

McMASTER QUANTITATIVE METHOD

EQUIPMENT, SUPPLIES AND REAGENTS:

Tongue depressors/applicator sticks
Balance
50 –100 ml beaker
Flotation solution
1 cc syringes or transfer pipets
Advanced equine counting chamber – McMaster Slide

Saturated Salt Flotation Solution

Add salt to water with constant stirring until no longer going into solution
Approximately 350 g / 1 liter (0.8 lbs/ 1 quart)

PROCEDURE:

1. Place beaker on balance and tare it.
2. Using tongue depressor, weigh out 3 gm of feces into beaker.
3. Add approximately 15 ml flotation solution.
4. Mix well with tongue depressor to break lumps.
5. Bring up to 45 ml with flotation solution.
6. Continue mixing with applicator sticks for several minutes
7. While mixture is still stirring, draw about 1 ml fecal suspension into syringe or transfer pipet
8. Load one side of counting chamber carefully to avoid producing bubbles.
9. Repeat sampling and loading procedure for second side of chamber.
10. Let preparation stand 5 min (examine it at least by 20 min).
11. Place chamber on microscope and examine with 10X objective.
12. Count eggs in both sides of chamber.
13. Calculate eggs per gram:
 $45 \text{ ml final volume, epg} = (\text{side 1} + \text{side 2}) \times 50$

Note: The minimum detection limit is 100 eggs per gram.

COMMENTS:

The McMaster counting chamber is available from Chalex Corporation (formerly Advanced Equine Products), 5004-228th Ave SE, Issaquah, WA 98029 USA, Tel: 425-391-1169, Fax: 425-391-6669, Email: chalexcorp@att.net, Website: www.vetslides.com. Cost is approximately \$20 per chamber. The volume under each grid for this chamber is 0.15 ml. Counting chambers with etched grids are less expensive than those with higher contrast green grids.

Also if you have a smaller amount of feces, this table may help:

<u>Feces, gm</u>	<u>partial vol. Zn SO₄ soln for breaking up fecal pellets, ml</u>	<u>total volume of Zn SO₄ soln for stirring 5 mins., ml</u>
1	5	15
2	10	30
3	15	45
4	20	60

CENTRIFICAL FLOTATION FOR FECAL SAMPLES

MATERIALS:

Centrifuge: Hand crank bench-top with horizontal rotor and shields for 16x100 mm tubes
Compound binocular microscope: should have at least 10X and 40X objectives with 10X eyepieces, best if has a calibrated ocular micrometer
Paper cups, unwaxed, or equivalent 3-4 oz. cups
2-ply cheesecloth, cut as needed
Water
Squirt bottles for solutions
Flotation solution – Saturated Salt Solution (specific gravity 1.2 or above)
Round-bottom glass tubes, 16x100 mm
rack or equivalent to hold the tubes up right
Wooden applicator sticks
Tongue depressor
Microscope slides: glass, 3x1 in.
Coverslips, 18 mm²

PROCEDURE:

1. Place 1 g of feces in a paper cup and break up feces with tongue depressor add sufficient water to fill the cup to a depth of about 1 cm (the seam line on the paper cup)
2. Continue mixing, breaking up the feces with care to mix thoroughly.
3. Pour the mixture through two layers of cheesecloth (i.e., two single sheets) into another paper cup.
4. The cup can be rinsed with fresh water. If this is a qualitative test, this step is unnecessary.
5. Rinse the fecal matter on the cheesecloth with a slight stream of water from the squirt bottle.
6. Fill the tube to about 1 cm from its top and centrifuge at about 800xg for 1 minute.
7. Decant discarding the supernatant or liquid.
8. Add about 4 ml of flotation solution, and re-suspend the pellet vigorously with an applicator stick.
9. Fill the tube to near the top with flotation solution, then centrifuge for 3 minutes.
10. Remove the tube from the centrifuge and place in a rack and fill the tube with the flotation solution such that the meniscus bulges slightly above the lip of the tube.
11. Apply an 18 mm² coverslip to the top of the tube and press it down against the rim with a clean applicator stick.
12. Let sit 5 minute to allow the eggs to float to the coverslip.
13. Remove the coverslip by lifting it straight up so that a drop adheres to it, and place the coverslip on a slide bearing the animal's identification number.
14. Examine with a compound microscope, using the ocular micrometer to determine sizes of observed objects.