BACTERIA COUNTING TECHNIQUE
Updated September 2013

I. Reference:

Gall, L. S., W. Burroughs, P. Gerlaugh and B.H. Edgington. 1949. J. Animal. Sci. 8:431.
Gall, L. S., et al. 1949. J. Animal. Sci. 8:441.
Moir, R. J. 1951. Aust. J. Agr. Res. 2:322.
Moir, R. J. and M. Somers. 1957. Aust. J. Agr. Res. 8:253.

II. Principle:

Rumen samples are taken and preserved in 50% formalin solution as done for the protozoa counting procedure. The organisms are diluted with water. A known volume of the diluted bacteria are mixed with nigrosine dye and spread evenly over a prescribed area of a slide. The slide is rapidly dried on a hot plate. The bacteria are counted under an oil immersion microscope. As all plant material and protozoa are stained the bacteria will appear as white spots. All organisms are counted from 50 representative fields and related to the volume of the sample and the area covered. One must record all dilutions, volumes, area, calibration, fields, and counts.

III. Apparatus and Reagents:

A. Sampling equipment
B. Storage container
C. Microscope, oil immersion
D. Clean microscope slides
E. 100 ml graduated cylinders
F. .1 ml pipette
G. Inoculum loop, 3 mm
H. 50% formaldehyde in water
I. Saturated methyl alcohol solution of water soluble Migrosine.

IV. Sample collection and storage:

A. Rumen samples are taken by the standard methods and an aliquot of each is stored in an air tight container with a 50% formaldehyde (CH_2O)(v/v) solution in a ratio of 1:1.

V. Counting Method:

A. Preserved sample is mixed very well to insure a uniform sample and free all bacteria that is bound to feed particles.
B. An aliquot of 0.1 ml is placed in a graduated cylinder and brought to 100 ml with water. Allow the particulate matter to settle out

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drawn on a clean sheet of paper. After cleaning with alcohol, dry a microscope slide and superimpose upon the circle.

D. With a pipette transfer 0.01 ml of the diluted rumen fluid to the slide and center over the circle.

E. With the 3 mm inoculum loop, transfer one loop full of the migrosine dye solution to the slide.

F. Immediately mix the dye and the sample thoroughly with the loop. When well mixed, spread the material evenly over the entire area of the circle.

G. Place the slide on a plate and dry rapidly. Once dried, the slide may be stored indefinitely.

H. Immersion oil is placed on the slide and with great care the slide is focused on the microscope. The bacteria will appear as white spots on a dark red to black background.

I. The slide should be observed under low power to insure that the slide is uniform. The bacteria of 50 randomly selected fields across several diameters of the slide are counted. This procedure is necessary to reduce the effect of an uneven distribution on the slide.

J. Under normal operation, the environment will add to the count. Therefore, a blank slide should be prepared with water in place of the diluted rumen material. The count of 50 fields from the blank is subtracted from the count of each sample slide.

VI. Calculations:

A. The ratio of the area of the 50 fields and the area of the circle is proportional to the ratio of the corrected number of bacteria per 50 fields and the number of organisms per sample transferred to the slide.

B. Once, the number of bacteria transferred to the slide in 0.01 ml is known the dilution factors are used to express the count as bacteria per ml of ruminal fluid.

VII. Comments:

A. This procedure is more of an art than a science and requires some time for each individual to determine the best conditions and methodology. The following are offered as suggestions.

1. The amount of dye used may need to be increased to achieve best results.
2. It is difficult to evenly spread the material over the entire area of the circle. Therefore, a circle made with a soft wax pencil upon the slide seems to work very well. This method is open to question, but appears to give a more uniform slide.

n distribution of the material before drying.

4. Drying should be rapid, however, this step is very sensitive and required close attention. Drying should be even, without disrupting the uniformity of the slide.