INTENDED USE
Calcofluor White Stain is recommended for use in qualitative procedures as a rapid non-specific fluorochrome stain for the initial microscopic detection of fungal elements, yeast, Acanthamoeba cysts, Microsporidia, and Pneumocystis jirovecii in clinical specimens.

SUMMARY
In 1961, Darken reported the uptake of a fluorescent brightener, Calcofluor White, by actively growing cultures of yeast and higher fungi. Hageage and Harrington described the use of Calcofluor White to demonstrate hyphae and yeast in clinical specimens. Monheit et al. applied this stain to frozen sections of lung and soft tissues for the intraoperative diagnosis of fungal infection. Baselski and Robinson reported the use of Calcofluor White in the detection of Pneumocystis carinii cysts in bronchoalveolar lavage (BAL) specimens. Wilhelmus et al. demonstrated the fluorescence of Acanthamoeba cysts in corneal scrapings by the Calcofluor White Stain. Weber et al. reported chemical fluorescence optical brightening agents, such as calcofluor white, also stain Microsporidia spores.

Calcofluor white is a non-specific fluorochrome with an ability to bind with chitin and cellulose. Evans blue dye is incorporated in the stain to minimize background material and helps emulsify solid, viscous materials that may mask fungal elements. Depending on the sample type and the organism to be identified, the use of potassium hydroxide is at the discretion of the user.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
DANGER! Potassium Hydroxide is a poison and may be harmful or fatal if swallowed. Potassium Hydroxide is corrosive and may cause burns or irritation to skin, eyes, and respiratory tract. Avoid breathing vapors and eye/skin contact. This product is for in vitro diagnostic use and should be used by properly trained individuals. Refer to the Safety Data Sheets for additional information.

STORAGE
This product is ready for use and no further preparation is necessary. The product should be stored in its original container at 15-30°C until used. Protect from light.

USER QUALITY CONTROL
This product should not be used if the color has changed, the expiration date has passed, or there are other signs of deterioration. 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.

SPECIMEN COLLECTION AND PREPARATION
Specimens should be collected and handled following recommended guidelines.

PROCEDURE
Materials Provided: Calcofluor White Stain and/or Potassium Hydroxide, 10%.
Materials Not Provided: Loop sterilization device, supplemental media, quality control organisms, glass slides and coverslips, inoculating loop, swabs, collection containers, incubators, alternative environmental systems, fluorescent microscope, stirrer, slide warmer, 100% Ethanol, 95% Ethanol, Xylene substitute, Methanol.

A fluorescent microscope with an excitation filter ranging between 340-490 nm is recommended for optimum results. Consult fluorescent microscope manufacturer for filter recommendations suitable for the fluorescence of calcofluor white.

Fungal elements and yeast:
1. Place specimen on clean glass slide and allow to dry on warmer at 60°C.
2. Add 1 drop of Potassium Hydroxide (if needed) and gently mix.
3. Add 1 drop of Calcofluor White Stain and mix.
4. Coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

Acanthamoeba cysts:
1. Place the specimen on a clean glass slide and allow to air-dry.
2. Appropriate specimens for slide preparation are corneal scrapings or biopsy, conjunctive or corneal ulcer, contact lens paraphernalia, and concentrated water samples of at least 100 ml.
3. Fix slide in methanol for 2 minutes.
4. Paraffinized sections (6 µm thick) may also be used.
5. Soak slide in a xylene substitute for 1-2 minutes to remove paraffin.
6. Dip slide in 100% alcohol (#0003303) twelve times.
7. Dip slide in 95% alcohol (#0003356) twelve times.
8. Rinse gently in deionized water and proceed with step 4.
9. Add 1 drop of Potassium Hydroxide and gently mix.
10. Add 1 drop of Calcofluor White Stain and mix.
11. Stain for five minutes and remove excess stain by rinsing in deionized water. Coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

Microsporidia:
Although the most common specimen is a fresh or preserved (formalin or SA) stool specimen, other specimens such as tissues, duodenal aspirates, concentrated urine, sputum, CSF, nasal pharyngeal, bronchoalveolar lavage, and conjunctiva are appropriate. A thin specimen (10 µl) should be placed on a clean glass slide and heat fixed on a slide warmer at 65-75°C until dry.
1. Fix slide in methanol for 2 minutes.
2. Add 1 drop of Potassium Hydroxide and gently mix.
3. Stain for one minute and remove excess stain by rinsing in deionized water. Coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

Pneumocystis carinii:
1. Concentrated bronchoalveolar lavage (10-25 µl) or tissue samples are the specimens of choice as decreased sensitivity is observed with induced sputum.
2. Fix slide in methanol for 2 minutes.
3. Add 1 drop of Potassium Hydroxide and gently mix.
4. Add 1 drop of Calcofluor White Stain to the slide and mix.
5. Stain for one minute and remove excess stain by rinsing in deionized water. Coverslip and examine the specimen using a fluorescent microscope. Observe for fluorescence and typical morphology.

EXPECTED RESULTS
Fungal elements, yeast: Bright blue or apple green fluorescence with typical morphology.
Acanthamoeba cysts: Cysts (10-25 µm) appear corrugated and double walled with bright blue or apple green fluorescence and bright orange cytoplasm.
Microsporidia: Blue or apple green fluorescence; intestinal Microsporidia; spores range in size from 0.9-1.5 µm to 1.2-2.0 µm; cell wall is brightened but staining is not specific.
Pneumocystis jirovecii: Brilliant blue or apple green fluorescence; cystic are 5-8 µm in diameter and contain up to 8 crescent or pleomorphic shaped sporozoites; cell wall and double parenthesis structures inside the cysts stain intensely.
Bacteria: Weak to no fluorescence; typical coccoid or bacillary shape.
Trichophyton mentagrophytes: Bright blue or green fluorescence.
Escherichia coli: Weak to no fluorescence.
LIMITATIONS OF PROCEDURES
1. Calcofluor white is a fluorescent brightener that aids in the detection of certain microorganisms by oil morphological definition. Definitive identification may require additional biochemical and serological testing or identification by an alternative staining technique.
2. Studies indicate that the capsule of Cryptococcus will not stain with calcofluor white. Alternate techniques such as direct examination using indigo 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. Both Calcofluor White Stain and Potassium Hydroxide must be added for *Pneumocystis carinii*, Acanthamoeba, and Microsporidia to adequately fluoresce.
5. Brightener induced fluorescence fades with prolonged viewing, especially in thinner sections. Fluorescence may be restored by restaining.
6. If a specimen contains excessive nonspecific debris or yeast, further examination for Microsporidia should be performed by another staining method. All positives for Microsporidia should be confirmed by the Modified Trichrome Stain (Microsporidium Trichrome Blue Stain #0004150).
7. KOH slide preparations are not permanent since even fungi will eventually be destroyed by potassium hydroxide.

BIBLIOGRAPHY

CONTACT
Alpha-Tec Systems, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing, as well as O&P collection systems and concentration devices for Parasitology. For Technical Assistance, email Technical@AlphaTecSystems.com, and for Customer Service, email Sales@AlphaTecSystems.com, or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

WARRANTY
This product is warranted by Alpha-Tec Systems, Inc. to perform as described in the labeling and literature supplied. Alpha-Tec Systems, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall Alpha-Tec Systems, Inc. be liable for any consequential damages arising out of aforesaid express warranty.

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PRODUCT CODES
0004501 Potassium Hydroxide, 10%, 25 ml
0004503 Calcofluor White Stain, 25 ml
Directions For Use for the following:

Calcofluor White Stain
Potassium Hydroxide, 10%

Manufactured by Alpha-Tec Systems, Inc.
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Vancouver, WA 98683 USA

GLOSSARY OF SYMBOLS

LOT
Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Number / Lotnummer / Lotnummer / Šaržna številka / Número de lote

REF
Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo

IVD
In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik embart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro

Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rapresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Gemaachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado

Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada

Manufacturer / Fabricant / Fabricante / Produttore / Hersteller / Fabrikant / Fabrikant / Proizvajalec / Fabricante

Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / laktak forsiktighet / Previdno / Atenção

Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperature comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opsoan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevali med navedenimi temperaturami / Armazene entre as temperaturas indicadas

Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenido sufficiente per <n> tests / Enthält ausreichend für <n> Untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebina zadostuje za <n> testov / Contêm quantidade suficiente para <n> testes

Consult instructions for use / Consulter la notice d’utilisation / Consulte las instrucciones para el uso / Consultare le istruzioni per l’uso / Bitte beachten Sie die Anwendungsanweisungen / Raadpleeg instructies voor gebruik / Konsultera bruksanvisningen innan användning / Glej navodila za uporabo / Consulte instruções para o uso