PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Department of Microbiology
Modified Kinyoun Acid Fast Stain Procedure
For Aerobic Actinomycetes

I. Purpose and Test Principle

Modified Kinyoun acid-fast stain is helpful for distinguishing some members of the aerobic actinomycetes. Some of these organisms contain cell wall mycolic acids that retain the primary stain, carbol fuschsin, even after acid decolorization. The modified acid-fast stain uses a weaker decolorizer (1% H_2SO_4) than does the mycobacterial stain (3% HCl). Methylene blue is used as a counter stain for background material and offers contrast to the acid-fast cells. This method of staining for acid fastness is useful for distinguishing partial or weakly acid-fast organisms, such as *Gordonia*, *Nocardia*, *Rhodococcus*, and *Tsukamurella*, from acid-fast negative organisms, such as *Streptomyces,* and fully acid-fast *Mycobacterium* species.

Smears can be made directly from specimens or prepared from culture isolates. The modified acid-fast stain used on direct specimens may more accurately reflect the true partially acid-fast nature of the organisms than do modified acid-fast stains prepared from colonial growth. Evaluation of Gram stain morphology of a suspected aerobic actinomycete should be the initial step in organism identification. Gram stain morphologies of the aerobic actinomycetes vary by genus; organisms appear as gram-positive rods ranging in shape from coccoid to bacillary. Filamentous and branching forms may be present, depending on the species involved and the stage of growth. A sufficient number of fields should be reviewed to allow determination of the most prevalent morphology and to detect any branching forms. Care should be taken not to confuse perpendicular aligning of the organisms with true branching. Smears made from colonial growth of filamentous isolates may fragment and appear as bacillary or coccoid forms. The mycolic acids in the cell wall reduce the ability of the crystal violet to enter the cell wall. This may result in a "beaded" or speckled Gram-positive appearance. When branching, "beaded" Gram-positive rods are observed, a Modified Kinyoun stain should be performed to aid in identification.

II. Specimen Information

A. Culture isolates with typical colonial and microscopic morphology of an aerobic actinomycete.
B. Clinical specimens, such as wounds, body fluids, and lower respiratory samples, may be tested when branching, beaded, Gram-positive rods are observed on the Gram stain. Specimens that are rated as Q0 due to superficial contamination do not warrant a follow up Modified Kinyoun stain.

III. Reagents & Equipment

A. Glass slides, wooden applicators, and sterile water or saline
B. Carbol fuchsin
C. 50% alcohol
D. 1% aqueous sulfuric acid
E. Methylene blue

Staining reagents should be stored at room temperature and may be used until the expiration date listed on bottles.

Controlled document. Do not copy.

IV. Procedure

A. Label a glass slide with the culture accession number, patient's last name and first initial and current date.
B. Prepare smear by harvesting a small amount of bacterial growth with a wooden applicator and emulsifying it in a drop of sterile water or saline on the glass slide. Do not make the smear too thick.
C. Heat-fix the smear until dry.
D. Place smear and control slides on a staining rack.
E. Flood the smears with Kinyoun carbol fuchsin and allow staining for 5 min.
F. Pour off excess stain.
G. Briefly rinse the smears for 3-5 s with 50% alcohol and immediately rinse with water.
H. Decolorize smears with 1% aqueous sulfuric acid for 2-3 min.
I. Rinse smears with water.
J. Flood smears with methylene blue and counterstain for 30-60 s.
K. Rinse smears with water, dry, and examine under oil immersion.

V. Interpretation

With the modified acid-fast stain, the background should be blue; slides that have a pink background may be inadequately decolorized and should be repeated. The smear should be scanned for areas where individual cells can be seen or areas where single layers of cells allow clear differentiation of cell borders. The acid-fast reaction of tightly packed clumps of organisms may not represent the true partially acid-fast nature of the cells; be wary of large clumps of cells which all appear to be acid-fast positive. Acid-fast cells will be clearly red; cells that stain purple or light pink may or may not be truly acid-fast.

Most *Nocardia* species are weakly or partially acid-fast (varies from 10 to 20% per oil immersion field). Both *Gordonia* and *Rhodococcus* species vary from no acid fastness to 10-20% per oil immersion field. *Tsukamurella* species vary from 20-100% per oil immersion field. *Streptomyces* species are usually negative. Occasionally, coccoid forms of *Streptomyces* may appear partially acid-fast; hyphae, however, are acid-fast negative. *Mycobacterium* species stain 100% acid fast by the Modified Kinyoun method. When cells stain strongly acid-fast, a regular Kinyoun stain using 3% acid-alcohol should be performed to determine if the isolate is a *Mycobacterium* spp.

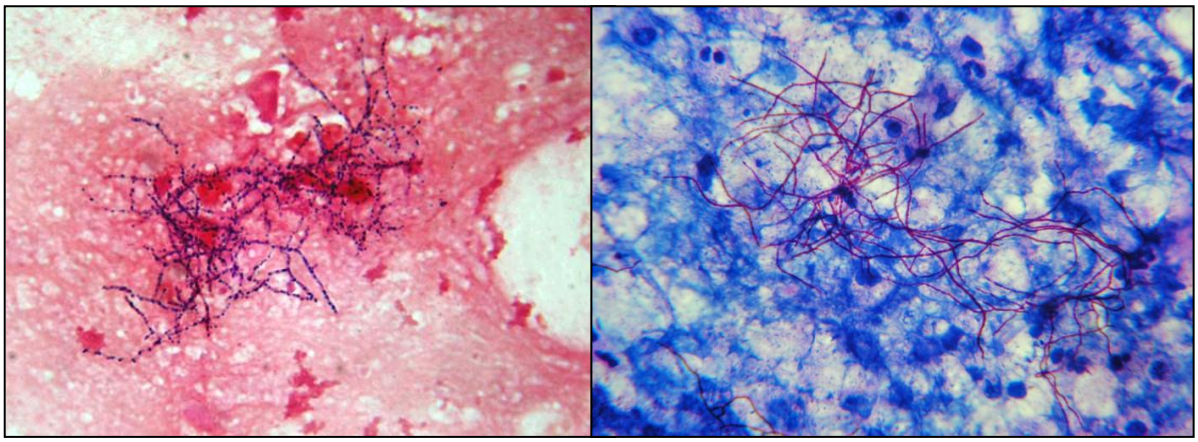
A. Clinical Specimens

Careful attention should be paid to the cellular material present in the sample. *Nocardia* is frequently seen in association with polymorphonuclear leukocytes. Phagocytized gram-positive or acid-fast organisms can sometimes be seen within macrophages and mononuclear cells; in the modified acid-fast smear these may appear as "beaded" cells with strongly acid-fast granules within non-acid-fast or weakly acid-fast rods.

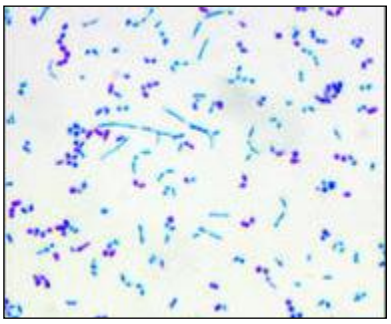
Controlled document. Do not copy.

Bronchial Washing with *Nocardia*

Gram Stain | Modified Kinyoun Stain



B. Culture Isolates
A stain that shows an unambiguous acid-fast positive reaction may frequently show only a few clearly red cells, with a majority of blue cells. Frequently, only the beads appear acid-fast positive. If modified acid-fast stain results are ambiguous, transfer of the organism to a lipid-rich medium, such as Lowenstein-Jensen medium or Middlebrook 7H11 agar, and repeat staining may give a more clear-cut stain result. Acid-fastness may become more evident as colonies age.



Nocardia farcinica
Modified Kinyoun Stain

VI. Quality Control

Because of the difficulty of standardizing this technique, it is imperative that positive and negative controls be stained simultaneously with patient smears. Control slides can be prepared from suspensions of the control organisms grown on Lowenstein-Jensen or 7H11 agar. Prepare a 0.5 McFarland suspension of each organism in sterile saline and use a 0.01 mL calibrated loop to prepare the smears. Do not prepare smears too thick. Heat-fix the control slides and stain simultaneously with patient smears. Smears should be evaluated by experienced laboratory personnel, and the quality of the stain itself should be evaluated before results are reported. Document all QC results in LIS.

Control strain	Expected Results
Nocardia farcinica ATCC 3308	Positive
Streptomyces albus ATCC 17900	Negative

VII. Limitations

A. Because of the difficulties of interpretation of the modified acid-fast smear, results of this stain should be considered preliminary and must be used only in conjunction with results from other tests.

Controlled document. Do not copy.

VIII. References

A. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

B. Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. Manual of Clinical Microbiology, 10th ed., Vol. 1, ASM Press, Washington, D.C.

C. Larone, D.H. 2011. Medically Important Fungi: A Guide to Identification, 5th ed., ASM Press, Washington, D.C.

D. blogspot.com for images of bronchial washings with *Nocardia*

IX. Document Control

Effective: 06/14/2001
Reviewed by director (AR): 09/24/2012
Reviewed by Joseph Schappert: 03/10/2010
Reviewed by supervisor (JC): 06/14/2001, 04/01/2002, 03/2003, 05/2004, 07/2005, 06/2006, 06/2007, 05/2008, 0/2009, 04/2011, 03/2013, Jason Ammons 07/2015

Updates: 09/20/2012 Added rinse with 50% reagent alcohol and changed decolorizer to 1% aqueous sulfuric acid instead of 0.5% sulfuric acid in ETOH.