# H2S gas collection blocks

**Crystal D. Buckner, Virgil R. Bremer, Sarah J. Vanness, Nerissa A. Ahern, Terry J. Klopfenstein, and Galen E. Erickson**
**University of Nebraska-Lincoln**
**Created summer 2007 – Updated September 2013**

**Material Needed**

Hard plastic fecal cup with no cracks, and a lid
18" Clear plastic tubing 5/16" OD 3/16" ID
In line sprayer screen ~1"
Epoxy
Silicone
¾" PVC male plug
1 X ¾ reducing bushing
1" insulating bushing
Duct Tape

1. Cannula plugs: cut 1" diameter hole in the middle of the rubber plug for a 1" reducing bushing.
2. Drill 9/32" hole in ¾" PVC male plug for tubing to pass through.
3. Screw ¾" plug into 1" reducing bushing. Push reducing bushing through cannula and place 1" insulating bushing on the back side. The insulating bushing prevents the reducing bushing from getting pushed out of the cannula by feed or rumen contractions.
4. Cut two 2/3" dense rubber tubing (~5/16" ID). Fit one of these on the tube and insert the tube through male plug, allowing it to sit on the outside of the plug ~1".
5. Epoxy, well, the rubber piece to the inside of the male plug and to the plastic tube. Epoxy the other dense rubber tube to the outer wall of the plastic tube that resides on the "outside" of the cannula plug.
6. Cut an 18" length of a clear plastic tube (5/16" OD, 3/16" ID)
7. Drill a 9/32" hole through the middle of a clean hard plastic fecal cup.
8. Sandpaper down the edges of this drilled hole.
9. Cut two pieces (approximately 2/3" each) of a dense rubber tubing (~5/16" ID)
10. Insert the plastic tube through one hole in the fecal cup and slide the two rubber pieces over this tube. Then push the plastic tube through the second hole of the fecal cup and allow the plastic tube to sit outside of the fecal cup ~1/2".
11. Epoxy well the rubber pieces to the inside walls of the fecal cup and to the plastic tube (do quickly). Epoxy a 1" sprayer screen to the outside of the fecal cup. Wrap duct tape over the edges of the sprayer screen to make it adhere to the curvature of the fecal cup.
12. Let all of this sit up for at least 24 hours. Remove the duct tape.
13. After the 24 hour time, silicone a fecal cup lid to the cup itself. Let this sit for 4-6 hours.

# In-Vivo Analysis of Hydrogen Sulfide

**Sarah J. Vanness, Crystal D. Buckner, Virgil R. Bremer, Nerissa A. Ahern, Terry J. Klopfenstein and Galen E. Erickson**
**University of Nebraska-Lincoln**
**Created Summer 2007, Revised December 2013**

I. Reference:

Siegel, 1964. A direct Microdetermination for Sulfide. Analytical Biochemistry 11:126-132

Kung, Limn. 1998 Inhibition of Sulfate Reduction to Sulfide by 9, 10-Anthraquinone in In Vitro Ruminal Fermentations. Journal Dairy Science 81:2251-2256

II. Personal Protective Equipment
- A. Lab coat
- B. Safety glasses/ goggles
- C. Latex gloves

III. Reagents:

A. 7.2 N Hydrochloric Acid (HCL)
   a. 658.8mL HCL
   b. q.s. to 1 Liter with $ddH_2O$

B. 1.2 N Hydrochloric Acid (HCL)
   a. 109.8mL HCL
   b. q.s. to 1 Liter with $ddH_2O$

C. .02 M N,N-dimethyl-*p*-phenylenediamine sulfate (DPD reagent)
   a. 0.23428g N,N-dimethyl-*p*-phenylenediamine sulfate
   b. q.s. to 50 mL with 7.2 N Hydrochloric Acid (HCL)

D. .03 M ferric chloride
   a. 0.40548g ferric chloride (Ferric chloride reagent)
   b. q.s. to 50 mL with 1.2 N Hydrochloric Acid (HCL)

E. Distilled water (pH 8 ± 0.05)
   a. Use dilute sodium hydroxide (20% of a 1M solution) to increase the pH of $dH_2O$ to pH 8
   b. If pH goes above 8.05 use $dH_2O$ to lower the pH

F. Sodium Sulfide solution for standards
   a. Weigh 0.500g of granular sodium sulfide
   b. Dissolve and q.s. into 500mL of $ddH_2O$ in a volumetric flask, then transfer into a capped bottle
   c. Only keeps for a couple of days

IV. Standards:

Mix the following individual standards using the pre-made sodium sulfide solution with the pH 8 $dH_2O$. Pipette each of the sodium sulfide quantities into separate 100mL volumetric flasks. Prepare standards prior to gas collection.

| Standards µmol S/ mL (for spec) | Sodium sulfide |
|---|---|
| 0 | 0.00mL |
| .0208 | 0.50mL |
| .0416 | 1.00mL |
| .0832 | 2.00mL |
| .1248 | 3.00mL |
| .1664 | 4.00mL |

V. Procedure:

1. Prepare reagents in 50 mL volumetric flasks before collecting gas samples.
2. Prepare sodium sulfide solution and standards before $H_2S$ collection.
3. Place 5 mL of pH 8 water in a 30 mL glass serum bottle with a rubber stopper and metal clasp.
4. Remove 5 mL of gas, with syringe/needle, out of bottle before taking rumen gas sample.
5. Flush the tubing line, of the gas collection block, with syringe before collecting gas sample, by taking several 20 mL+ gas draws from the line with a syringe and a modified tip.
6. Collect 10-20 mL of gas from the tube in the steer, but only inject 5 mL of gas sample into serum bottles with a syringe/needle, which is bubbled directly into the water.
7. Pipet 5 mL of each standard into a glass test tube.
8. Bottles with water and gas are shaken vigorously for 15 seconds to ensure that the hydrogen sulfide is dissolved in the water.
9. The metal clasps are removed from the bottles and 0.5 mL of DPD reagent is added to each sample and the standard solutions.
10. Add 0.5 mL of the ferric chloride reagent immediately to the samples and standards.
11. Replace the stoppers on the vials and swirl (vortex the test tubes) to ensure that the reagents are mixed thoroughly.
12. Allow the samples and standards to develop in the dark for 25 minutes.
13. Pipette 200 µL each of the standards, samples, and a blank three times on a 96 well plate. After the development of the samples and standards, each sample is then plated with the standards and blank ($dH_2O$) plated in the first wells.
14. The samples are read on a spectrophotometer at 670 nm.