

remel

SAF FIXATIVE

INTENDED USE

Remel SAF (sodium acetate-acetic acid-formalin) Fixative is a liquid medium recommended for use in qualitative procedures for the transportation and preservation of intestinal parasites in stool specimens. Concentration and permanent stained smear procedures can be performed from SAF.

SUMMARY AND EXPLANATION

SAF Fixative was originally developed by Junod as a multipurpose medium for preservation of intestinal parasites.¹ In a study of over 900 specimens, Yang and Scholten found advantages in using SAF in comparison to similar fixatives such as PVA and Schaudinn's fixatives, both of which contain mercuric chloride. SAF does not contain mercuric chloride, making it a safer alternative. Also, SAF lends itself to multiple procedures, including wet mount examination, concentration method, and permanent stained smears.^{2,3}

PRINCIPLE

SAF Fixative can be used for both concentration method and permanent stained smear. It does not contain mercuric chloride and is, therefore, considered a safer fixative than Schaudinn's.⁴ Microscope slides used to prepare permanent stains should be coated with albumin before application of stool material that has been preserved in SAF Fixative.⁵

REAGENTS (CLASSICAL FORMULA)*

Sodium Acetate (CAS 127-09-3)	15.0 g
Formalin, 5% (CAS 50-00-0)	40.0 ml
Acetic Acid, 2% (CAS 64-19-7)	20.0 ml
Demineralized Water (CAS 7732-18-5)	925.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

WARNING! Contains formaldehyde which can cause cancer. May cause allergic respiratory and skin reaction. Causes eye, skin, and respiratory tract irritation. May be harmful if swallowed, inhaled, or absorbed through the skin. May cause central nervous system depression. This substance has caused adverse reproductive and fetal effects in animals. Refer to Material Safety Data Sheet for more information.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and test materials after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used.

PRODUCT DETERIORATION

This product should not be used if (1) the appearance has changed from clear and colorless, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Refer to collection instruction sheet included with this product. Consult appropriate references when necessary.⁴⁻⁶

1. Substances and medications, such as mineral oil, barium, bismuth, antibiotics, antimalarials, and nonabsorbable anti-diarrheal pre-parations, interfere with the detection of intestinal protozoa. Intestinal protozoa may be undetectable for one to several weeks after administration of any of these substances.

Specimen collection should be performed after seven days in the case of antibiotics or barium.⁵

2. A series of three specimens is considered a minimum for an adequate examination. Stool specimens should be collected every other day or within a time frame of no more than 10 days. If a series of 6 specimens is ordered, they should be submitted within a 14 day period.⁵
3. Specimens should not be contaminated with water or urine. Water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms.⁵
4. Adequate mixing of the specimen and preservative is mandatory. The fixative should be mixed with the specimen in a 3 to 1 ratio. Generally, fixation time should be no less than 30 minutes.⁵

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Paper towels or other absorbent material, (2) Applicator sticks, plain and cotton-tipped, (3) Disposable glass or plastic pipettes, (4) Incubator or slide warmer, (5) Trichrome Stain (R40217) or iron-hematoxylin stain reagents, (6) Coplin jars, staining rack, forceps, (7) Glass microscope slides, coverslips, mounting medium, (8) Microscope with a calibrated ocular micrometer, immersion oil.

PROCEDURE

Specimens should be considered potentially infectious and handled accordingly.

1. Fresh stool specimens should be placed in SAF Fixative by adding ½ teaspoon to a 15 ml vial. Allow a minimum of 30 minutes for fixation before processing preserved stool specimen (sample).
2. Process SAF-fixed stool specimen as follows: mix the vial several times by inversion to ensure the sample is well-mixed with the fixative.
3. Strain approximately 3-4 ml of resuspended sample through a filtration funnel into a 15 ml centrifuge tube.
4. Using 0.85% saline, wash as much of the sample through the funnel until the volume in the centrifuge tube is 15 ml.
5. Centrifuge the tube at 2,000 rpm (500 x g) for 10 minutes.
6. If the supernatant fluid is cloudy or dark, decant and add more saline. Resuspend the sample and repeat step 5.
7. Decant the supernatant.
8. Final sediment should be approximately 0.5-1.0 ml. To increase the volume of sediment, repeat steps 3-5. To decrease the volume, remove the excess sediment, add saline to 15 ml (total volume) and repeat step 5.
9. Prepare a smear from the sediment in the following manner. Use smear for permanent stain.
 - a. Place one drop of Mayer's Albumin on a labeled slide.
 - b. Add an equal amount of sediment and mix thoroughly.
 - c. Spread the mixture across the slide in bands of varying thicknesses.
 - d. Allow the slide to dry 5-10 minutes at room temperature until 'tacky'.
 - e. Immerse the slide in 70% ethanol to coagulate the albumin (about 30 minutes).
 - f. Proceed with staining following established laboratory procedures.
10. Concentrate the remaining sediment following established laboratory procedures.

INTERPRETATION

This transport medium serves as a vehicle for preserving intestinal parasites while transporting the specimen to the laboratory.

QUALITY CONTROL

All lot numbers of SAF Fixative have been tested using the following quality control organism and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Giardia intestinalis</i> ATCC® 30888	30 minutes @ 25°C	Well-fixed smear, typical morphology observed

LIMITATIONS

1. Protozoa observed in wet preparations should be confirmed by permanent stained smears. This is particularly important in the case of *Entamoeba histolytica* vs. *Entamoeba coli*.⁴
2. Smears prepared from SAF fixed specimens demonstrate better organism morphology when stained with iron-hematoxylin than with trichrome stain.⁴
3. Results obtained will largely depend on proper and adequate specimen collection and fixation. Improperly fixed specimens will result in protozoan forms that are non-staining or predominantly red.⁴
4. Slides used for making smears should be free of grease.⁴
5. *Entamoeba coli* cysts are difficult to fix properly and may be difficult to identify on the stained slide. For this reason, it is possible to have fixatives that meet quality control criteria and yet, do not always yield good morphology for this particular organism. Use of a longer fixation time (60 minutes) may produce better morphology after staining.

BIBLIOGRAPHY




1. Junod, C. 1972. Bul. Soc. Pathol. Exot. 65:390-398.
2. Yang, J. and T. Scholten. 1977. Amer. J. Clin. Path. 67:300-304.
3. Scholten, T.H. 1972. J. Parasitol. 58:633-634.

4. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock. 2011. Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.
5. Clinical and Laboratory Standards Institute (CLSI). 2005. Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline. 2nd ed. M28-A2. CLSI, Wayne, PA.
6. Garcia, L.S. 2001. Diagnostic Medical Parasitology. 4th ed. ASM, Washington D.C.

PACKAGING

REF R21730, SAF Fixative, 15 ml/Vial..... 12 Vials/Pk
REF R21921, SAF Fixative, 15 ml/Vial..... 120 Vials/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

IFU 21730, Revised June 19, 2012

Printed in U.S.A.