Wisconsin Fungal Case Presentations

WCLN Webinar
February 10, 2016

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Disclosure

Raymond P. Podzorski, Ph.D., D(ABMM)
February 10, 2016

No relevant financial relationships to disclose.

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New Pulmonary Nodule-12/2013

- 68 y/o ♀
- Severe rheumatoid arthritis
- Takes multiple immunosuppressive medications
- Quit smoking in 2006 (48 years)
- Right lower lobe (RLL) pulmonary nodule noted on pre-surgical workup
- Images from September 2013 showed no RLL nodule (ct scan)

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Past Medical History

- Bladder cancer
- Hysterectomy
- Cholecystectomy
- Cataract surgery

4

Procedures

- CT of chest w/contrast 12/14/2013
- PET CT scan 12/17/2013
- Demonstrated a 2.5 x 3.3 cm mass at the bottom of the right lower lobe
- Enlarged mediastinal lymph nodes also noted
- Enhanced metabolic activity noted in the large mass and the mediastinal lymph nodes

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Procedures

- Video Mediastinoscopy 1/4/2014
  - Biopsies collected
  - Frozen sections showed inflammation and necrotizing granulomas
  - Tissue for routine bacterial culture, fungus culture and AFB culture
    - Tissue Gram stain, rare PMNs, NOS
    - AFB stain, NOS

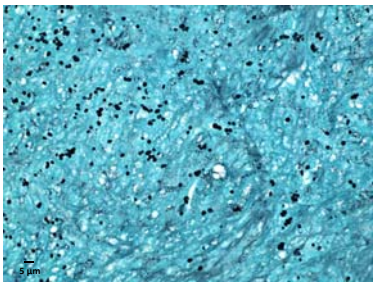
6

Pathology Report of Mediastinal Lymph Node

- Multiple caseating granulomas
- Numerous yeast, 3-4 µm
- Yeast consistent in size

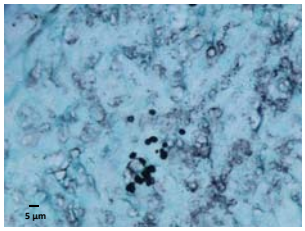
7

Mediastinal Lymph Node GMS Stain 60X



8

Mediastinal Lymph Node GMS Stain 100X



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Yeast Histopathology

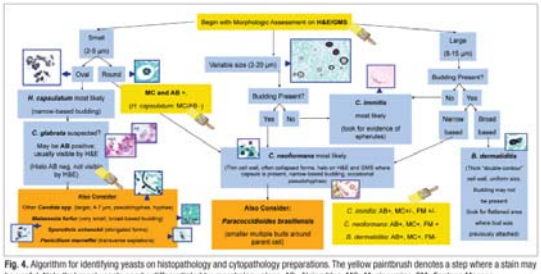

Fig. 4. Algorithm for identifying yeasts on histopathology and cytopathology preparations. The yellow paintbrush denotes a step where a stain may be useful. Note that most yeasts can be differentiated by morphology alone. AB=Alcian blue, MC=Mucicarmine, FM=Fontana Masson.

CAP Today, December 2010, Trail of Clues Leads to Infectious Organisms (Fig. by Dr. Bobbie Pritt, Mayo Clinic)

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Sensitivity of laboratory tests for diagnosis of pulmonary Histoplasmosis (Percent positive)

Test	Acute or subacute, pericarditis, rheumatological pulmonary,	Chronic pulmonary	Mediastinal
Antigen	25–75	15	0
Fungal stain	10	40	<25
Culture	15	50–85	<25
Serology	95	100	67

Adapted from *TRENDS in Microbiology Vol.11 No.10 October 2003 489.*

In acute pulmonary disease, the sensitivity of antigen detection ranges from about 25% in patients with local manifestations to over 75% in those who present within the first month of exposure.

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Table 1. Comparison of Diagnostic Tests in All Cases

	Disseminated cases (n = 158)				Pulmonary cases (n = 60)		
All tests	AIDS (n = 56)	OIC (n = 87)	NIC (n = 15)	All (n = 158)	Acute cases (n = 6)	Subacute cases (n = 46)	Chronic cases (n = 8)
Culture	34/48 (70.8)	57/75 (76.0)	7/9 (77.8)	98/132 (74.2)	0/3 (0)	14/26 (53.8)	4/6 (66.7)
Pathology	18/25 (72.0)	32/43 (74.4)	8/8 (100)	58/76 (76.3)	0/2 (0)	8/19 (42.1)	3/4 (75.0)
Antigen	53/56 (94.6) [13.80–7.67][a]	81/87 (93.1) [10.48–7.62]	11/15 (73.3) [6.92–7.65]	145/158 (91.8) [11.32–7.88]	5/6 (83.3) [2.41–2.26]	14/46 (30.4) [0.53–1.23]	7/8 (87.5) [0.93–0.83]
Antibody	15/19 (78.9)	37/53 (71.2)	8/9 (88.9)	60/80 (75.0)	4/6 (66.7)	39/41 (95.1)	5/6 (83.3)

NOTE. Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.
[a] Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody tests were positive in 2 (18.2%) of 11 patients who had undergone organ transplantation, 12 (85.7%) of 14 who were receiving tumor necrosis factor antagonists, and 20 (62.5%) of 32 with other causes for immunocompromise.

Table 2. Comparison of Diagnostic Tests in Proven Cases

	Disseminated cases (n = 111)				Pulmonary cases (n = 23)[a]	
Proven tests	AIDS (n = 38)	OIC (n = 62)	NIC (n = 11)	All (n = 111)	Subacute (n = 17)	Chronic (n = 5)
Culture	34/38 (89.5)	57/61 (93.4)	7/8 (87.5)	98/107 (91.6)	14/17 (82.4)	4/4 (100)
Pathology	18/23 (78.3)	32/38 (84.2)	8/8 (100)	58/68 (85.3)	8/12 (66.7)	3/4 (75.0)
Antigen	35/38 (92.1) [14.46–7.34][b]	58/62 (93.5) [11.90–7.44]	7/11 (63.6) [5.11–7.74]	100/111 (90.1) [12.10–7.82]	7/18 (38.9) [0.62–1.23]	4/5 (80.0) [0.63–0.54]
Antibody	9/13 (69.2)	16/19 (84.2)	6/7 (85.7)	41/56 (73.2)	12/13 (92.3)	3/3 (100)

NOTE. Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.
[a] None of the acute pulmonary cases were proven.
[b] Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody test results were positive in 2 (20%) of 10 patients who had undergone organ transplantation, 7 (87.5%) of 8 who were receiving tumor necrosis factor antagonists, and 14 (66.7%) of 21 with other causes for immunocompromise.

Diagnosis of Histoplasmosis, CID 2011:53, 448-454, Hage et al

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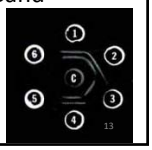
Laboratory Testing

- Histoplasma Urine Antigen – random 2/3/2014, None Detected
- EIA Histoplasma Ab screen - Equivocal
- CF Histoplasma mycelial Ab – negative
- CF Histoplasma yeast - 1:8
- Histoplasma Immunodiffusion – M Band

Sample Immunodiffusion
NOT FROM THIS CASE



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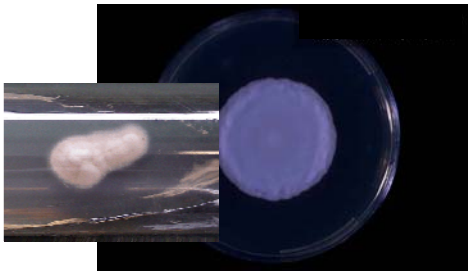
Mediastinal Lymph Node Colony After 19 Days of Incubation 30° C



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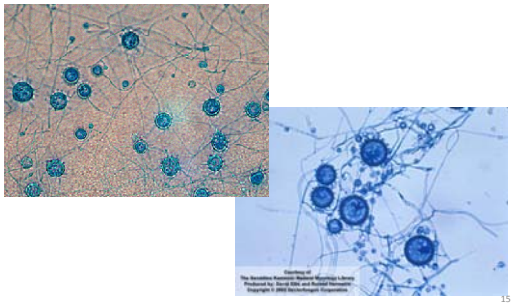
Scotch-Tape Preparation of Culture



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Identification of *H. capsulatum*

- SLIDE CULTURES SHOULD NOT BE PERFORMED
- Conversion of filamentous form to yeast form in culture (incubate 37° C)
- Exoantigen test
- DNA probe
- Nucleic Acid sequencing
- MALDI-TOF MS

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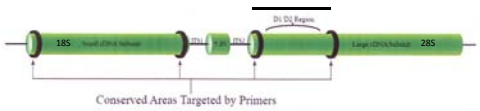
Fungal Sequence Identification



Region of fungal rRNA gene complex PCR amplified and cycle-sequenced by this assay.

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Nucleic Acid Sequence

```
>dbj|AB176510.1| Ajellomyces capsulatus gene for 28S rRNA, partial sequence,
isolate:IFM 49110 Length=574 Score = 1051 bits (569), Expect = 0.0
Identities = 571/572 (99%), Gaps = 0/572 (0%) Strand=Plus/Plus
Query 1   ACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAAGAGCTCAAATTTGAAATCC 60
Sbjct 3   ..............................................................  62
Query 61  GGCCCCCCTGGGGGCCTGAGTTGTAATTTGCAGAGGATGCTTCGGGCGCGACCGCGGTCC 120
Sbjct 63  ..............................................................  122
Query 121 AAGTCCCCTGGAACGGGGCGTCGTAGAGGGTGAGAATCCCGTCTCCGGCCGGCCGGTCTC 180
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Query 181 GCCCGTGTGAAGCTCCTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCCAAATGGGTGG 240
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Query 361 CGCTTGCGATCAGAGTCGGCCGCGGGGGTTCAGCGGGCATTCGTTGCCCGTGCAATCCCC 420
Sbjct 363 .........C.....................................................  422
Query 421 CGCGGCCGGGCCAGCGTCGGTTTCGACGGCCGGTCAAAGGCCCCCGGAATGTGTCGCCTC 480
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Query 481 TCGGGGCGTCTTATAGCCGGGGGTGCAATGCGGCCAGTCGGGACCGAGGAACGCGCTCCG 540
Sbjct 483 ..............................................................  542
Query 541 GCACGGACGCTGGCTTAATGGTCGTCAGCGAC 572
Sbjct 543 ................................ 574
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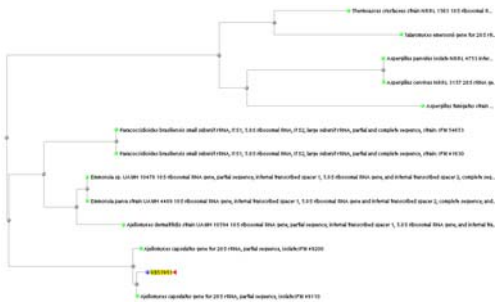
Distance Tree



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Background *Histoplasma capsulatum*

- Single species in genus, 3 varieties of species
- Endemic in the lower Mississippi river valley and Ohio river valley
- Found in soil enriched with guano of various birds or bats, often found around chicken coups
- Spread though inhalation of microconidia in disturbed soil infected with *H. capsulatum*
- Among Medicare beneficiaries, most common endemic mycosis in the US
- Estimated that 500,000 people are newly infected each year in US

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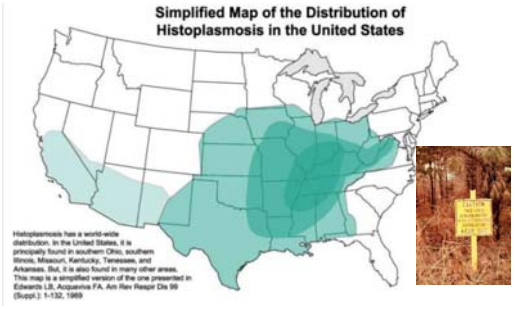
US Distribution of *H. capsulatum*



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Hunting for Histo?

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Painful Hip

- 78 y/o ♂
- Widower, lives alone, does have "lady friends"
- Non-insulin dependent diabetes
- Silicosis diagnosed in 1976
- Atrial fibrillation
- Prostate cancer, post seed implantation
- Not immunosuppressed
- Painful loose left total hip (done 1993/ re-done 2008) Fluoroscopy procedure aspirated 1.5 ml clear yellow fluid in February 2011

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Hip Fluid February 2011

- Cytospin Gram stain
  - rare PMNs, NOS
- Hematology
  - No crystals
  - Nucleated cell count 1,115/µl
  - Differential –
    - 45% PMNs
    - 43% Lymphocytes
    - 3% Monocytes
    - 7% Synovial cells
    - 2% Macrophage

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Hip Fluid February 2011

- 4 days later culture grew 7 colonies
  - "Very round", small yeast
  - Rapid urea +
  - Rapid trehalose –
  - Set up DNA sequencing

26

Nucleic Acid Sequence

>gb|FJ534909.1| Cryptococcus neoformans var. grubii strain CBS 8710 large subunit ribosomal RNA gene, partial sequence Length=629 Score = 1146 bits (620), Expect = 0.0 Identities = 620/620 (100%), Gaps = 0/620 (0%)
Strand=Plus/Plus

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Query 541 GCAGCTCGCCTTTATGGCCGGGGTTCGCCCACGTTCGAGCTTAGGATGTTGACAAAATGG 600
Sbjct 544 ..................................................................... 603
Query 601 CTTTAAACGACCCGTCTTGA 620
Sbjct 604 .................... 623
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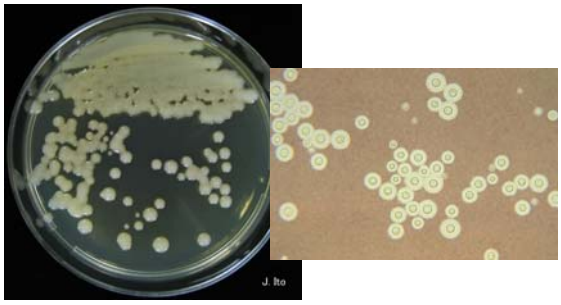
27

Distance Tree

28

Cryptococcus neoformans



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History March 2011

- March 17 hip hardware removed
  - Replaced with an antibiotic-impregnated temporary femoral stem
  - Tissue collected and submitted for culture
  - Few days later tissue grew rare *C. neoformans*

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Background *Cryptococcus spp.*

- Genus *Cryptococcus* contains many species (19-30)
- *C. neoformans* and *C. gattii* are the main human pathogens
- *C. neoformans* found in soil enriched with guano from pigeons (and other birds) and aged pigeon guano
- *C. neoformans* worldwide distribution
- *C. gattii* found around flowering *Eucalyptus camaldulenis* trees and coniferous trees native to the pacific northwest
- Spread though inhalation of fungus in disturbed soil infected with *C. neoformans* or plant debris infected with *C. gattii*
- Prior to late 1990's *C. gattii* found predominantly in the tropics and sub-tropics

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Centers for Disease Control and Prevention
MMWR Morbidity and Mortality Weekly Report
Weekly / Vol. 59 / No. 28 July 23, 2010

Emergence of *Cryptococcus gattii* — Pacific Northwest, 2004–2010

- *C. gattii* previously *C. neoformans var. gattii*
- 1999 *C. gattii* outbreak on Vancouver Island, BC (as of 2007 a total of 218 cases in the area)
- December 2004 a *C. gattii* case in Oregon
- As of July 2011, 96 *C. gattii* cases in Oregon, Washington, Idaho, and California
  - 46 patients had no travel history to Canada

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North American of Geography of *C. gattii*

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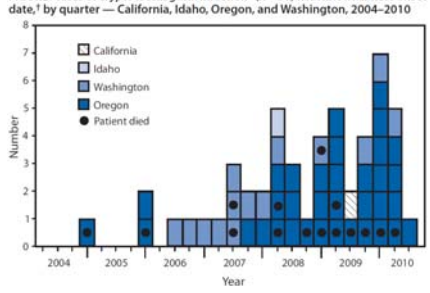
FIGURE. Cases of *Cryptococcus gattii* infection* (n = 51) with known illness onset date,† by quarter — California, Idaho, Oregon, and Washington, 2004–2010



Source: *Cryptococcus gattii* Public Health Working Group.
* Defined as illness occurring on or after January 1, 2004, in a U.S. resident with a culture-confirmed isolate of *C. gattii*.
† Includes estimated date for one patient each in 2007, 2008, and 2010, and two patients in 2009.

59:865-868, 2010 MMWR

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Possible Areas Endemic for *Cryptococcus gattii*

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Differences Between *C. gattii* and *C. neoformans*

Characteristic	*Cryptococcus gattii*	*Cryptococcus neoformans*
Ecological Niche	*Eucalyptus* trees; Native trees of the Pacific NW (e.g. Douglas fir)	Bird Guano
Location	Tropical & subtropical; Pacific NW; possibility for further spread	Worldwide
Immune Status of Host	Immuno-competent (>50%)	Immuno-compromised (>80%)
Lung Manifestations	Commonly nodules	Commonly infiltrates
Brain Lesions	More common	Less common
Hospital Stay and Duration of Therapy	Longer	Shorter

January 18, 2011, ASM

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Table 2. Laboratory tests for the differentiation of *C. gattii* from *C. neoformans*

Test	Differentiates	Does not differentiate
Serum tests:		
a) Cryptococcus antigen		X (positive for both species)
b) β-1-3-D-glucan		X (negative for both species)
Direct specimen stains:		
a) Gram		X
b) India Ink		X
c) Calcofluor		X
d) Mucicarmine		X
e) GMS		X
Appearance:		
a) Colony morphology		X
b) Cornmeal-Tween 80 morphology		X
c) Temperature and media requirements		X
Commercial biochemical identification systems e.g. VITEK 2 ID-YST, MicroScan		X
Biochemical tests:		
a) Urease		X (positive for both species)
b) Melanin production (phenyloxidase)		X (positive for both species)
c) CBG agar	X	
Serological typing of isolates	X (no longer commercially available)	
PCR	X (target-dependent)	
DNA sequencing	X	

January 18, 2011, ASM

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L-Canavanine Glycine Bromothymol Blue Agar

FIG. 1. Reactions of various cryptococci on CGB agar. The left plate depicts a positive reaction with *C. gattii*; the middle plate shows a weak reaction around the inoculum, which is interpreted as negative; and the right plate shows a negative reaction with *C. neoformans*.

Available from REMEL

47:3669-3672. 2009 JCM

38

Distance Tree

39

Nucleic Acid Sequence

```
gb|CP000287.1|Cryptococcus gattii WM276 chromosome B, complete sequence Length=2187695 Score =
1123 bits (608), Expect = 0.0 Identities = 616/620 (99%), Gaps = 0/620 (0%) Strand=Plus/Plus
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Query 121     TGCTGGACCGTGTCTAAGTCCCTTGGAATAGGGTATCAAAGAGGGTGACAATCCCGTACT 180
Sbjct 1521249 ............................................................ 1521308
Query 181     TGACACGATCACCAGTGCTCTGTGATACGTTTTCTACGAGTCGCGTTACTTGGGAGTGTA 240
Sbjct 1521309 ............................................................ 1521368
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Sbjct 1521669 ............................................................ 1521728
Query 601     CTTTAAACGACCGTCTTGA 620
Sbjct 1521729 ................... 1521748
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I Got Wacked!

- 16 y/o ♂
- In normal state of good health
- Modest acne
- Mows lawns part time
- Presented with an asymptomatic non-healing "skin tag"-like lesion on left index finger

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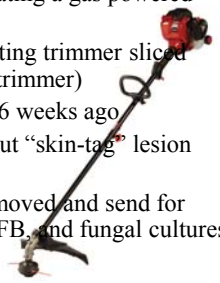
I Got Wacked!

- Injured finger while operating a gas powered string trimmer
- Spinning string from rotating trimmer sliced finger (he was operating trimmer)
- Accident occurred about 6 weeks ago
- Original wound healed, but "skin-tag" lesion remained
- "Skin-tag"-like lesion removed and send for histology and bacteria, AFB, and fungal cultures



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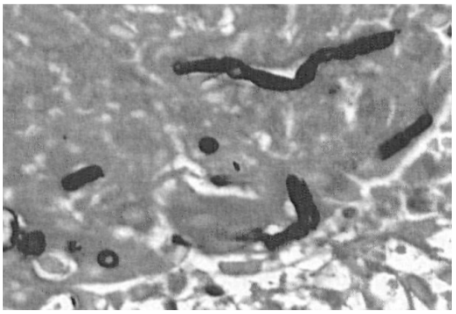
"Skin-Tag" GMS Stain 60X



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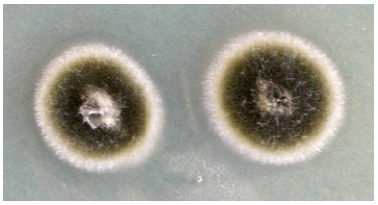
"Skin-Tag" Fungal Culture after 4 Days



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Scotch-tape Preparation of Fungal Culture

Alternaria sp.

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Table 7. Major Causes of Human Skin, Nail, and Hair Infections by Nondermatophytic Fungi

Skin and Nail Infections	Hair Infections
Acremonium spp.	*Piedraia hortae*
Alternaria spp.	*Trichosporon* spp.
Aspergillus spp.	
C. albicans	
E. dermatitidis	
Fusarium spp.	
Hortaea werneckii	
Lasiodiplodia theobromae	
Neoscytalidium spp.	
Onychocola canadensis	
Pyrenochaeta unguis-hominis	
Scopulariopsis breviculis	

CLSI M54-A, 2012, Principles and Procedures for Detection of Fungi in Clinical Specimens-Direct Examination and Culture; Approved Guideline

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Background *Alternaria spp.*

- Genus *Alternaria* contains 44 species
- Very common in the environment
- Most are plant pathogens, but a few can be found in the soil
- Commonly considered a saprophytic contaminate of clinical specimens, may occasionally can cause a true infection
- Infections in subcutaneous tissue, eye, nail, skin, nasal sinuses, and peritonitis
- Exposure may play a role in asthma and hypersensitivity pneumonitis

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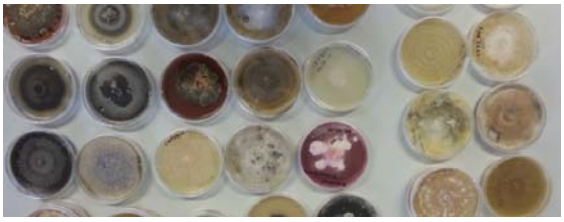
The End



St Mary's Hospital
LABORATORY SERVICES

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