# Top five papers in mycology: the lab perspective

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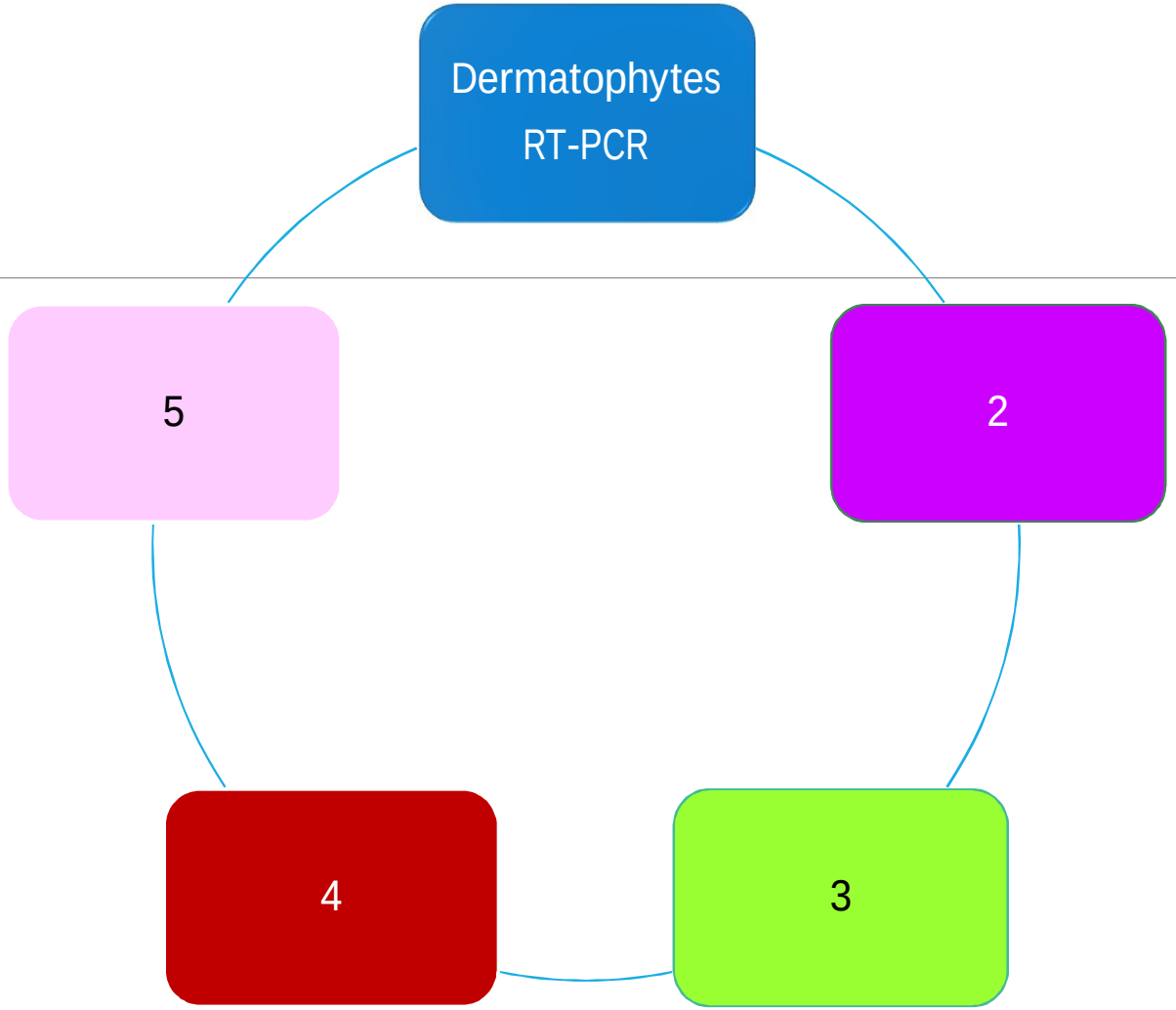
# 5 topics

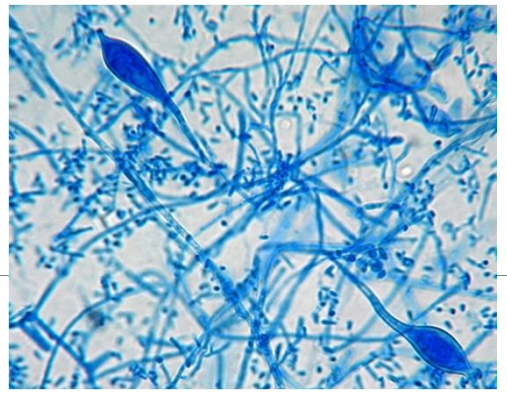


ORIGINAL ARTICLE

*Mycoses. 2018;61:119–126*

## Evaluation of multiplex real-time PCR for identifying dermatophytes in clinical samples—A multicentre study

Shany Sherman[1] | Maya Goshen[2] | Orit Treigerman[3] | Keren Ben-Zion[2] | Marie-Jeanne Carp[2] | Noam Maisler[2] | Inbal Binsky Ehrenreich[2] | Aviva Kimchi[4] | Sara Lifshitz[4] | Gill Smollan[5] | Batya Davidovici[1,6] | Michael David[1,6] | Emmilia Hodak[1,6] | Rina Segal[1,6]



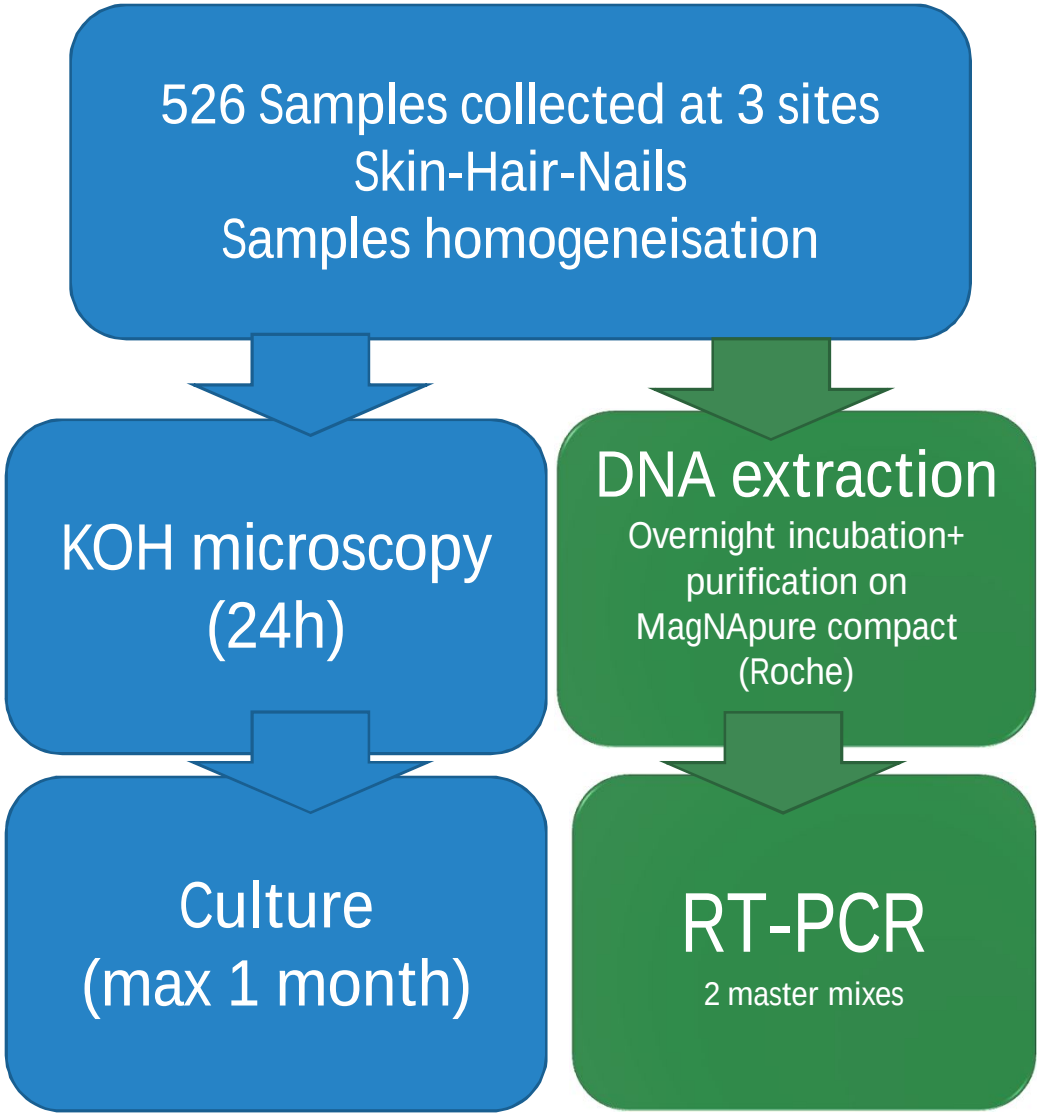
*In house*, Multiplex Real-Time PCR (LightCycler 480)
Comparison with culture+microscopy
526 SKIN HAIR & NAILS samples
Collection on 3 sites in Israël
Retrospective study

## Objectives

1. To develop and test a multiplex RT-PCR for identification of the most common dermatophytes in Israël

2. To implement a new diagnostic algorithm

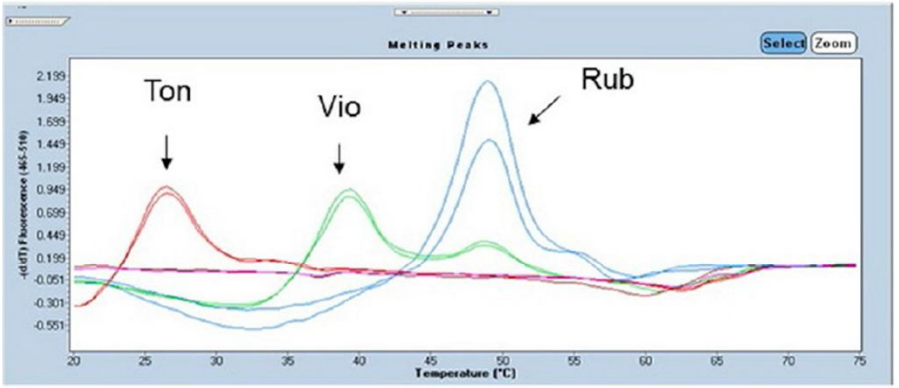
# Materiels & Methods



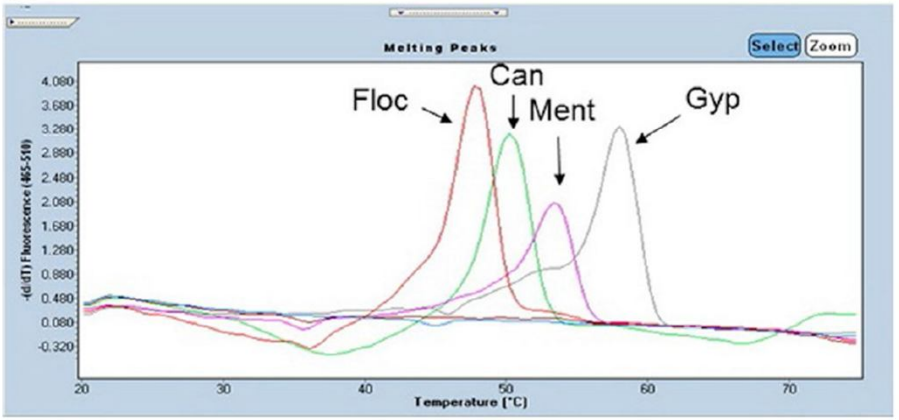
# RT-PCR (ITS1-ITS2)
## with Melting curve analysis

| Master mix | Peak temp °C | Genotype |
|---|---|---|
| MMX1 | 26°C | *Trichophyton tonsurans* |
| MMX1 | 40°C | *Trichophyton violaceum* |
| MMX1 | 49°C | *Trichophyton rubrum* |
| MMX2 | 46°C | *Epidermophyton floccosum* |
| MMX2 | 50°C | *Microsporum canis* |
| MMX2 | 53°C | *Trichophyton mentagrophytes* |
| MMX2 | 59°C | *Microsporum gypseum* |

(A)




(B)

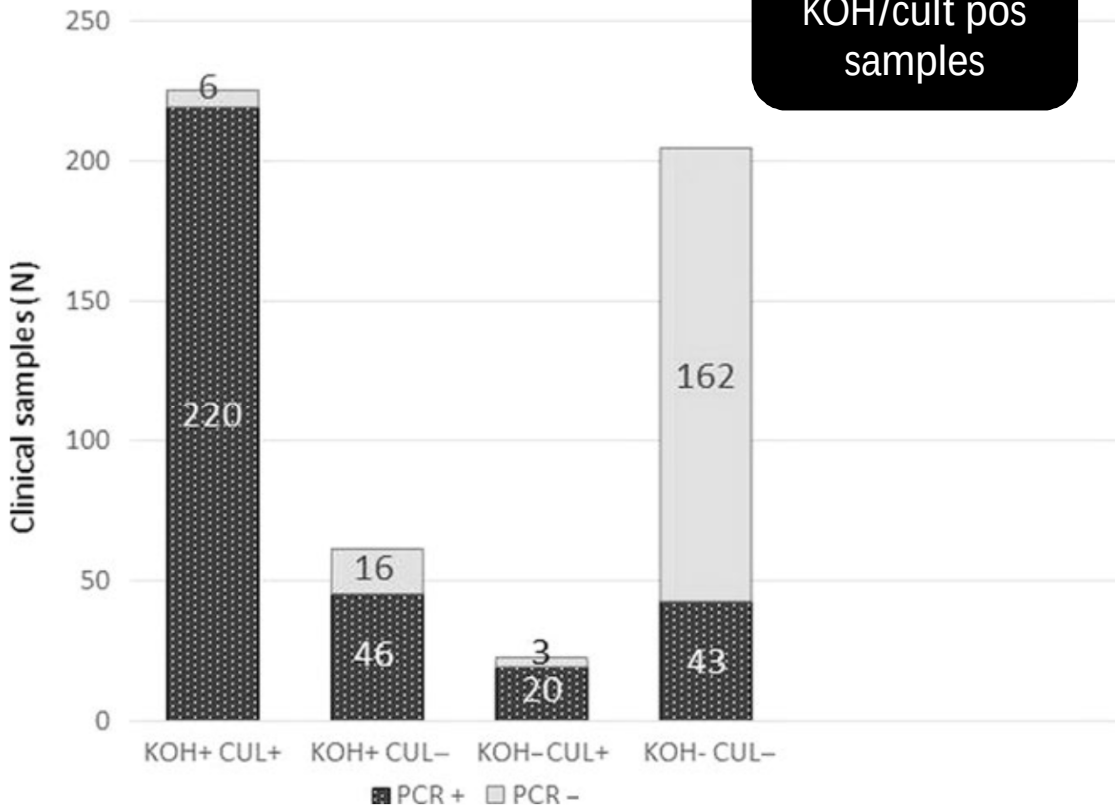


# RESULTS from 3 sites (N=526)

PCR POS in 96% KOH/cult pos samples

☹ Specificity: cross reaction between *M. canis* & *M. audouinii*

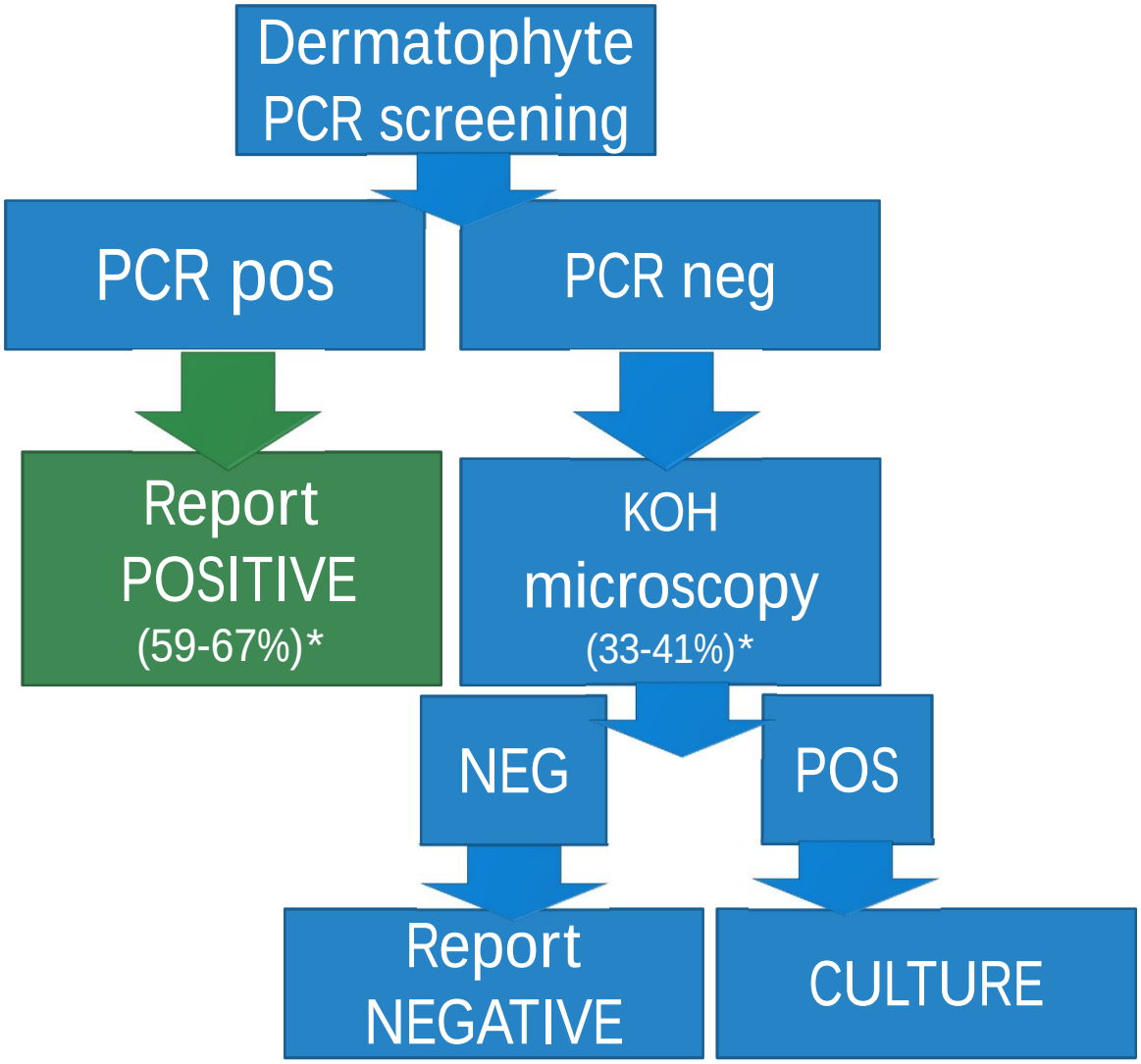
*-Gold standard: KOH and/or cult positive*
*-PCR sensitivity: 92%*
*-PCR specificity: 79%*
*-Additional positive PCR cases not detected in culture: 10-30%*



# Comparison with other studies

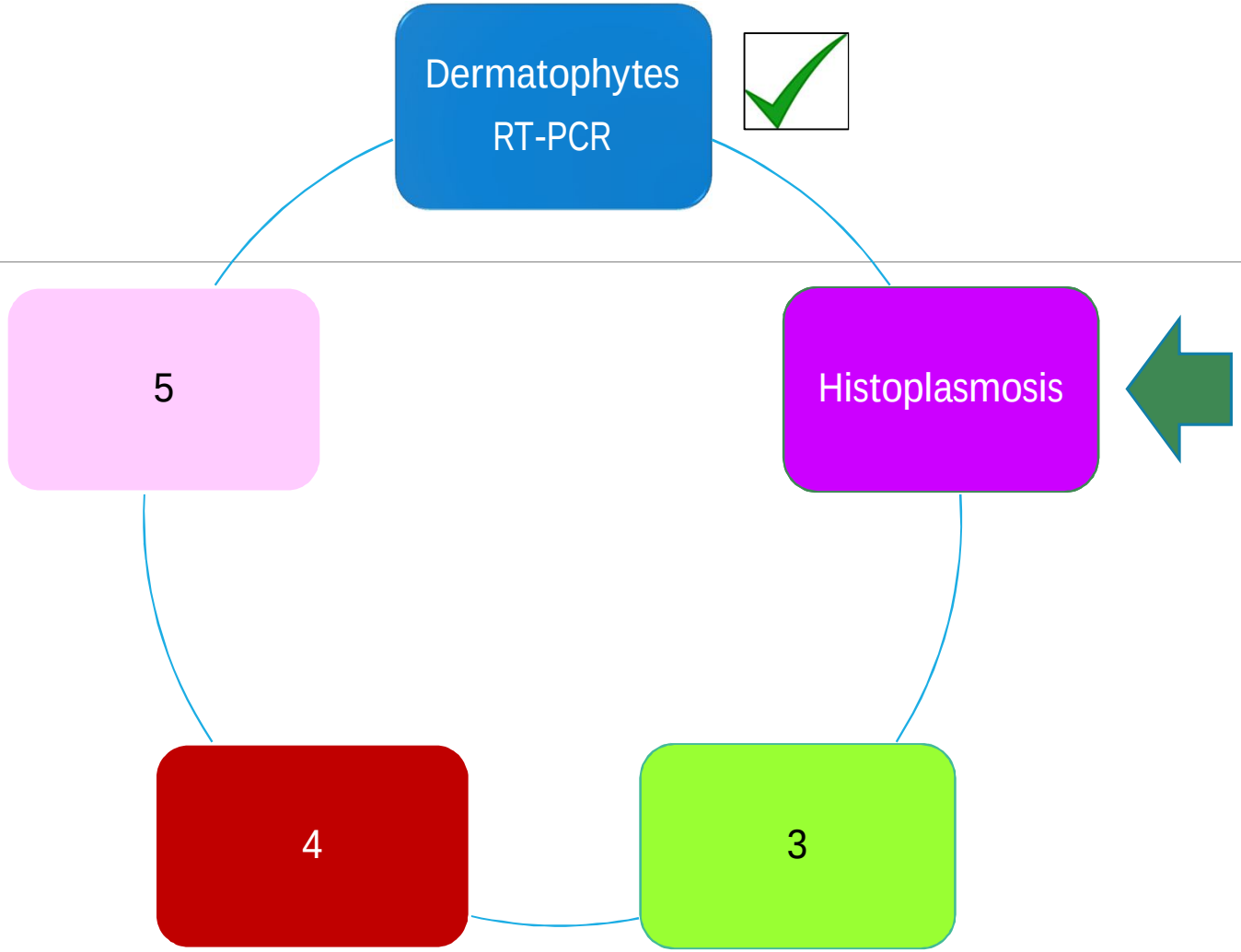
| Reference | No. of samples | Dermatophyte strains in PCR | KOH | Culture n (%) | PCR, n (%) | p/c parameter* |
|---|---|---|---|---|---|---|
| Arabatziz et al[3] | 92 | 6 | 40 | 30 (33%) | 47 (51%) | 1.56 |
| Brillowska-Dabrowska et al[20] | 118 | 1 (*T. rubrum*) | NA | 27 (23%) | 49 (42%) | 1.83 |
| Bergmans et al[8] | 120 | 11 | 57 | 45 (38%) | 74 (62%) | 1.64 |
| Wisselink et al[16] | 1437 | 5 | 0 | 307 (21%) | 697 (49%) | 2.27 |
| Alexander et al[15] | 862 | 1 (*T. rubrum*) | 862 | 470 (55%) | 446 (52%) | 0.95 |
| Bergman et al[17] | 202 | 2 | 0 | 79 (39%) | 103 (51%) | 1.30 |
| Sherman et al, present study | | | | | | |
| RMC | 223 | 7 | 126 (out of 213) | 127 (57%) | 149 (67%) | 1.17 |
| HMO | 200 | 7 | 103 | 88 (44%) | 118 (59%) | 1.34 |
| Military | 103 | 7 | 59 | 37 (36%) | 68 (66%) | 1.84 |

# New Algorithm proposed




* performances of the tests in the present study

# 5 topics



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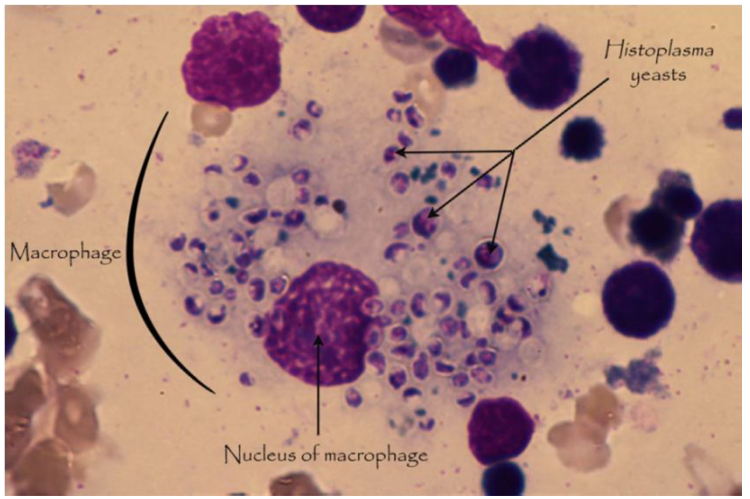
MINIREVIEW

# Laboratory Diagnostics for Histoplasmosis

**Marwan M. Azar,[a] Chadi A. Hage[b]**

Department of Pathology, Section of Microbiology, Massachusetts General Hospital, Boston, Massachusetts, USA[a]; Department of Medicine, Thoracic Transplantation Program, Indiana University, Indianapolis, Indiana, USA[b]




## Background

Histoplasmosis: the most endemic mycosis in South America

Wide spectrum of disease: pulmonary to disseminated, acute or chronic

Emerging imported cases in Europe: Liège: 2 cases in 2017!

## Objectives

1. To synthesize the currently available laboratory diagnostics for histoplasmosis,
2. To assess the assays performance in **various clinical contexts**.

# Laboratory diagnosis of Histoplasmosis

| | |
|---|---|
| 1. Diagnostic microscopy/histology: | sensitivity 9-43% |
| 2. Culture (up to 6 weeks) | sensitivity 15-85% |
| 3. Immunodiagnostic tests | sensitivity: 50-81% |
| 4. PCR | sensitivity: |

**EORTC/MSG Dimorphic Fungi**

✓Proven : culture or histology
✓Probable: appropriate clinical presentation, a predisposing condition, and mycological evidence, such as the presence of antigenuria

## Culture

**Gram stain**: low sensitivity prefer **Calcofluor white**

**Culture** (Sabouraud) 30°C: 2-3 weeks (up to 6 weeks): gold standard

**ID**: microscopy (DD Sepedonium), prefer PCR/Seq for confirmation

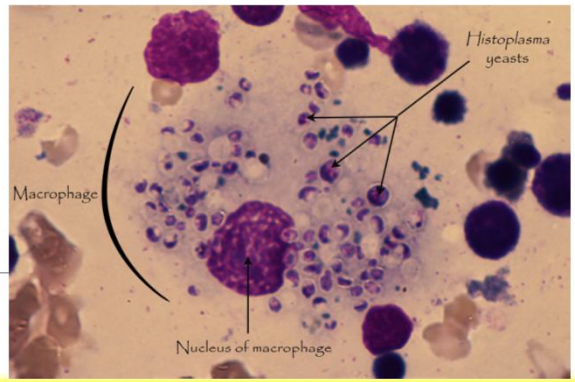
**TABLE 1** Summary of diagnostic test for histoplasmosis[a]

| | % histoplasmosis result by type | | | |
|---|---|---|---|---|
| Test | Acute pulmonary | Subacute pulmonary | Chronic pulmonary | Progressive disseminated |
| Culture | 0–20 | 53.8 | 66.7 | 74.2 |

In patients with HIV/AIDS, respiratory cultures may be positive in up to 90%, while blood cultures may be positive in up to 50%

## Histology (PAS/Gomori)

Yeats inside macrophages, numerous but can be scarse (non-HIV )
DD: *other yeast or parasites*

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| Culture | 0–20 | 53.8 | 66.7 | 74.2 |
| Pathology | 0–42 | 42.1 | 75.0 | 76.3 |

Nonviable organisms may be found in in mediastinal or lung granuloma tissues for many years after initial infection→ incomplete granulomas and/or fibrosis

**Antigen (blood/Urine)**

Reference test: MiraVista EIAs 3rd generation (USA)

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| Pathology | 0–42 | 42.1 | 75.0 | 76.3 |
| Antigen | 82.8–83.3 | 30.4 | 87.5 | 91.8 |

- AIDS patients: Ag detection in urines has a higher sensitivity (95%)
- Ag in urines: equal sensitivity than in blood (*Sherman Mycoses 2017*)
- Histoplasmosis meningitis: sensitivity of Ag in CSF : 40-65%
- Monitoring of Ag clearance in serum: in HIV/AIDS patients: <2ng/ml →antifungal discontinuation
- Drawback: cross reactivity with other Ag: other dimorphic, Aspergillus sp.

**Serology** (4-8 weeks)

Immunodiffusion (ID)
Complement Fixation
EIA

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| Antigen | 82.8–83.3 | 30.4 | 87.5 | 91.8 |
| Serology | 64.3–66.7 | 95.1 | 83.3 | 75 |

- More useful for subacute and chronic forms (Ag less performing)
- CF/ titer of 1:8 is positive, indicating previous exposure to *H. capsulatum*.
  titer of 1:32 or a 4-fold rise in antibody titer from acute- to convalescent-phase serum is strongly suggestive of active infection
  titers decrease SLOWLY with disease resolution but incompletely
  CF>ID in sensitivity. Both are > EIA for specificity.

**Molecular (PCR)**

**No test FDA approved!**

- Can be performed in blood, serum, tissue, ….
- Highly specific BUT comparison with reference tests must be performed→new reference method?
- Vairable sensitivity, 100%specificity in studies

| Molecular method[a] | *H. capsulatum* molecular target[b] | No./type of patients[c] | No. of clinical samples | Specimen source(s) (*n*)[d] | Comparator test | Sensitivity (%) | Specificity (%)[e] |
|---|---|---|---|---|---|---|---|
| LAMP (32) | *hcp100* gene locus | 6 HIV+ with PDH, 10 controls | 16 | Urine | Culture | 67 | 100 |
| Nested PCR (31) | *hcp100* gene locus | 15 HIV+ with PDH*, 12 controls* | 40 | Bone marrow (11), hepatic biopsy sample (9), bronchial aspirations (6), BAL fluid (4), lymph node (2), gut biopsy (2), blood (2), CSF (2), serum (2) | Culture | 100 | 100 |
| PCR-EIA (34) | *H. capsulatum*-specific gene sequence (99 bp) | 51 with positive urine *Histoplasma* antigen, 25 controls | 76 | Urine | Culture | 80 | 100 |
| | | | | | Urine antigen (1–19.9 U) | 0 | NR |
| | | | | | Urine antigen (>20 U) | 18.5 | NR |
| Real-time PCR (30) | 192-bp region of *GAPDH* gene | Suspected fungal infection, N NR | 797 (15 culture-positive samples) | Bronchial washings (346), BAL fluid (212), pleural fluid (157), tracheal secretions (35), tissue (14), sputum (13), lung washes (6), blood (4), bone marrow (5), peritoneal fluid (3), other body fluids (2) | Culture | 73 | 100 |
| FISH (35) | Ribosomal 18S subunit | 3 HIV+ with clinical diagnosis of invasive mycosis, 30 controls | 33 | Blood culture | Culture | 100 | 100 |
| PCR (35) | rRNA | 3 HIV+ with clinical diagnosis of invasive mycosis, 30 controls | 33 | Blood | Culture | 100 | 100 |
| Real-time PCR (44) | *H. capsulatum*-specific gene sequence (99 bp) | 9 with histoplasmosis | 9 | FFPE | Culture | 89 | ND |
| Real-time PCR (33) | Internal transcribed spacer region of rRNA gene complex | Suspicion for clinical mycoses, N NR | 348 (71 culture-positive samples) | Bone marrow (108), CSF (55), blood (48), BAL fluid (43), intestinal biopsy (31), liver biopsy (30), lymph nodes (25), skin biopsy (8) | Culture | 96 | 96 |
| PCR (36) | *RYP1* gene | 15 HIV+ with histoplasmosis, 6 controls | 21 | Blood | Diagnosis of histoplasmosis (specific comparator not reported) | 87 | 100 |
| Nested PCR (37) | Conserved regions of NAALADase genes | 5 with proven (4) or probable (1) histoplasmosis per EORTC criteria | 9 | Serum (4), FFPE (4), BAL fluid (1) | Diagnosis of histoplasmosis per EORTC criteria | 77 | ND |
| Real-time PCR (37) | Conserved regions of NAALADase genes | 5 with proven (4) or probable (1) histoplasmosis per EORTC criteria | 9 | Serum (4), FFPE (4), BAL fluid (1) | Diagnosis of histoplasmosis per EORTC criteria | 33 | ND |
| Nested PCR (38) | *hcp100* gene locus | 7 with acute pulmonary histoplasmosis | 7 | Serum | Serology (EIA; titer range, 1:320–1:2,560) | 86 | ND |
| Simplex PCR (38) | *H. capsulatum*-specific gene sequence (1281–1283 [220] bp) | 7 with acute pulmonary histoplasmosis | 7 | Serum | Serology (EIA; titer range, 1:320–1:2,560) | 86 | ND |

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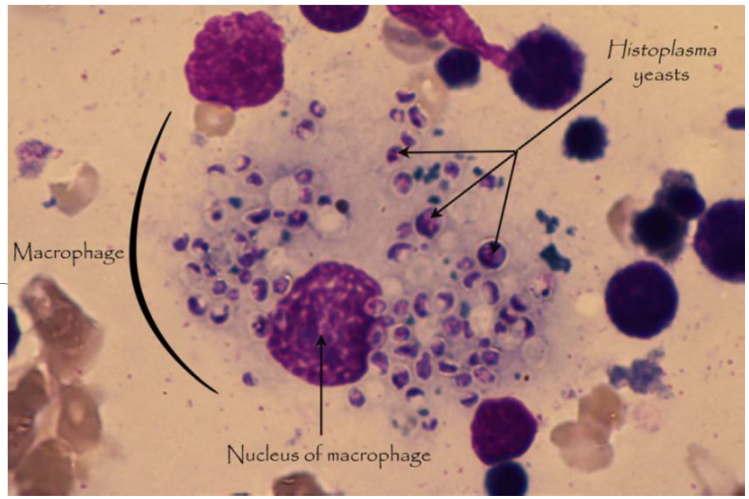
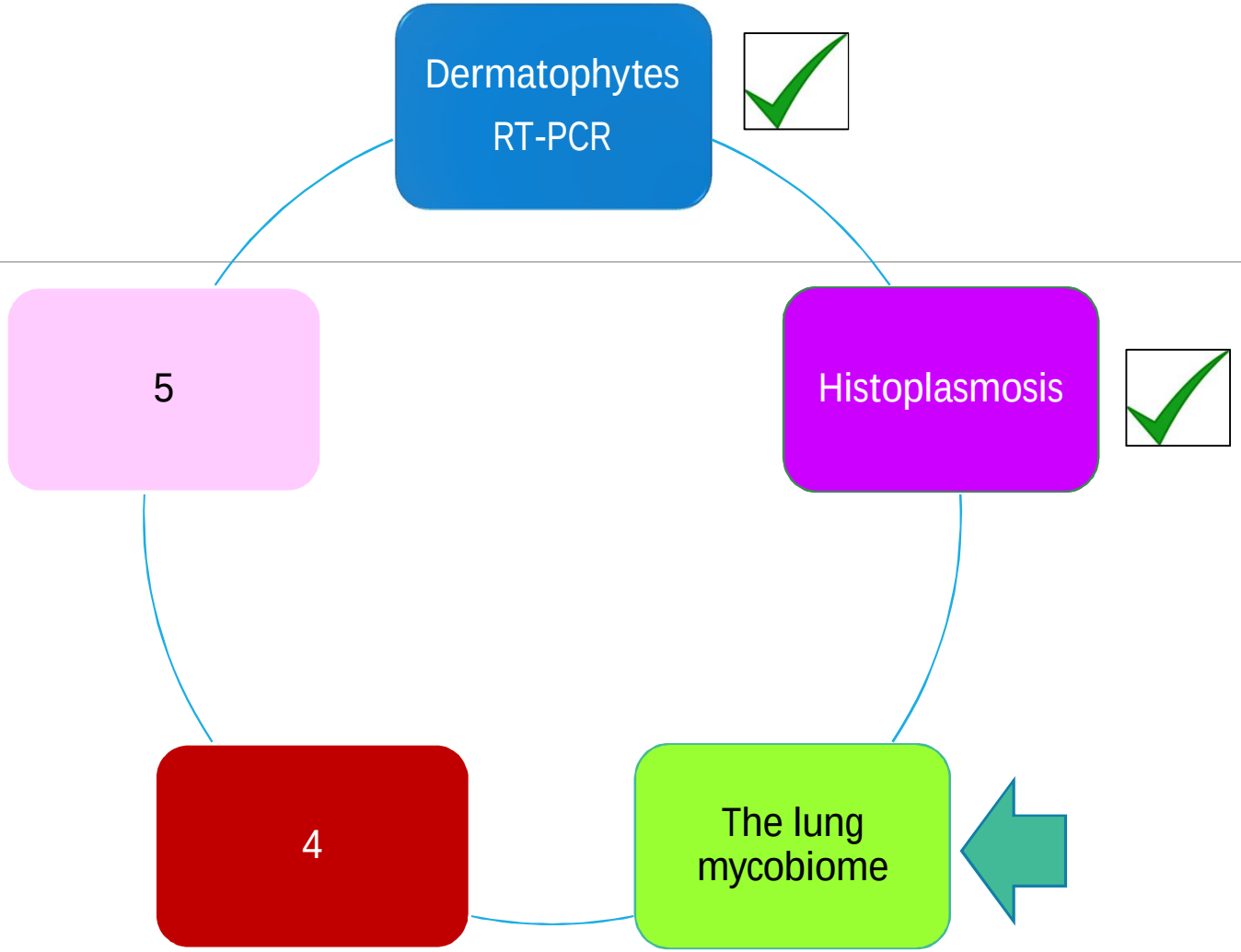



## Conclusion

Consider the clinical presentation and talk with your microbiologist!

# 5 topics


Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
4
5

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 13 February 2015
doi: 10.3389/fmicb.2015.00089

# The lung mycobiome: an emerging field of the human respiratory microbiome

**Linh D. N. Nguyen[1], Eric Viscogliosi[1] and Laurence Delhaes[1,2]***

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[2] Parasitology-Mycology Department, Hospital University Center, Faculty of Medicine, Lille, France

Objective
Review the knowledge of this emerging field

WHAT IS KNOWN in lung mycobiome

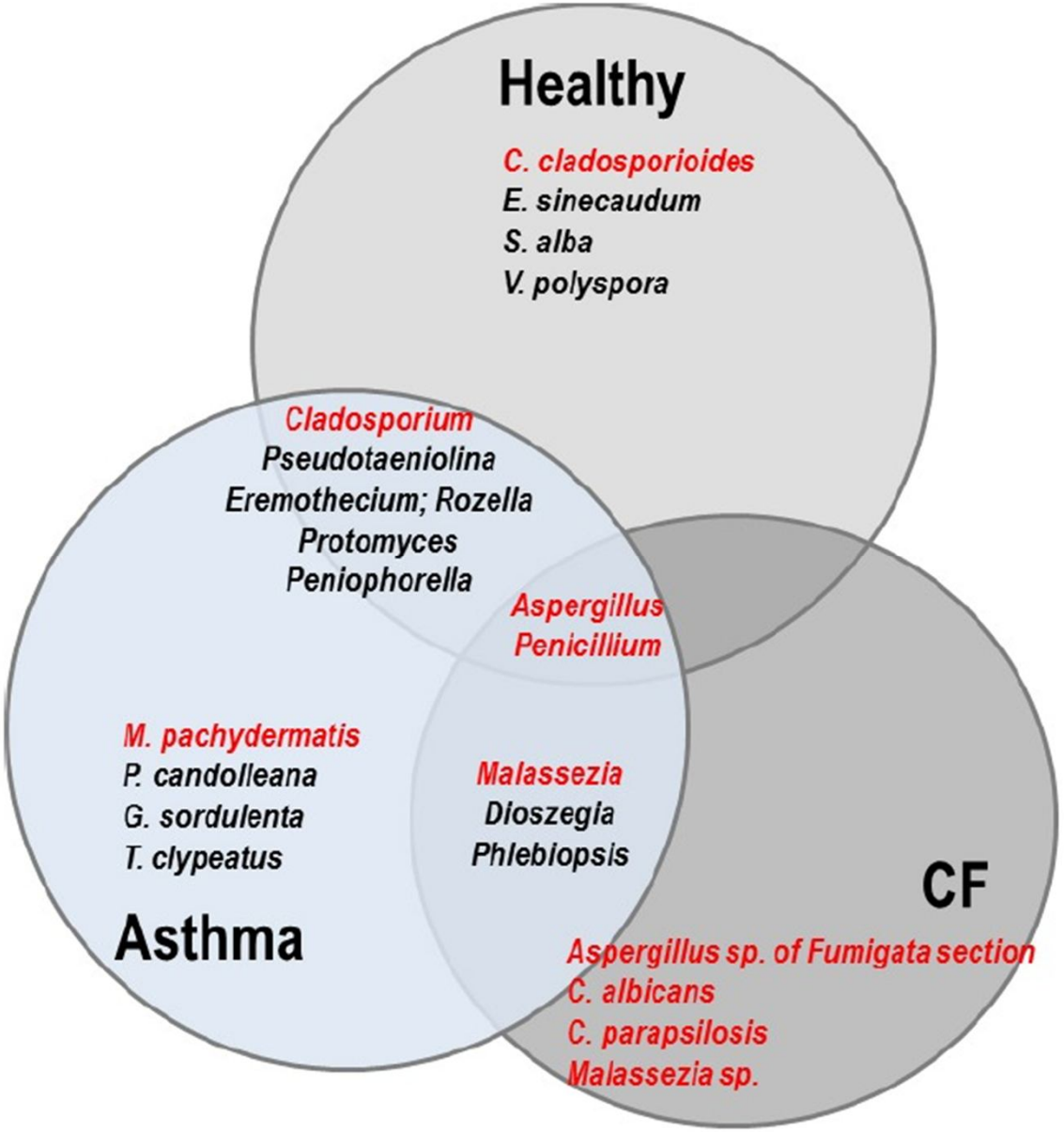
1. Fungi are present even in healthy people
2. Composition is highly variable between individuals
3. Fungi are <<<bacteria or viruses the lung
4. CRD are associated with a decrease of fungal diversity

the lung mycobiome (previously named the fungal microbiota or microbiome) has drawn closer attention. There is growing evidence that the lung mycobiome has a significant impact on clinical outcome of chronic respiratory diseases (CRD) such as asthma, chronic obstructive pulmonary disease, cystic fibrosis, and bronchiectasis. Thanks to advances in culture independent methods, especially next generation sequencing, a number of fungi

especially the gut, has also been unraveled. By interacting with the bacteriome and/or virome, the respiratory mycobiome appears to be a cofactor in inflammation and in the host immune response, and therefore may contribute to the decline of the lung function and the disease progression. In this review, we report the recent limited explorations of

# The lung mycobiome

- Most frequent phila: Ascomycota and Basidiomycota
- **Healthy people**: various genus dominated by environmental agents such as *Cladosporium, Eurotium, Aspergillus, Penicillium* …
- Data from NGS studies reveal that cultures do not reflect the reality!
- Limitations in detecting the dynamics of interactions between different populations: viruses and fungi or mold impact in CRD.

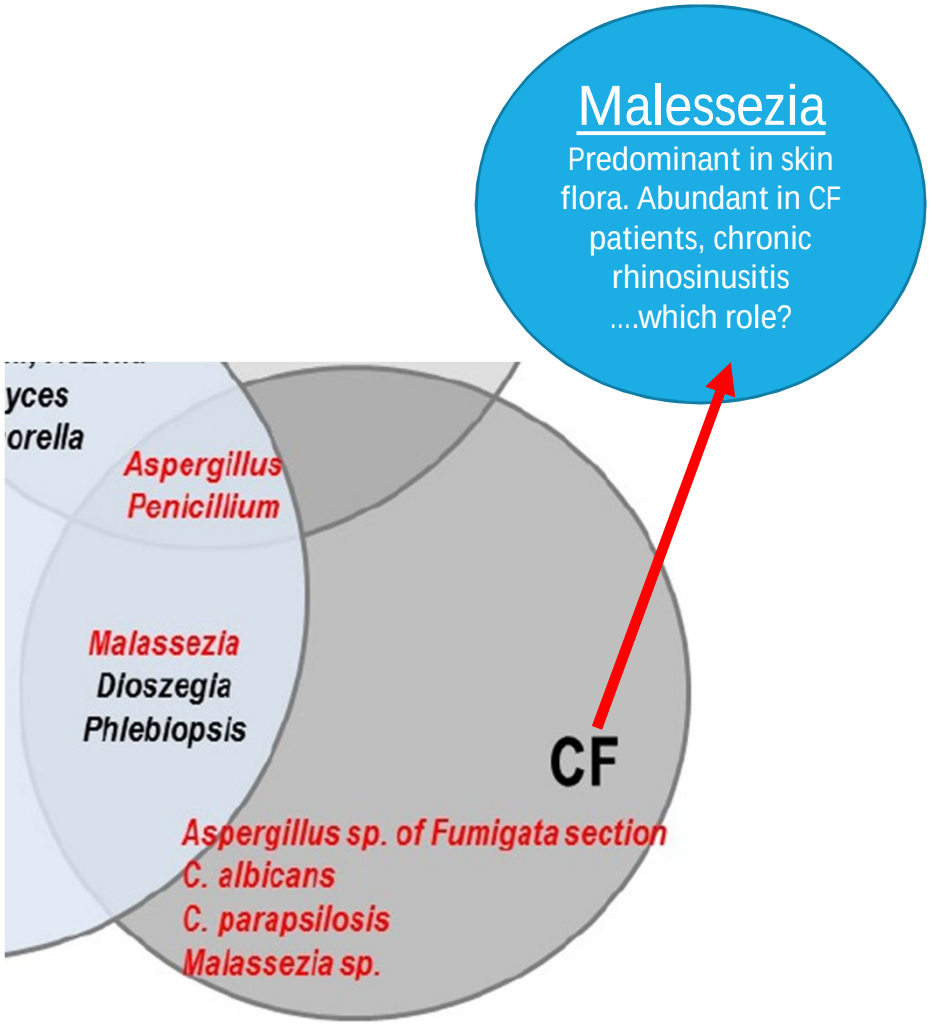


Studies from Dehlaes and Charlson , 2012,
*in CF and transplant patients: *C. albicans*, *Aspergillus* spp., *Penicillium*, *Cryptococcus* ,*Eurotium*, in which *Candida* species dominated
+ Reduced fungal diversity in these population.

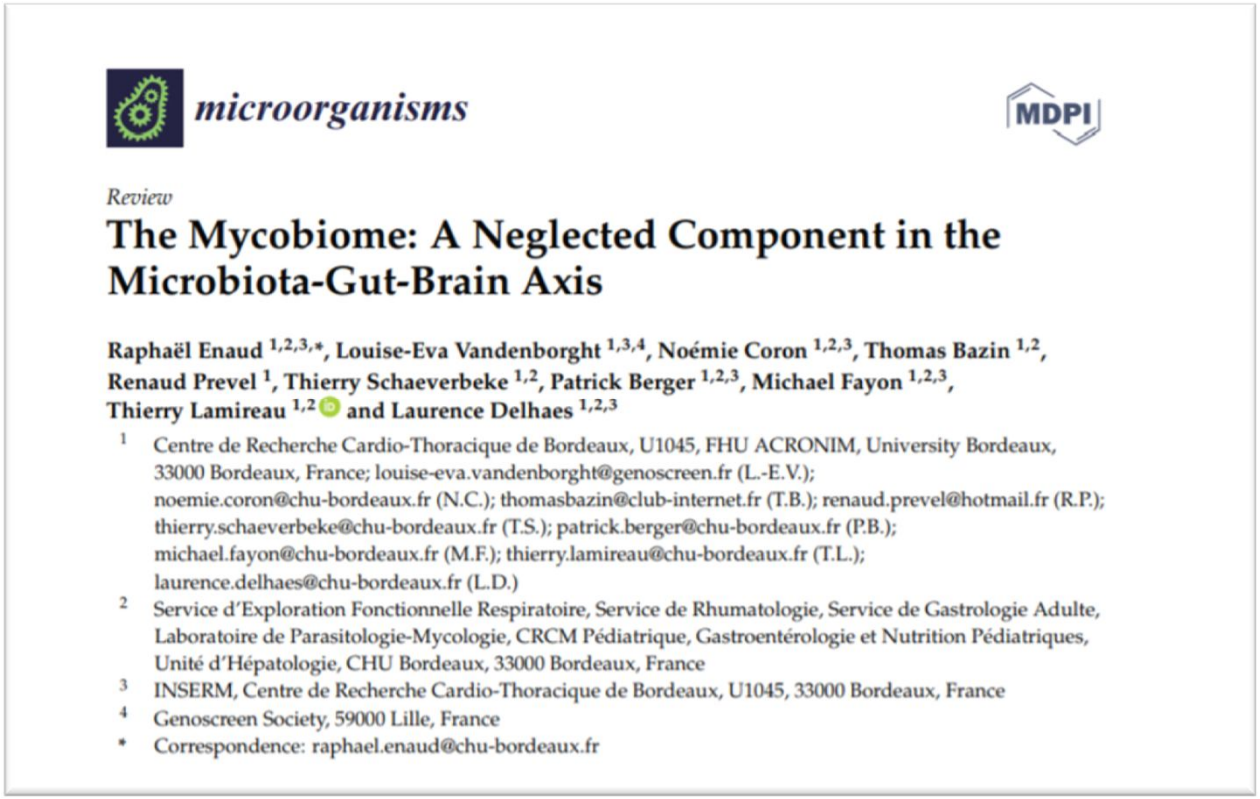
Which role play fungal agents in CF?

- *C. albicans* has been related to lung function decline in CF
- "Climax-attack community"
  - *Climax: Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus* spp., *Scedosporium* spp.
  - Attack: *S. pneumoniae*, *H. influenza*, *Rhinovirus*, *Adenovirus*

Perspective: To choose a treatment that establish a « climax » microbiome in the lung in CF patients?




*Delhaes, PloSONE, 2012*
*Charlson , Am. J. Respir. Crit. Care Med. 2012*



*microorganisms*

MDPI

*Review*

# The Mycobiome: A Neglected Component in the Microbiota-Gut-Brain Axis

**Raphaël Enaud [1,2,3,*], Louise-Eva Vandenborght [1,3,4], Noémie Coron [1,2,3], Thomas Bazin [1,2], Renaud Prevel [1], Thierry Schaeverbeke [1,2], Patrick Berger [1,2,3], Michael Fayon [1,2,3], Thierry Lamireau [1,2] and Laurence Delhaes [1,2,3]**

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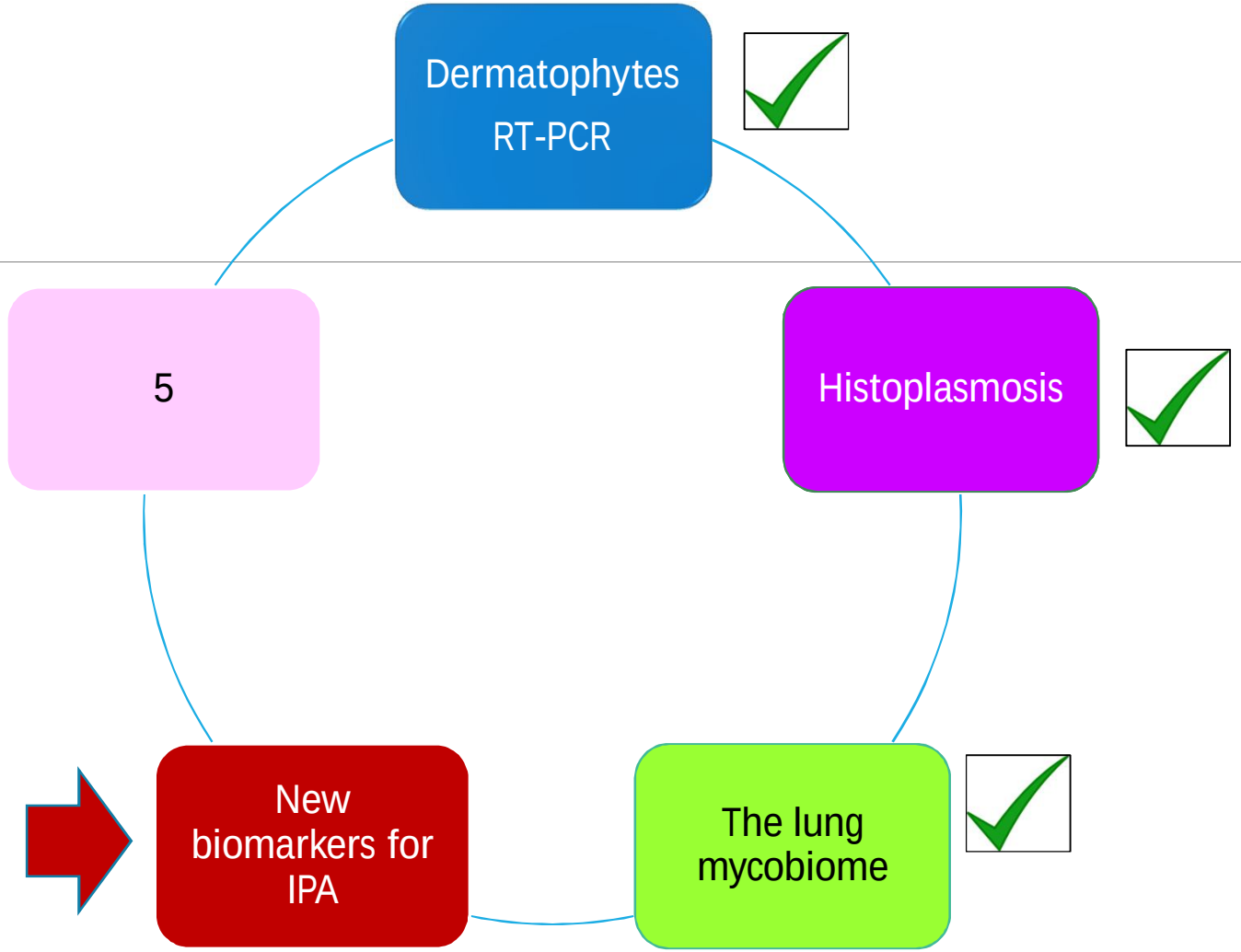
Your next reading...

# 5 topics


Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
New biomarkers for IPA
5

frontiers in Microbiology

ORIGINAL RESEARCH
published: 29 November 2017
doi: 10.3389/fmicb.2017.02362

# Evaluation of Bronchoalveolar Lavage Fluid Cytokines as Biomarkers for Invasive Pulmonary Aspergillosis in At-Risk Patients

*Samuel M. Gonçalves[1,2], Katrien Lagrou[3,4], Cláudia S. Rodrigues[1,2], Cláudia F. Campos[1,2], Leticia Bernal-Martínez[5], Fernando Rodrigues[1,2], Ricardo Silvestre[1,2], Laura Alcazar-Fuoli[5], Johan A. Maertens[3,6], Cristina Cunha[1,2] and Agostinho Carvalho[1,2]*

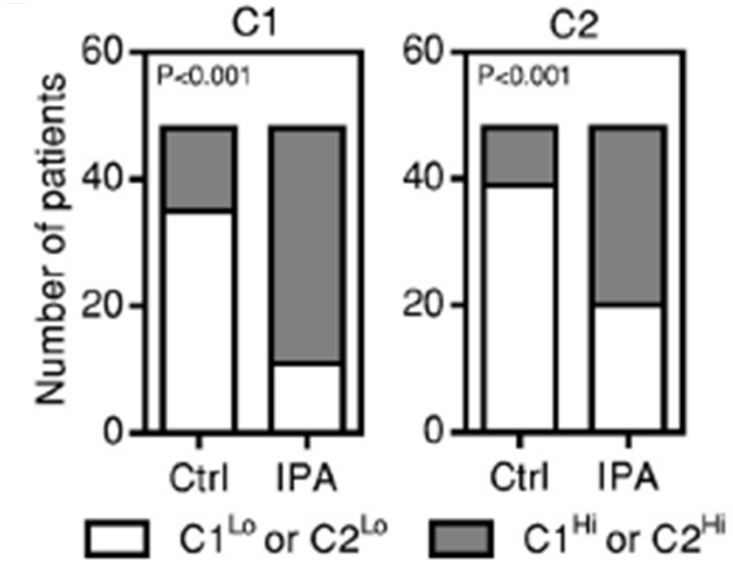
## MATERIELS AND METHODS

- KULeuven, ≥18 years-old
- Nested case-control study with 113 patients at risk of IPA
- Probable & proven cases vs control (no IPA: GM/Cult-neg)
- ANALYSES
  - 32 analytes (cytokines) in BAL+serum
  - GM in BAL
  - Genotyping of rs2305619 in PTX3 and rs16910526 in CLEC7A (dectin-1)

## OBJECTIVES

To determine whether a signature of alveolar cytokines could be associated with the development of IPA and used as a diagnostic biomarker

# Two clusters of cytokines are discriminant in IPA vs controls




C1: TNFa, IL-23, IL-6, IL-17

C2: IL-8, IL-1b

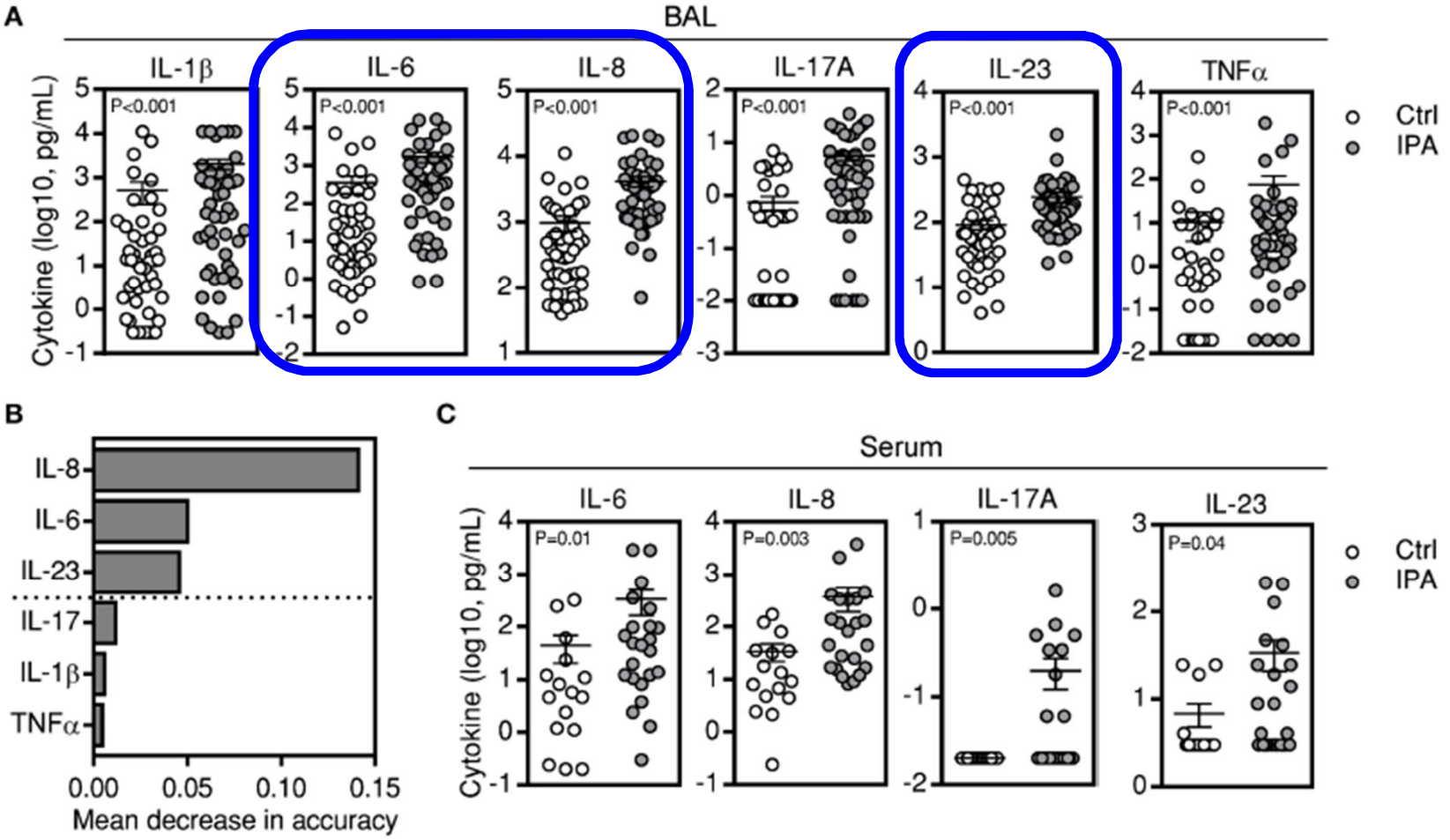
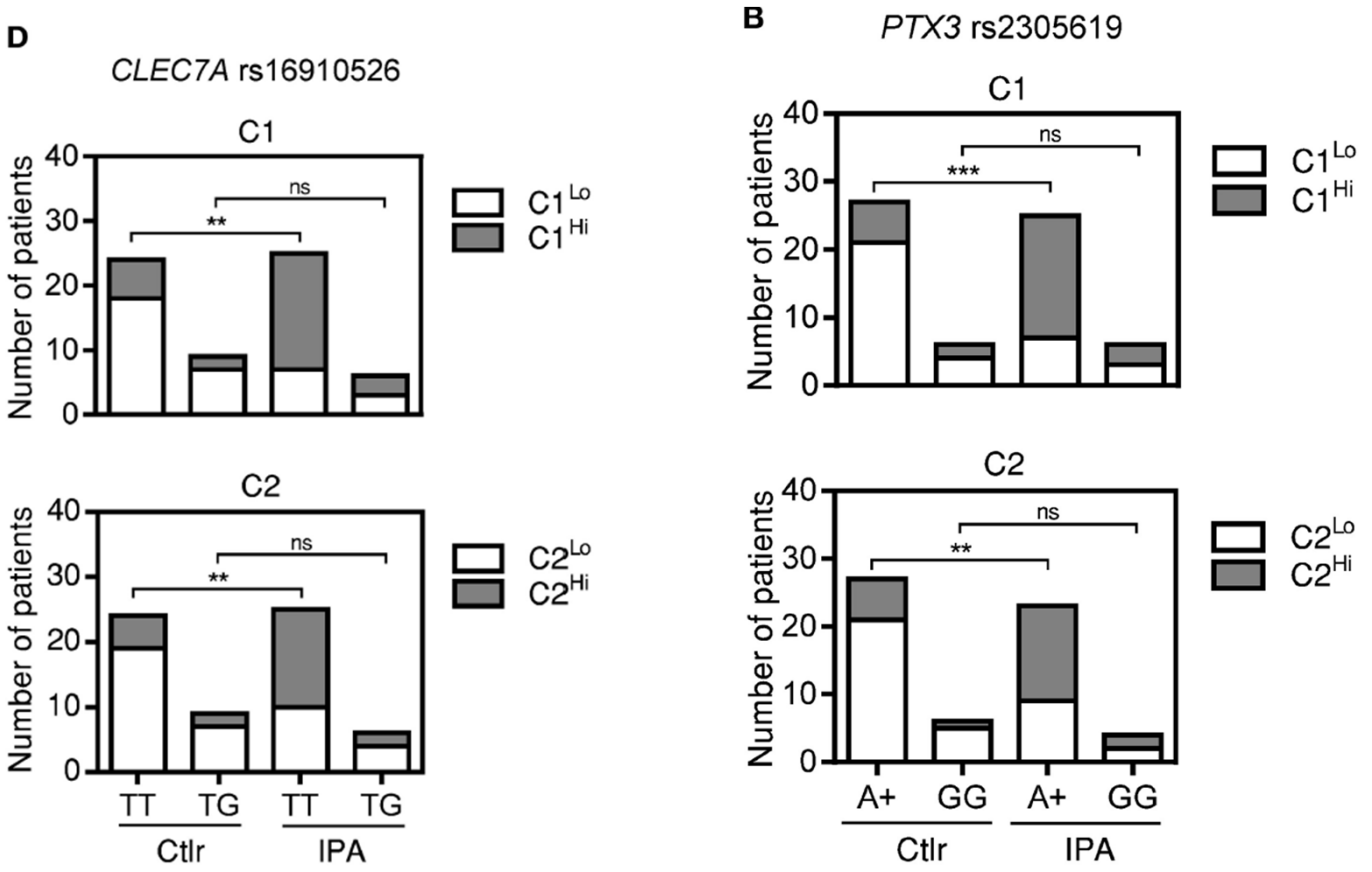

A
BAL
IL-1β
IL-6
IL-8
IL-17A
IL-23
TNFα
P<0.001
Cytokine (log10, pg/mL)
Ctrl
IPA
B
IL-8
IL-6
IL-23
IL-17
IL-1β
TNFα
0.00
0.05
0.10
0.15
Mean decrease in accuracy
C
Serum
IL-6
IL-8
IL-17A
IL-23
P=0.01
P=0.003
P=0.005
P=0.04
Cytokine (log10, pg/mL)
Ctrl
IPA

**TABLE 2 |** Performance of BAL cytokines as diagnostic biomarkers for IPA.

| Cytokine | Cut-off[†] | Sensitivity | Specificity | PPV | NPV | NRI |
|---|---|---|---|---|---|---|
| | | (95% CI) | | | | |
| IL-1β | 27.1 | 70 (55–83) | 68 (55–81) | 70 (60–79) | 69 (58–78) | 0.34 |
| IL-6 | 89.8 | 74 (63–85) | 79 (68–89) | 78 (67–87) | 73 (63–81) | 0.51 |
| IL-8 | 904 | 90 (81–98) | 73 (60–85) | 78 (68–85) | 88 (75–94) | 0.63 |
| IL-17A | 0.66 | 72 (58–84) | 81 (70–90) | 80 (68–88) | 74 (64–82) | 0.53 |
| IL-23 | 103 | 76 (66–90) | 77 (67–90) | 78 (67–86) | 76 (65–84) | 0.53 |
| TNF-α | 0.94 | 80 (70–90) | 69 (55–81) | 73 (63–81) | 77 (65–86) | 0.49 |

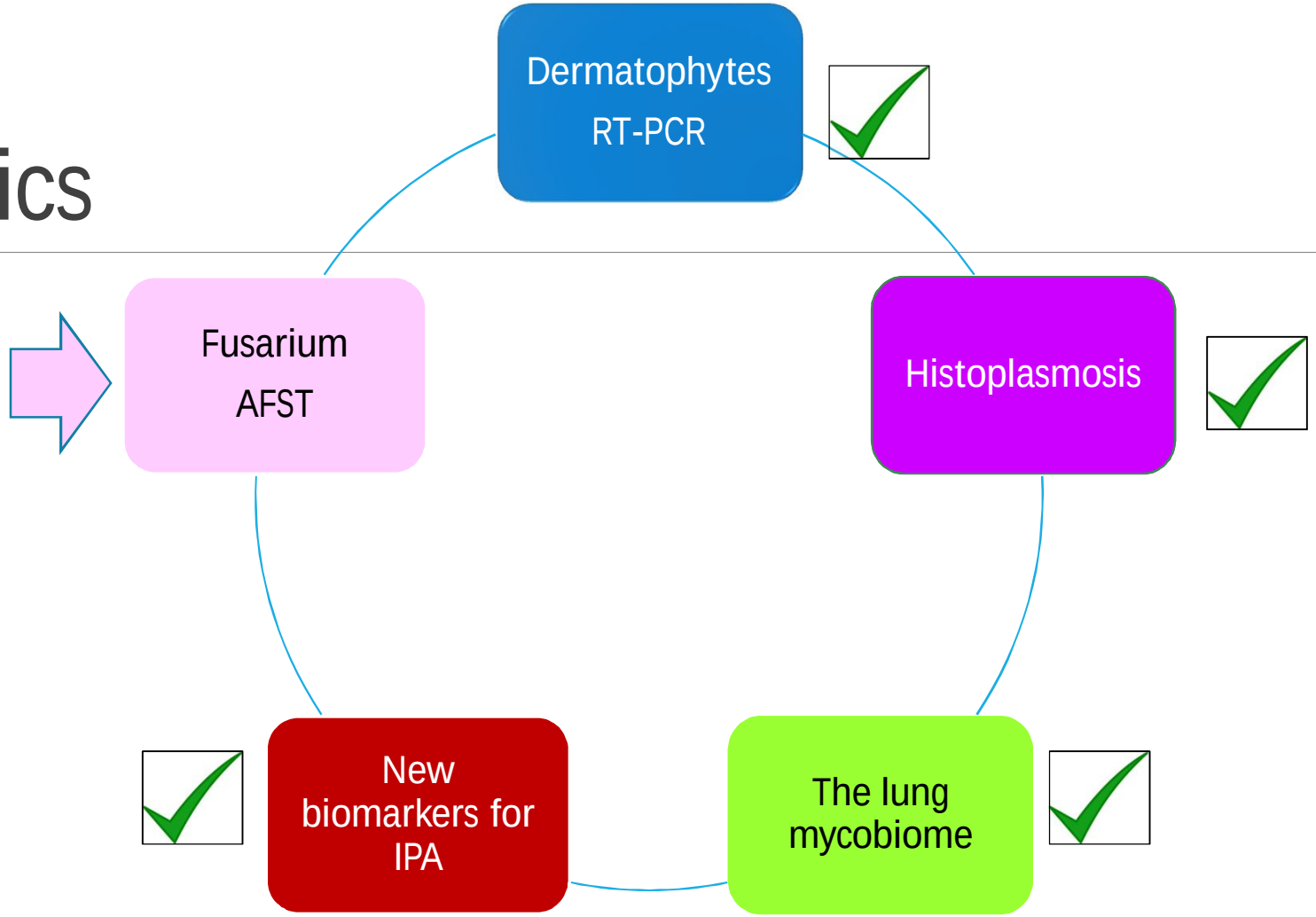
[†] *Cut-off values of cytokines are expressed as pg/mL. Statistically-derived optimal cut-off was determined by Youden's index (maximum sensitivity and specificity given by the inflection point of the* $AUC^{ROC}$*). The net reclassification index (NRI) was used to compare the performance of each cytokine cut-off with the known diagnosis of IPA. IL, interleukin; TNF, tumor necrosis factor; PPV, positive predictive value; NPV, negative predictive value.*

# Genetic variants of dectin-1 receptor and PTX3 impairs discriminatory value of cytokines in IPA




**p<0,01

# 5 topics


Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
New biomarkers for IPA
Fusarium
AFST

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SUSCEPTIBILITY

# Comparative Evaluation of Etest, EUCAST, and CLSI Methods for Amphotericin B, Voriconazole, and Posaconazole against Clinically Relevant *Fusarium* Species

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## BACKGROUND

- Opportunistic fungus: superficial to invasive infections
- F. solani Species Complex > F. oxysporum SC > F. fujikuroi SC
- AmB and VOR: drugs of choice
- AFST is species specific
- No cut-off defined for Fusarium
- ECVs recently defined for Fusarium

## Objective

To compare the in vitro EUCAST and CLSI reference methods vs E-test for *in vitro* susceptibility testing of Fusarium sp against AmB , VOR , POS

# Mat & methods

20 clinical isolates of Fusarium

Molecular identification: TEF1 and rPB2 target genes

Etest: Inoculum concentration: 0.5 McFarland standard (equivalent $10^6$ to $5.10^6$ CFU/ml).

RPMI 1640 agar with 2% glucose.

After a period of 15 min, the E-test strips were applied

Incubation for 48 h at 35°C

EUCAST and CLSI methods: as described.

# Agreement Results

**TABLE 2** Comparison among the three methods for antifungal susceptibility testing of *Fusarium* spp.

| Method comparison | Drug | Median (range) difference | Agreement (%)[a] | | Paired *t* test *P* value | Pearson *r*[b] | Categorical agreement (%) |
|---|---|---|---|---|---|---|---|
| | | | ±1 dil. | ±2 dil. | | | |
| CLSI vs EUCAST | AMB | −1 (−1 to 1) | 100 | 100 | 0.234 | 0.78 | 85 |
| | VOR | 0 (−1 to 2) | 95 | 100 | 1.000 | 0.81 | 90 |
| | POS | 1 (−2 to 2) | 75 | 100 | 0.383 | 0.89 | 100 |
| EUCAST vs Etest | AMB | −1 (−3 to 2) | 80 | 95 | 0.007 | 0.86 | 100 |
| | VOR | 0 (−2 to 3) | 75 | 95 | 0.494 | 0.76 | 95 |
| | POS | −1.5 (−2 to 2) | 45 | 100 | 0.013 | 0.97 | 90 |
| CLSI vs Etest | AMB | −1 (−4 to 1) | 60 | 90 | 0.008 | 0.71 | 85 |
| | VOR | 0 (−2 to 3) | 80 | 95 | 0.479 | 0.77 | 95 |
| | POS | −1 (−3 to 3) | 70 | 85 | 0.097 | 0.89 | 90 |

[a]dil., dilution.
[b]$P < 0.0001$ for all comparisons.

# Conclusion

E-test overall resulted in 1-dilution-higher MICs than the reference methods, with most differences being within 2 dilutions, which may lead to errors if same breakpoints will be applied.

However, the categorical agreement was high (85%) using previously published ECVs.

Etest can be used for routine susceptibility testing of amphotericin B, voriconazole, and posaconazole for Fusarium species.

Perspectives: Further work is warranted in order to establish clinical breakpoints for Fusarium.

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