Department of Microbiology
Qualitative Fecal Fat Test Procedure

I. Purpose and Test Principle

True or neutral fats are esters of glycerol with three molecules of fatty acid. In the normal individual, the neutral fats are split in the intestine by pancreatic lipase to its component parts of glycerol and fatty acids. These products are then absorbed by the intestinal wall. Consequently, normal feces are devoid of appreciable amounts of neutral fats or split fats (the fatty acids derived from enzymatic activity on neutral fats).

In an individual with a pancreatic disorder, there may be deficient secretion of pancreatic enzymes. All the neutral fats entering the intestine are not split into their components and neutral fats will be found in the feces. Analysis of fecal fats is helpful in diagnosing or excluding pancreatic insufficiency but is of no value in differentiating the specific pancreatic condition.

In individuals with deficient fat absorption, the fatty acids derived from neutral fats are not absorbed as in the normal individual. In this case, the split fats in the feces are increased (there are small amounts of split fats normally present in feces). Steatorrhea caused by a malabsorption disorder would have normal neutral fat content and increased fatty acids.

Both neutral and split fat can be detected with Sudan III stain. Neutral fats stain directly with Sudan III, and the other two forms stain minimally or not at all. After the addition of acetic acid and heat, the fatty acids and soaps are converted to lipids that stain strongly with Sudan III.

II. Specimen Information

A. Collection
   At least 5 ml of liquid stool or 1 g of formed stool should be submitted in a clean specimen cup free of soap or detergent.
B. Transport & Stability
   If transportation time to the laboratory will exceed 1 h, specimens should be refrigerated or frozen. Refrigerated samples are stable for up to 24 h and frozen samples are stable for up to 1 week.

III. Reagents & Equipment

A. 3" X 2" mm glass slides
B. 24 X 50 mm cover slips
C. Glass test tubes with cap and tongs
D. 95% ethanol
E. Sudan III stain (prepare a 1% solution)
   1. Add 1 g Sudan III and 1 g of benzoic acid to 50 ml 70% ethanol and 50 mL Acetone.
   2. Allow stain to sit overnight to dissolve. Filter into brown reagent bottle.
   3. Store at room temperature. Outdate: 6 months.
F. 36% acetic acid
   1. Add 36 ml glacial acetic acid to 64 ml distilled water.
   2. Store solution at room temperature. Outdate: one year.

G. Microscope with 40X objective and calibrated ocular micrometer
H. Applicator sticks
I. Bunsen burner
J. Distilled water
K. Forceps

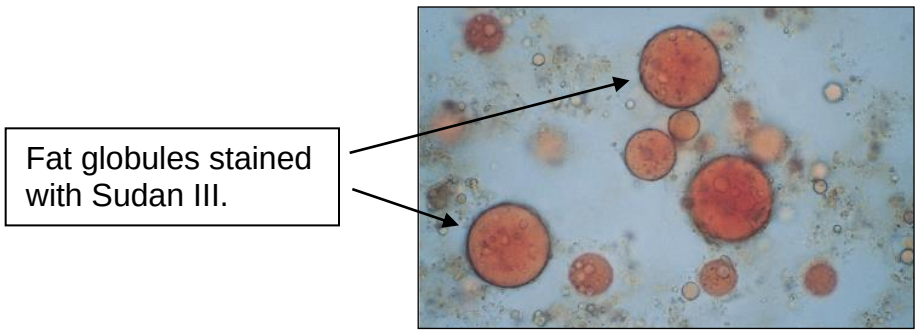
IV. Procedure

A. Obtain a small portion of the stool from different areas of specimen. Place the stool into a test tube and add an equal amount of water. Using a plastic transfer pipette, remove 0.25 mL of the suspension (about 6 drops) and transfer to a glass test tube for the neutral fat evaluation. Remove another 0.25 mL of the suspension and transfer to a separate glass test tube for the split fat evaluation.

B. Neutral fats
   1. Add 0.25 mL of ethanol to the first specimen suspension aliquot.
   2. Add 0.25 mL of Sudan III Stain.
   3. Cap the test tube and vortex the sample until the contents are well mixed. Allow the fecal suspension to sit for 5 minutes.
   4. Remix the stool with a plastic transfer pipette and place 4 drops on a large glass slide.
   5. Cover the specimen with a 24X50 cover slip.
   6. Scan the slide under 40X for orange globules. Count the number of large orange curved droplets.
   7. Proceed to the evaluation of split fats.

C. Split Fats
   1. Add 0.25 mL of 36% acetic acid to the other specimen suspension aliquot.
   2. Add 0.25 mL of Sudan III.
   3. Using test tube tongs, hold the test tube so that the opening is aimed away from yourself and others. Gently heat tube by passing it over a flame until bubbles start to appear. Remove tube from the heat and allow bubbles to subside. Repeat twice more. **Caution: heating the tube too rapidly may cause contents to erupt out of the tube.**
   4. Place 4 drops on a large glass slide. Cover with a 24x50 cover slip.
   5. Scan slide on 40X. Look for large orange globules

V. Interpretation and Reporting



A. Neutral Fats
   1. Interpretation & Reporting
      a. The presence of < 60 orange globules/hpf is considered normal. Report: **Normal amounts of neutral fats seen.**
      b. The presence of ≥ 60 orange globules/hpf is considered increased. Report: **Increased amounts of neutral fats seen**

B. Split Fats (Soaps and/or Fatty Acids)
   1. Interpretation & Reporting
      a. The presence of < 100 orange globules/hpf is considered normal. Report: **Normal amounts of soaps and/or fatty acids (total fats) seen.**
      b. The presence of ≥ 100 orange globules/hpf is considered increased. Report: **Increased amounts of soaps and/or fatty acids (total fats) seen.**

VI. Quality Control Testing

Each batch of patient samples should include control material to evaluate the Sudan III stain as well as the sample processing technique.

A. Neutral Fat Control
   Mayonnaise may be used to serve as the positive neutral fat control. Mayonnaise should be stored in the refrigerator until use. Process and stain as outlined above for both neutral and split fats. Numerous orange globules should be observed in both sample preparations.

B. Split Fat Control Testing
   Shavings from a bar of soap may be used for the positive split fat control. Process and stain as outlined above for both neutral and split fats. No orange globules should be apparent in the neutral fat preparation. Numerous orange globules should be observed in the split fat preparation.

Quality control results should be entered into the LIS. Notify the Microbiology supervisor or technical specialist if expected results are not obtained.

VII. Limitations

Very few, if any, neutral fat globules are seen in a normal stool specimen. If the patient has ingested mineral oil or castor oil, the neutral fats may appear falsely increased.

VIII. References

A. Henry, J.B. Clinical Diagnosis and Management 21st ed. 2007. W.B. Saunders Company. Philadelphia, p. 292.
B. Khouri, M.R., Huang C., and Shziau, Y.F. 1989. Sudan Stain of Fecal Fat: New Insight Into an Old Test. Gastroenterology; 96: 421-427.
C. Strasinger, S. Urinalysis and Body Fluids. 4th ed. 1987. F.A. Davis Co., Philadelphia. p. 196.
D. Garcia, L.S. Diagnostic Medical Parasitology 5th ed. 2007. ASM Press. Washington, D.C. p. 846.

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