**PROCEDURE: BactiDropTM Calcofluor White Stain**

1. **Principle**

Calcofluor white is a non-specific fluorochrome with the ability to bind with cellulose and chitin. Upon excitation with longwave ultraviolet light, this compound functions to delineate the cell walls of cellulose containing organisms.Prior to staining with calcolfluor white, potassium hydroxide is used to act as a clearing agent by dissolving tissue cells. Evans blue dye is incorporated in the stain to minimize background material.

1. **AVAILABILITY**

Monday – Friday, 7:30-3:30. On weekends when mycology tech is available.

1. **TEST CODES**

CALC

CALCO

1. **Specimen Requirements**
   1. Heat fixed prepared slides
   2. Performed on all specimens for which a fungus culture has been ordered. Smears are not performed on autopsy specimens.

1. **Materials AND EQUIPMENT**
   1. provided
      1. Bactidrop™ Calcofluor White 1.0g
      2. Evans Blue Dye 0.4g
      3. Bactidrop™ Potassium Hydroxide 100.0g

* 1. Materials Needed but not Provided
     1. Glass slides and coverslips.
     2. Fluorescent Microscope with filter ranging between 440-500nm with a peak of 440nm and a barrier filter of 500-520-nm is recommended for optimum results.
     3. Wooden applicator sticks.
     4. Quality control organisms.

1. **STorage and Handling**
   1. This product is ready for use and no further preparation is necessary. Store product in its original container at 20-25°C until used. Do not freeze or overheat. Protect from light.
   2. This product should not be used if (1) there is evidence of dehydration, (2) the color has changed, (3) the expiration date has passed, or (4) there are other signs of deterioration. The expiration date applies to the product in its intact container when stored as directed. Discard remaining portion of partially used ampule at end of workday.
2. **QUALITY CONTROL**
   1. Controls slides are prepared in the laboratory and performed with each use
   2. Positive control = C. albicans bright green fluorescence
   3. Negative control= E. coli ATCC 25922 weak to no fluorescence
   4. All lot numbers of BactiDropTM Calcofluor White have been tested using the following quality control organisms 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 RESULTS

Trichophyton mentagrophytes bright green fluorescence

ATCC® 9533

Eschericia coli weak to no fluorescence

ATCC® 25922

1. **PROCEDURE**
   1. Specimen Preparation
      1. Place specimen on a clean glass slide and allow to dry on slide warmer at 60°.
      2. Hair, skin scrapings and ground nail samples can be placed directly on the slide. Heat fixing is not required.
   2. Staining procedure
      1. Place dropper in the assembled, reusable ampule crusher provided. Hold dropper/crusher in an upright position and lightly tap the bottom to dislodge any bubble that might have formed. Grasp the middle of the dropper/crusher with the thumb and forefinger, and with the tip pointing away, press gently to crush the ample. Invert dropper and squeeze lightly to dispense in a dropwise fashion.
      2. Add 1 drop of Bactidrop™ Potassium Hydroxide (10%) and mix gently with a wooden applicator stick.
      3. Add 1 drop of Bactidrop™ Calcofluor White and mix gently with a wooden applicator stick.
      4. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.
2. **Interpretation of Results**
   1. Fungal elements, yeast- Bright apple green fluorescence with typical morphology. Various fungal elements will stain fluorescently including *Candida* sp., *Histoplasma* sp., *Blastomyces* sp. and *Aspergillus* sp. among others.
   2. Bacteria- weak to no fluorescence, typical coccoid or bacillary shape.
   3. The kit will also stain *Pneumocystis carinii* cysts and parasites such as *Acanthamoeba* sp.
   4. Keratin, collagen, elastin fibers and sperm cells are also stained and may provide structural guidelines for diagnosis or may be confused with fungal elements.
   5. Results should reported as follows:
      1. Many = organisms in all high dry fields
      2. Moderate = organisms in all low power fields
      3. Few = organisms present but not in all low power fields
3. **Limitations** 
   1. Calcofluor white is a fluorescent brightener that aids in the detection of certain microorganisms by means of morphological delineation. Definitive identification may require additional biochemical and serological testing, or confirmation by an alternate staining technique.
   2. Studies indicate that the capsule of *Cryptococcus* will not stain with calcofluor white. Alternate techniques, such as direct examination using India ink, are recommended for the detection of this organism.
   3. Various types of debris may fluoresce. Bacteria may also fluoresce but less brightly than fungi.
   4. Brightener-induced fluorescence fades with prolonged viewing, especially in thinner sections, but fluorescence may be restored by restaining.
4. **Precautions/Warnings**
   1. Bactidrop™ Bactidrop™ Calcofluor White may cause eye, skin and respiratory tract irritation.
   2. Bactidrop™ Potassium Hydroxide (10%) is corrosive. Causes eye and skin burns. May be harmful if swallowed. May cause severe respiratory tract irritation with possible burns. May cause severe digestive tract irritation with possible burns.
   3. For in vitro diagnostic use only.
5. **REFERENCES**

* 1. Darken, M. 1961. Science. 133:1704-1705.
  2. Hageage, G.J. and B.J. Harrington. 1984. Lab. Med. 15:109-112.
  3. Monheit, J.E., D.F. Cowan, and D.G. Moore. 1984. Arch. Pathol. Lab. Med. 108:616-618.
  4. Al-Doory, Y., R.J. Yankey, and M.L. Elgart. 1985. Lab. Mgment. 23:63-68.
  5. Cernak, P. Methodist Hospital, Houston, TX. 1987. Data on file. Remel, Lenexa, KS.
  6. Baselski, V.S. and M.K. Robison. 1989. Am. Clin. Lab. 8:36-37.
  7. Milligan, T. 1992. J. Clin. Microbiol. 30:754.
  8. Wilhelmus, K.R., M.S. Osato, R.L. Font, N.M. Robinson, and D.B. Jones. 1986. Arch. Opthamol. 104:1309-1312.
  9. Weber, R., R.T. Bryan, D.A. Schwartz, and R.L. Owen. 1994. Clin. Microbiol. Rev. 7:426-461.
  10. 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.
  11. Clark, G. 1981. Staining Procedures. 4th ed. Williams and Wilkins, Baltimore MD.
  12. Remel BactiDropTMCalcofluor White,PI June 18. 2014