MODIFIED PURINE ASSAY
Updated September 2015

I. References:

A. Zinn and Owens. 1986. Can. J. Animal. Sci. 66:157.
B. Aharoni and Tagari. 1991. J. Dairy Sci. 74:2540.

II. Personal Protective Equipment:

A. Lab coat
B. Safety glasses/goggles
C. Designated acid handling gloves

III. Reagents:

A. 2M perchloric acid ($HClO_4$)
1. Add 167 ml 12 M (70%) $HClO_4$ to ddH_2O in a 1 L volumetric flask.
2. q.s. to 1L with ddH_2O

NOTE: Handle acid in designated perchloric acid fume hood

B. 0.2 M Monobasic ammonium phosphate (H_6NPO_4)
1. Add 23 g of H_6NPO_4 to a 1L volumetric
2. q.s. to 1L with ddH_2O

C. 28.5 mM Monobasic ammonium phosphate (H_6NPO_4)
1. Add 143 mL of 0.2 M H_6NPO_4 or add 3.2775 g H_6NPO_4 to a 1L volumetric flask.
2. q.s. to 1L with ddH_2O

D. pH 2 ddH_2O
1. Adjust pH with H_2SO_4

E. .4 M Silver nitrate ($AgNO_3$)
1. Add 6.9 g $AgNO_3$ to 100 mL volumetric flask.
2. q.s. with pH 2 ddH_2O

Note: This needs to be made up daily, or at least every other day. It may not go into solution very well so be sure to stir while adding. Keep this in a light resistant container. Store in the refrigerator.

F. .5 N Hydrochloric acid (HCl)
1. Add 41.3 ml of concentrated HCl to water in a 1L volumetric flask.
2. q.s. to 1L with ddH_2O

G. Washing solution: (for 500 mL)
1. Add 3.25mL $HClO_4$
2. Add 21.75 mL 28.5 mM H_6NPO_4
3. q.s. to 500 mL with .2 M H_6NPO_4

NOTE: All used chemicals must be placed into waste jar for proper disposal by EHS.

III. Procedure: Split up into two days

Day 1

A. Weigh samples in duplicate into 25 or 50 ml screw cap tubes.
1. Use .5 g for duodenal and pool samples
2. 0.4 g for Dacron bag samples
3. 0.2 g for bacterial samples

(**Note**: sample weight can be adjusted if you are short on sample, but this change must be accounted for in step 9 and in the calculations).

B. Add 2.5 ml $HClO_4$ (2M perchloric acid) and tightly cap tube.

C. Vortex until sample is wet.

D. Incubate in 90-95°C water bath for 30 minutes. It is very important **not** to let the water bath get any hotter than 100 °.

E. After 30 minutes vortex the samples to break up the charred mass and put them back in the water bath for another 30 minutes.

F. Add 17.5 mL of 28.5 mM H_6NPO_4. Add half of the needed volume, vortex, and add the remaining half. Make sure there are no black clumps sticking to the sides of the culture tubes.

G. Vortex again and reinsert tubes in the water bath for 10 to 15 minutes

H. Filter the samples through Whatman #1 filter paper into 60 × 125 mm disposable glass culture tubes. If the screw top test tubes are used, samples can be sealed and stored for up to four days in the refrigerator.

I. Transfer 0.25 ml of filtrate (1 ml for Dacron bag samples) into a 16 × 125 mm tube and add 0.25 ml 0.4 M $AgNO_3$ (1 ml for Dacron bags) and 4.5 ml 0.2 M H_6NPO_4 (3 ml for Dacron bags). Allow samples to stand overnight in the refrigerator.
(**Note**: If you adjusted the samples weight in step 1, adjust this step accordingly so that you have the same concentration purine. For example, if you used half the amount of sample, double the filtrate and reagent volume used in this step.)

Day 2

J. Centrifuge for 10 minutes at approximately 1,000 x g and draw off supernatant liquid. Do not disturb the pellet.

K. Wash pellet with 4.5 mL of washing solution and 250 µL $AgNO_3$. Vortex and repeat step 10 (some samples will turn yellow).

L. Add 5 ml of 0.5 N HCl and vortex until thoroughly mixed

M. Cover tubes with marbles or foil and allow to incubate in 90-05°C water bath for 30 minutes

N. Centrifuge samples again

O. Don't disturb or re-suspend the pellet

P. Allow samples to cool for 10 minutes. Pipette 200 µl of standards, samples, and 0.5 N HCL (blank) in duplicate into a microtiter plate and spec at 260 nm.

IV. Standards:

A. Combine 0.151 g of Guanine ($C_5H_5N_5O$) and 0.135 g Adenine ($C_5H_5N_5$) in 250 ml of 0.5 N HCl = 1.144 mg/ml.

B. Pipette 10 ml of the above solution into 100 ml volumetric and q.s. with 0.5 N HCl = 0.0114 mg/ml. **This is your stock standard**.

C. Prepare the following working standards:

mL stock/100mL	Concentration (µg/mL)
0.1	0.114
0.3	0.342
0.5	0.570
1.0	1.144
2.0	2.288
3.0	3.432
4.0	4.576
5.0	5.720
10.0	11.440
15.0	17.460

D. Dacron bag samples usually run from 0.5 to 3 µg/ml and bacterial samples will be higher yet.

V. Calculations:

Once the samples have been analyzed you need to calculate the amount of N due to the microbes. In order to do this you must determine your dilution factor.

1. If you used 0.5 g of sample divide that by 20 ml (2.5 perchloric acid and 17.5 buffer) = 0.025 g/ml.

0.025 * 0.25 ml filtrate = 0.00625 / 5 ml total volume (.5 N HCl) = 0.00125

0.5 g sample / 0.00125 = 400 which is your dilution factor

Note: It is very important for you to go through these calculations yourself. If you adjusted the amount of sample used, you must adjust for it in these calculations. For instance, if you were using 1 ml of filtrate or 10 ml instead of 5 ml of HCl that you account for that.

Example of calculations if sample weight was cut in half:

0.25 g sample / 20 ml = 0.0125 g/ml
0.025 * 0.5 ml filtrate = 0.00625 / 5 ml total volume = 0.00125
0.25 g sample / 0.00125 = 200 which is your dilution factor.

2. Then to determine the amount of bug Nitrogen, take your µg/ml reading from the spec, and multiply that by your dilution factor, then take that number and divide by your sample weight * 1000. This gets your number to a mg/g basis. Now take the mg/g number and divide by .2 or your own determined purines to nitrogen ratio. If you then divide this number by 10, you have the percentage of N due to bugs.

$$\frac{\text{µg/mL (from spec)} * 400}{\text{Sample weight} * 1000} = \text{mg/g} \quad \frac{\text{(mg/g)} \;/\; \text{P:N ratio}}{10} = \text{\% bug N}$$