⁴           | 2.97 × 10⁴           | 1.40                   |
| 15 days               | 1,731                | 3.76 × 10⁴           | C                     |                        |
| 19 days               | 1,927                | 4.18 × 10⁴           | C                     |                        |
| 35 days               | 1,648                | 3.57 × 10⁴           | 2.13 × 10⁴           | 16.8                   |
| 40 days               | 1,301                | 2.82 × 10⁴           | 7.58 × 10³           | 37.2                   |
| 43 days               | 1,321                | 2.87 × 10⁴           | 5.93 × 10³           | 48.4                   |

* Viability expressed as colony-forming units (CFU) per milliliter.

### Table 2. Comparison of titers during incubation and storage of Mycoplasma mycoides (T₁ strain) vaccine

| Treatment of vaccine | LDHb (milliunits/ml) | LDH titer (= CFU/ml) | Plate titer (CFU/ml) | Dilution titer (organisms/ml) |
|----------------------|----------------------|----------------------|----------------------|-----------------------------|
| Incubation at 37 C   |                      |                      |                      |                             |
| 0 hr                 | 13.4±2.1             | 2.91 × 10⁶±4.64 × 10⁴ | 2.50 × 10⁷±4.10 × 10⁷ |                             |
| 21 hr                | 161±13.2             | 3.49 × 10⁶±2.86 × 10⁷ | 4.01 × 10⁷±4.32 × 10⁷ |                             |
| 45 hr                | 889±84.2             | 1.93 × 10⁷±1.83 × 10⁷ | 2.45 × 10⁷±1.91 × 10⁷ |                             |
| 69 hr                | 2,237±103.3          | 4.85 × 10⁷±2.24 × 10⁷ | 5.23 × 10⁷±3.26 × 10⁷ | 1.97 × 10⁸±5.35 × 10⁸      |
| Storage period at 4 C|                      |                      |                      |                             |
| 4 weeks              | 1,044±85.9           | 2.26 × 10⁸±1.87 × 10⁸ | 9.10 × 10⁸±3.87 × 10⁸ |                             |

* n = 14.

### Table 3. Mean lactate dehydrogenase (LDH) titer and dilution titer results estimated for 44 batches of T₁ vaccine

| Treatment of vaccine | LDH (milliunits/ml) | LDH titer (=CFU/ml) | Dilution titer (organisms/ml) |
|----------------------|----------------------|----------------------|-------------------------------|
| Incubation at 37 C   |                      |                      |                               |
| 0 hr                 | 12.2 ± 0.95          | 2.65 × 10⁷ ± 2.1 × 10⁷ |                               |
| 21 hr                | 161 ± 7.68           | 3.49 × 10⁷ ± 1.67 × 10⁷ |                               |
| 45 hr                | 888 ± 35.7           | 1.93 × 10⁷ ± 7.74 × 10⁷ |                               |
| 69 hr                | 2,180 ± 60.9         | 4.73 × 10⁸ ± 1.32 × 10⁸ | 1.72 × 10⁹ ± 2.48 × 10⁹      |
| Storage period at 4 C|                      |                      |                               |
| 4 weeks              | 1,178 ± 52.9         | 2.52 × 10⁸ ± 1.15 × 10⁸ | 1.45 × 10⁹ ± 3.93 × 10⁹      |

* n = 44.
organisms per milliliter, instead of 10 raised to a power by index, to effect a clearer comparison with the presentation of CFU titers and LDH titers.

Table 1 shows the average results for a portion of a single batch of vaccine grown and stored in three separate 8-oz screw-capped bottles. The ratio of LDH titer to CFU titer changed from the order of 1 during incubation at 37 C, to 48 after 43 days of storage at 4 C.

The mean \( n = 14 \) LDH and CFU titers (Table 2) estimated during incubation at 37 C were not significantly different \( (P = 0.05) \) when examined by Student's \( t \) distribution. Differences between the LDH titer or the CFU titer and the dilution titer, after 69 hr of incubation, were not significant \( (P = 0.05) \) by Behrens' test. The coefficients of variation calculated for the LDH titer, CFU titer, and dilution titer found at 69 hr were 17, 22, and 99, respectively.

The results in Table 3 show the LDH and dilution titers for 44 different preparations of the vaccine produced over a period of 9 months.

**DISCUSSION**

The results shown in Table 1 indicate that satisfactory results were obtained for viable counts by the LDH titer during incubation at 37 C but that it was not a reliable indicator of viability during storage at 4 C. After 4 weeks of storage at 4 C (Table 3), there was a loss of 92 of the viable organisms found by the dilution titer, in contrast to 53 of loss indicated by the LDH titer. This was probably due to the measurement of intact but nonviable organisms by the LDH method. Similarly, careful estimation of titers on individual growth curves, for incubation periods greater than 30 hr, usually showed a higher titer by the LDH method than that estimated by the CFU titer (Table 1; reference 1). The similar results obtained by both methods on this occasion, at 69 hr (Table 2), may be due to the different sampling technique used, i.e., the samples were decanted without prior mixing of the culture. If this was the reason, it suggests that the intact but nonviable organisms sink to the bottom of the broth culture.

The mean dilution titer was considerably higher than that found by LDH estimations and CFU titers (Table 2), but this was found not to be significant \( (P = 0.05) \). The coefficient of variation of the dilution titer suggests that this finding was probably due to the much larger variation experienced with this method. The coefficient of variation was similar for the LDH titer and CFU titer and much lower than that of the dilution titer. Thus, individual values of the dilution titer of the vaccine cannot be accepted with as much confidence as those of the other two methods.

The short shelf-life of the vaccine has sometimes necessitated the despatch of vaccine before the final dilution titer is known (2). It is now possible, by means of LDH measurements, to monitor this vaccine rapidly at all stages of its production, and the titer of the vaccine is now known before its release. The LDH estimations are particularly advantageous for standardizing inocula for seeding the broth for vaccine production and for checking that the organism is proceeding on a normal growth curve during incubation of the broth. For this latter purpose, individual vaccine titers, at different points of the growth curve, may be compared with the results given in Table 3.

The LDH titer can be assessed within 30 min of sampling the vaccine and therefore provides a useful addition to quality control procedures for the production of this vaccine.

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