Top five papers in mycology: the lab perspective

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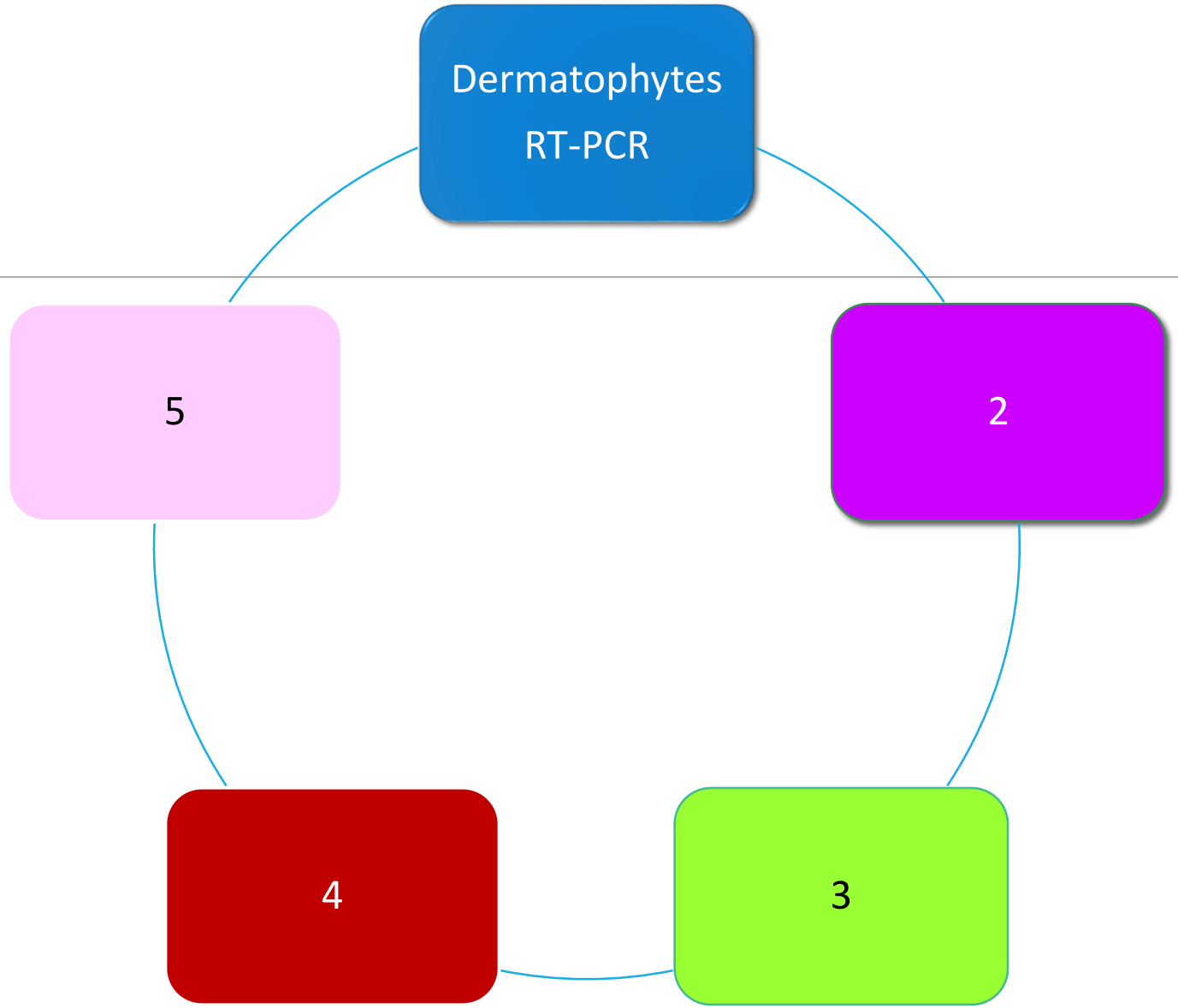
23 MARCH 2018

5 topics

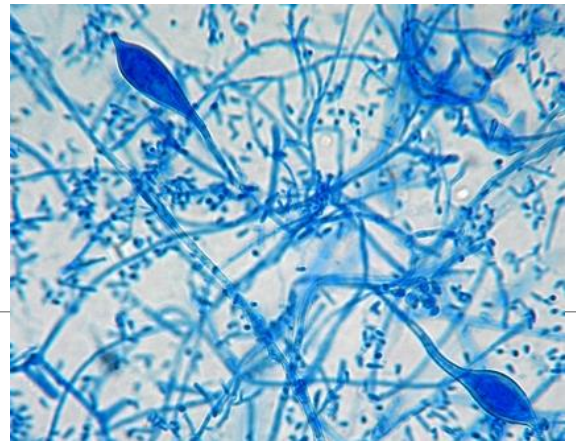


ORIGINAL ARTICLE

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]



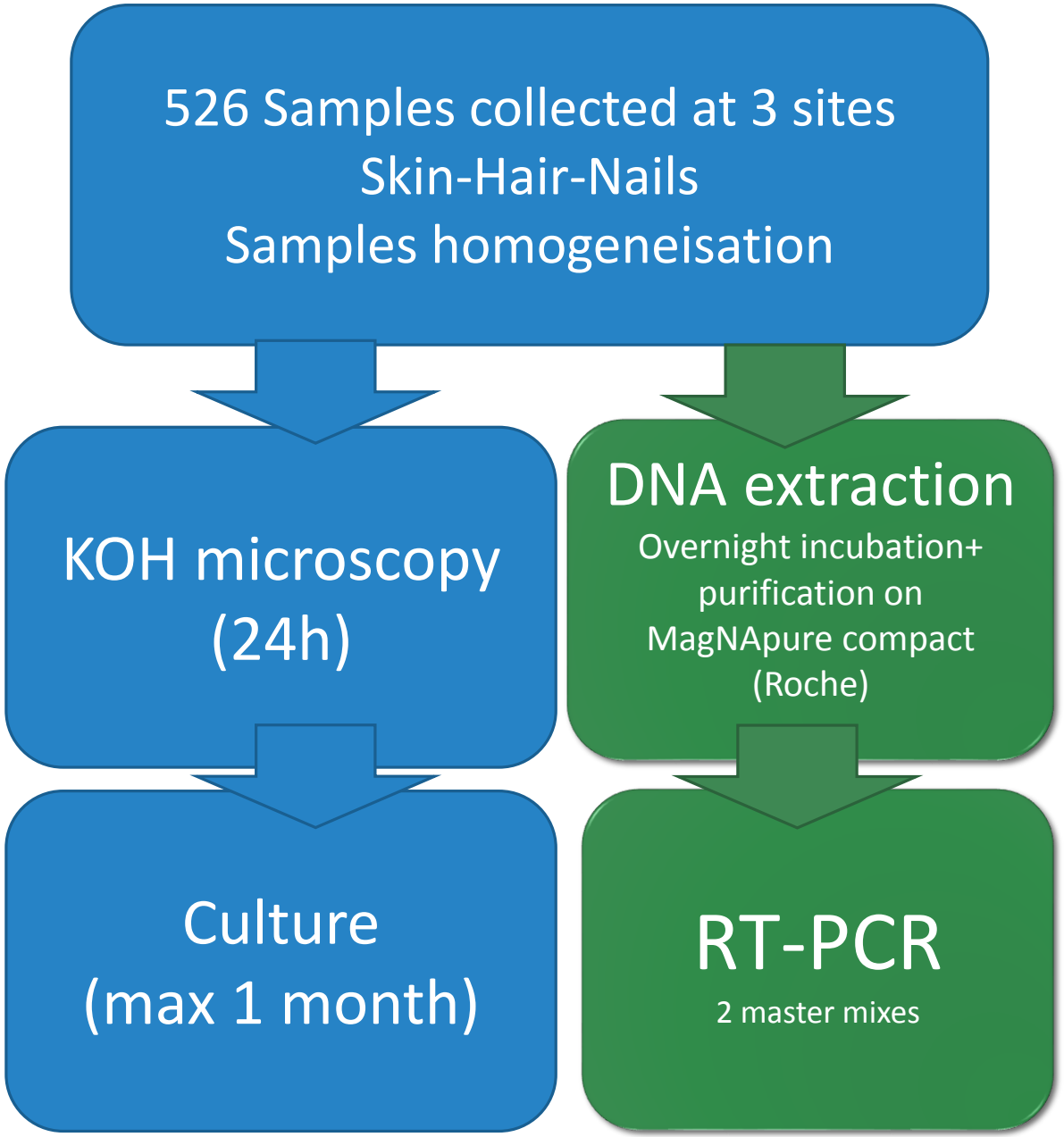
In house, Multiplex Real-Time PCR (LightCycler 480)
Comparison with culture+microscopy
526 SKIN HAIR & NAILS samples
Collection on 3 sites in Israël
Retrospective study

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

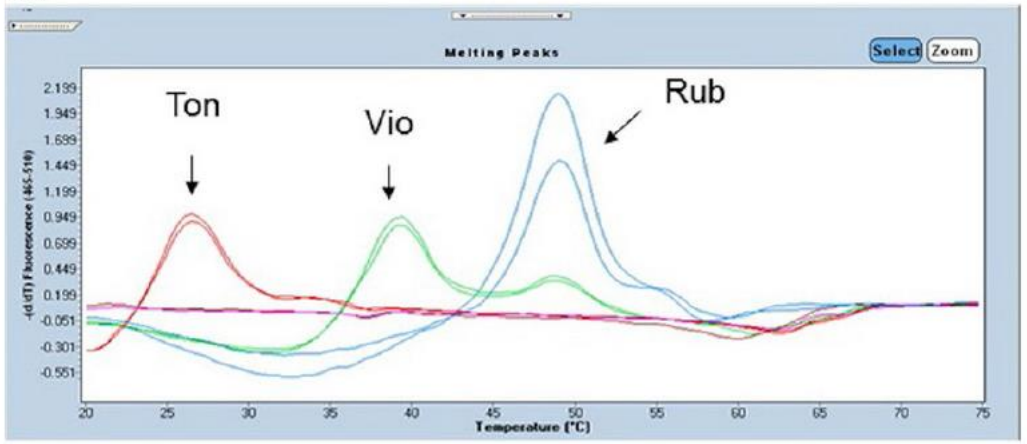
Materiels & Methods



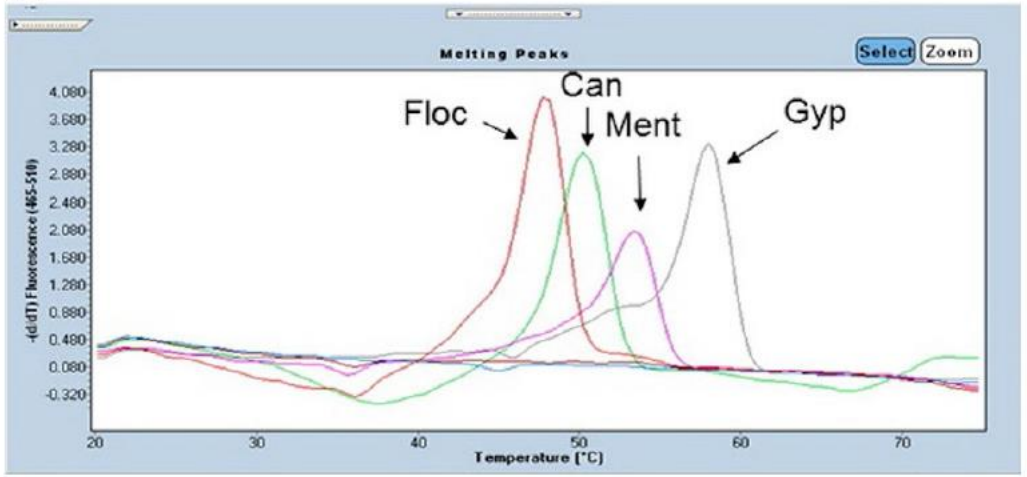
RT-PCR (ITS1-ITS2) with Melting curve analysis

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)

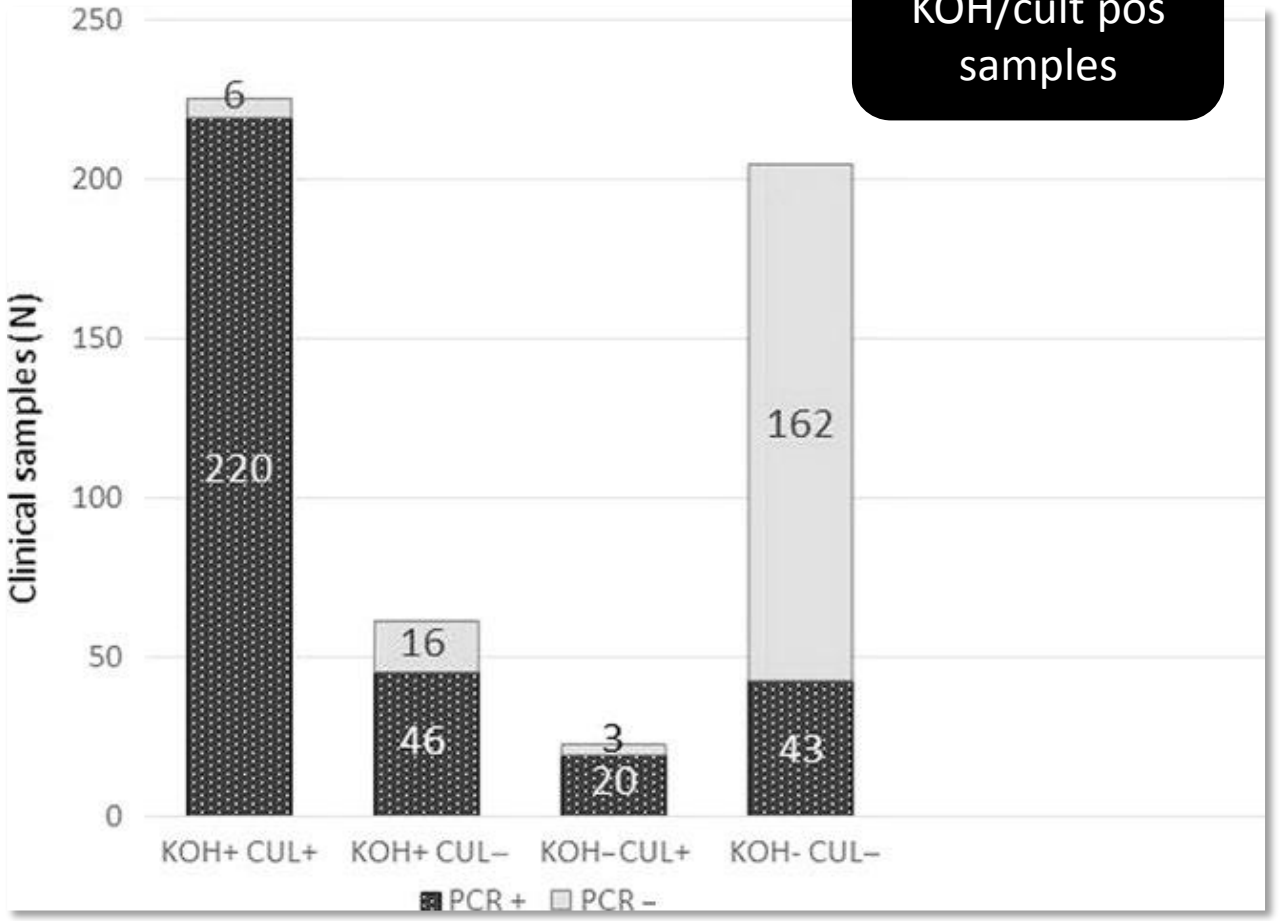


RESULTS from 3 sites (N=526)

PCR POS in 96% KOH/cult pos samples

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

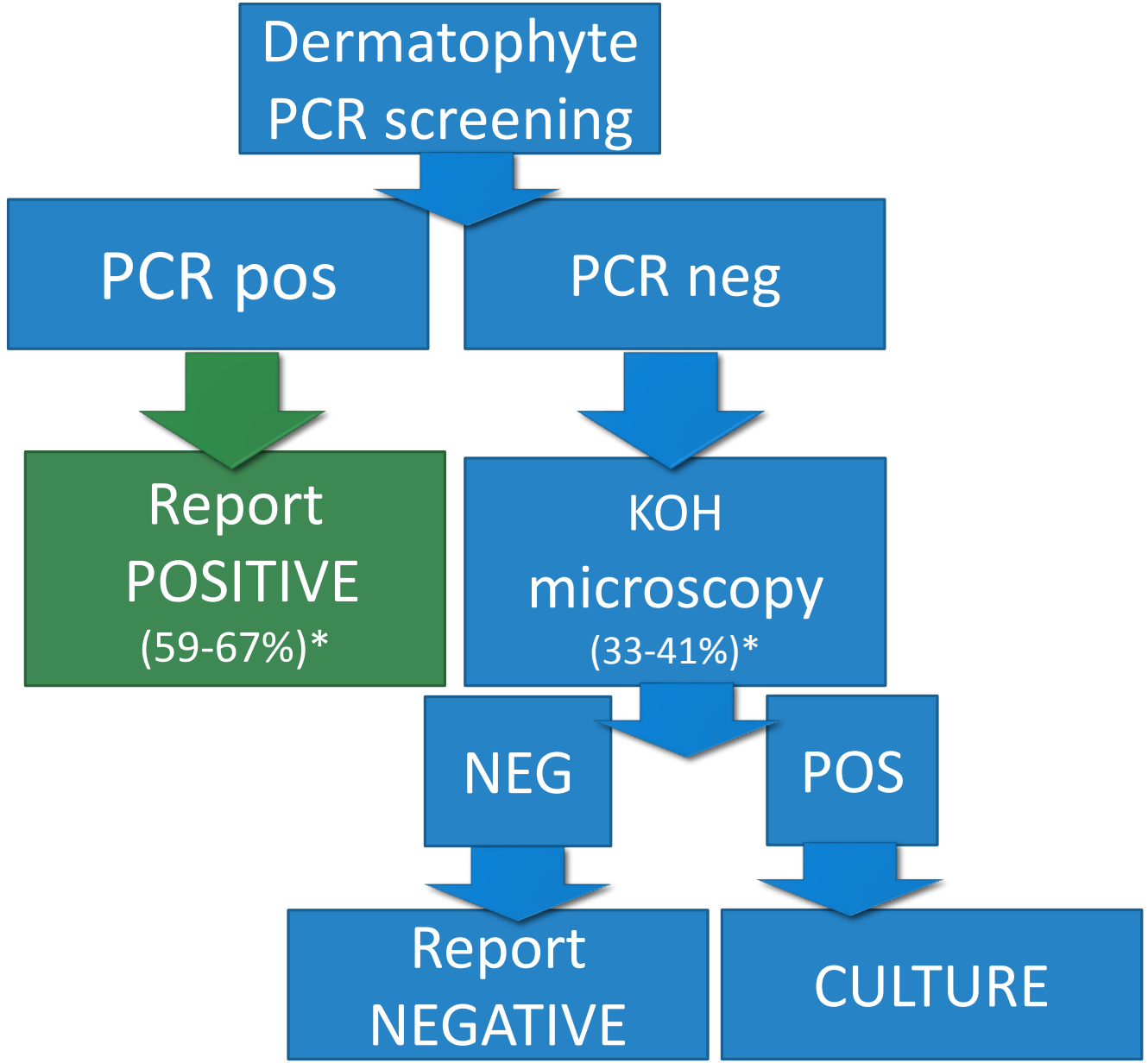
-Gold standard: KOH and/or cult positive
-PCR sensitivity: 92%
-PCR specificity: 79%
-Additional positive PCR cases not detected in culture: 10-30%



Comparison with other studies

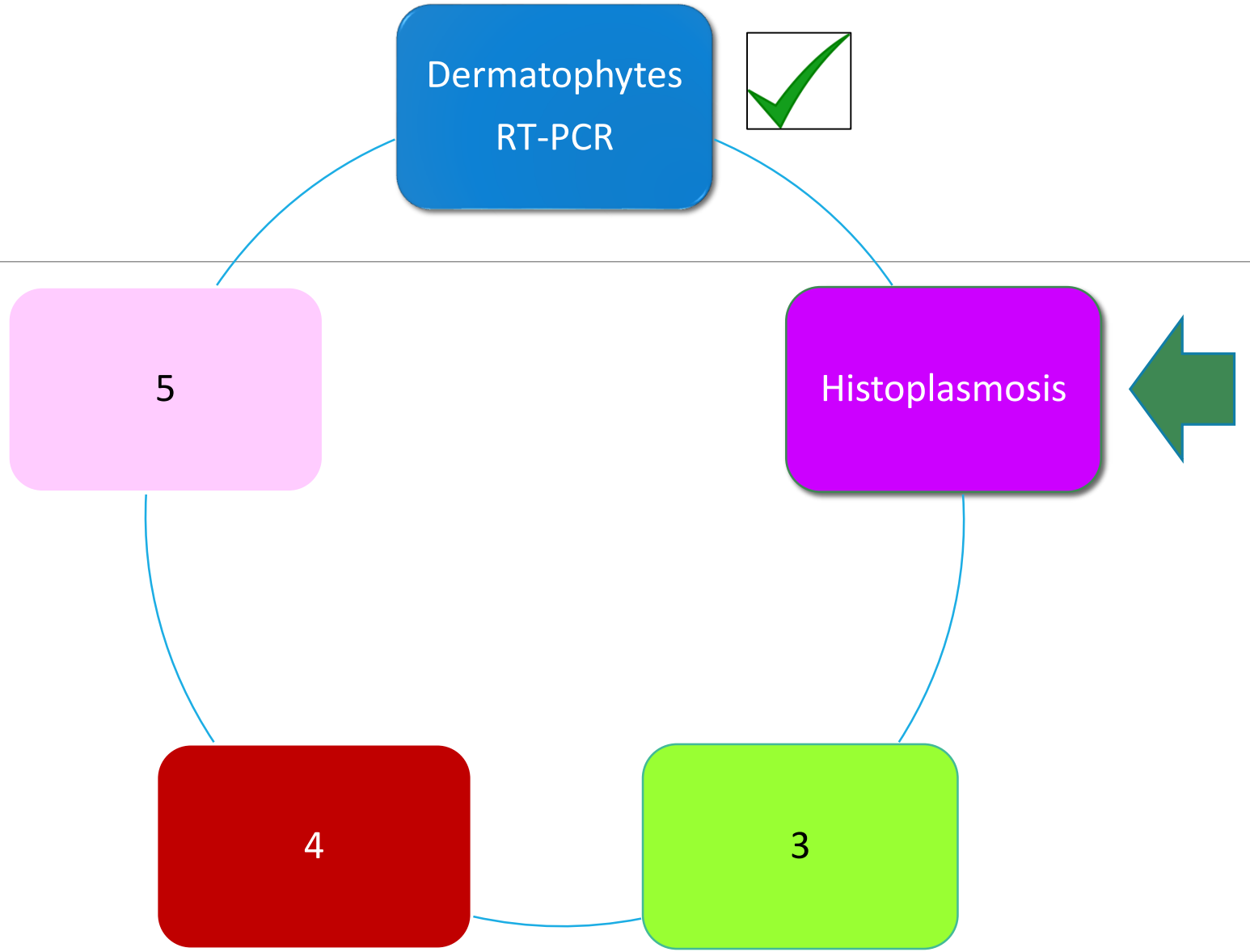
Reference	No. of samples	Dermatophyte strains in PCR	KOH	Culture n (%)	PCR, n (%)	p/c parameter*
Arabatziz et al[3]	92	6	40	30 (33%)	47 (51%)	1.56
Brillowska-Dabrowska et al[20]	118	1 (*T. rubrum*)	NA	27 (23%)	49 (42%)	1.83
Bergmans et al[8]	120	11	57	45 (38%)	74 (62%)	1.64
Wisselink et al[16]	1437	5	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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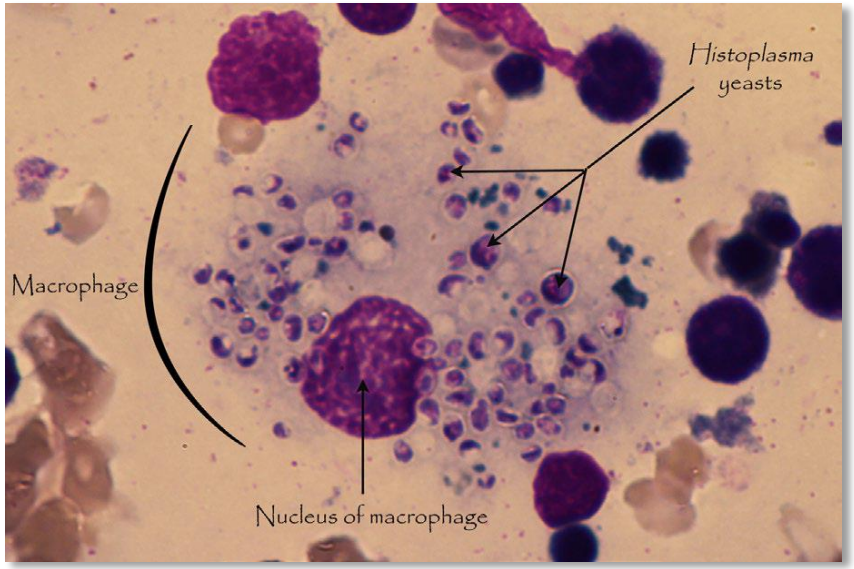
MINIREVIEW

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

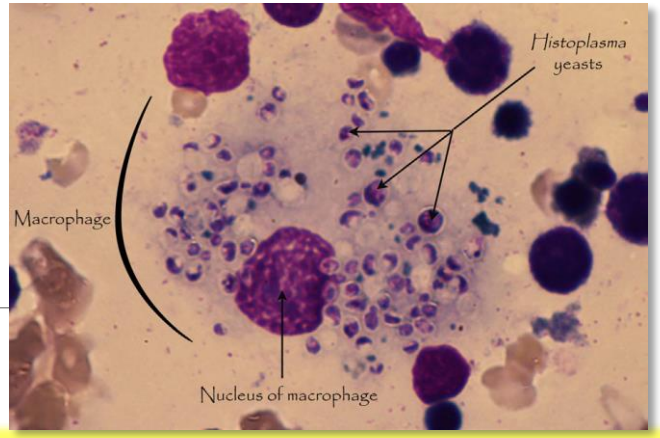
TABLE 1 Summary of diagnostic test for histoplasmosis[a]

	% 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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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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Pathology	0–42	42.1	75.0	76.3
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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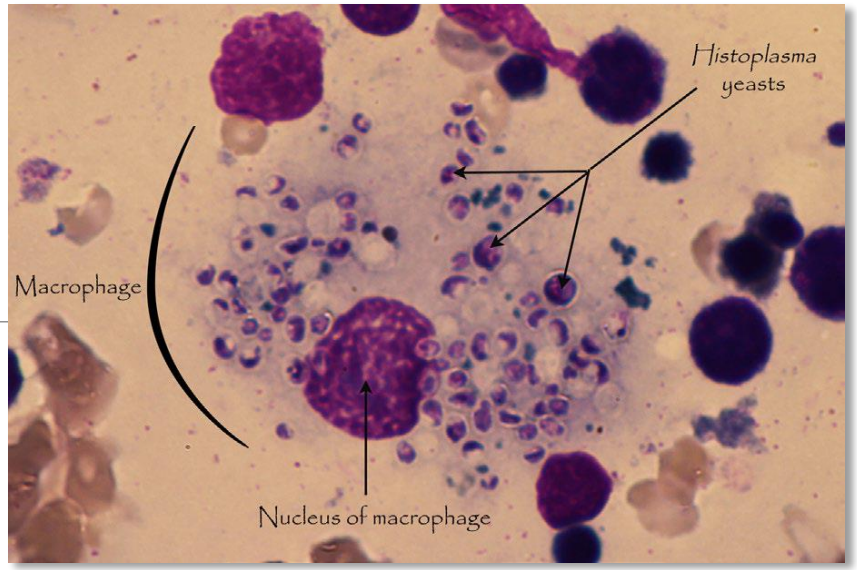

Conclusion

Consider the clinical presentation and talk with your microbiologist!

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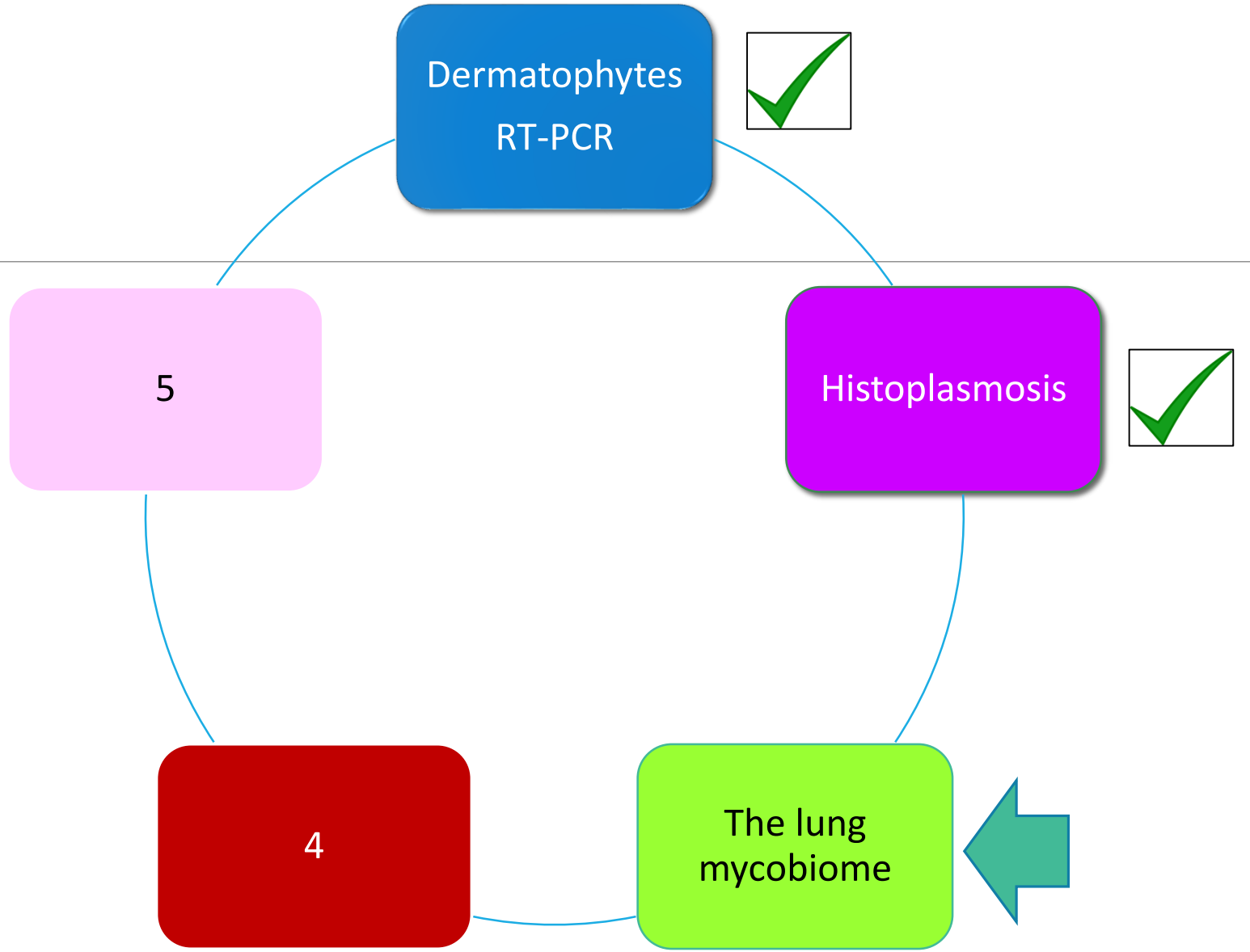
Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
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frontiers in MICROBIOLOGY

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]*

[1] Biology and Diversity of Emerging Eukaryotic Pathogens, Center for Infection and Immunity of Lille, INSERM U1019, CNRS UMR 8204, Lille Pasteur Institute, University of Lille Nord de France, Lille, France
[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

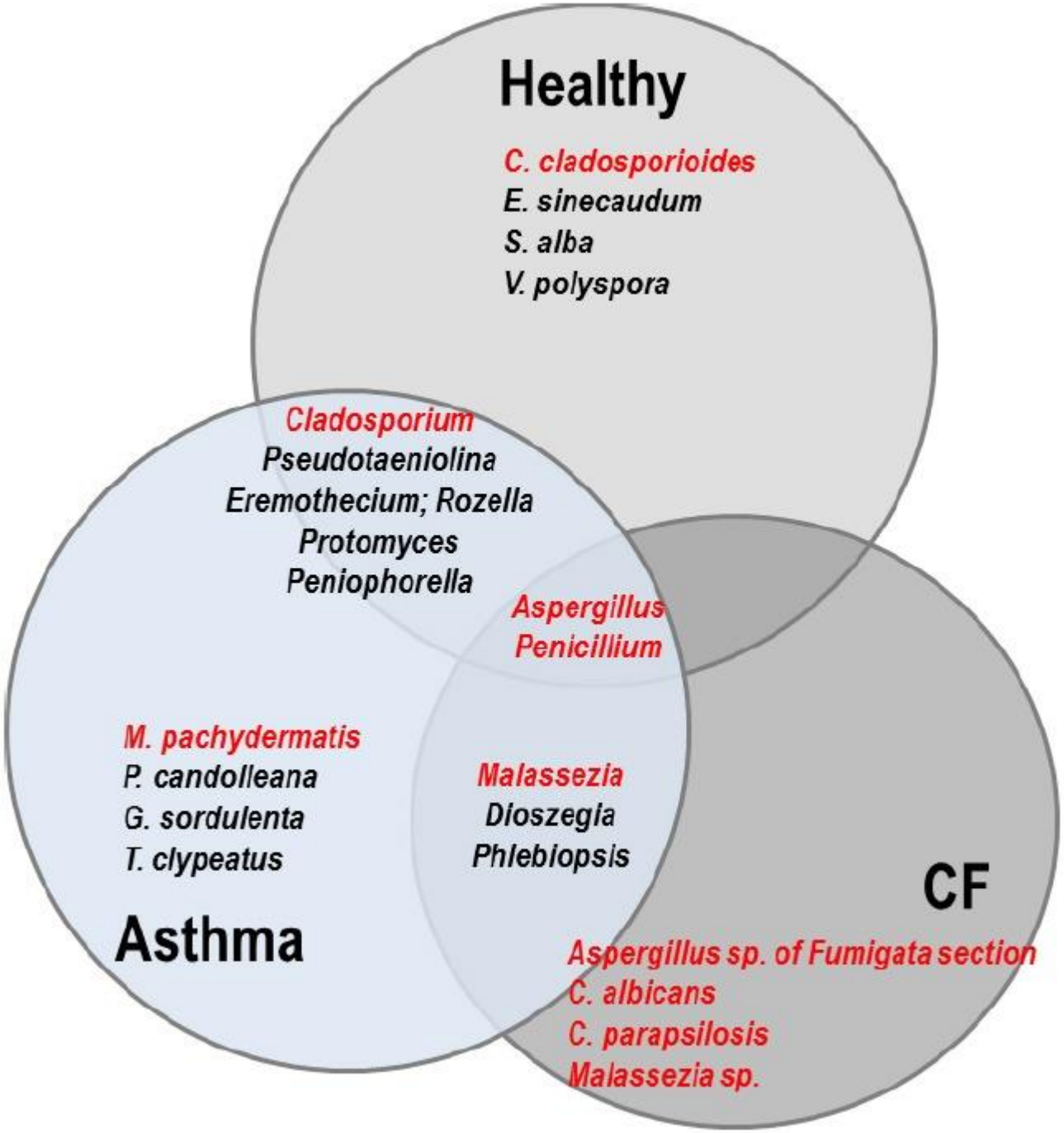
1. Fungi are present even in healthy people
2. Composition is highly variable between individuals
3. Fungi are <<<bacteria or viruses the lung
4. CRD are associated with a decrease of fungal diversity

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.



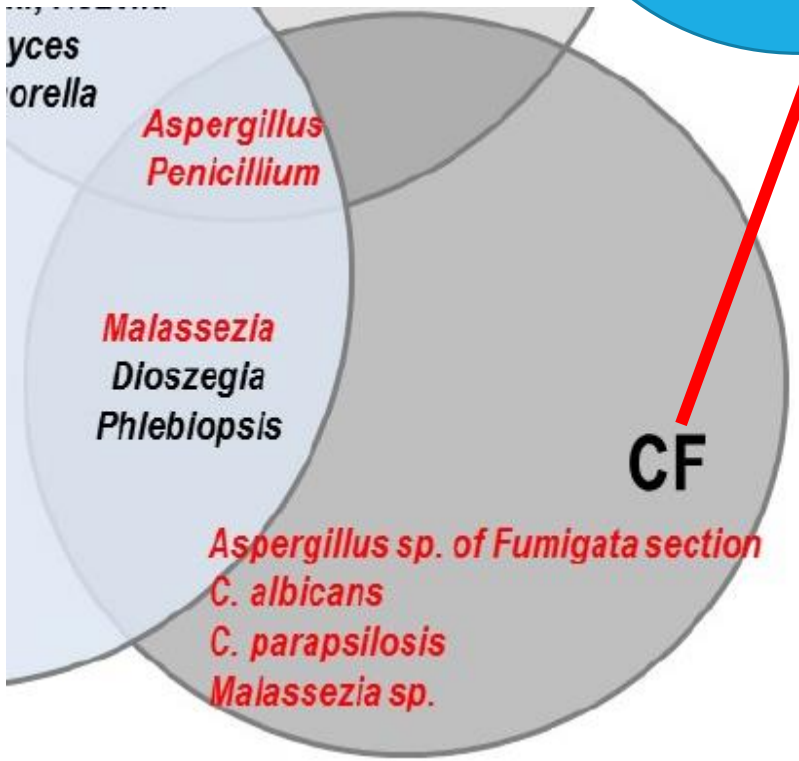
Studies from Dehlaes and Charlson , 2012,
*in CF and transplant patients: *C. albicans*, *Aspergillus* spp., *Penicillium*, *Cryptococcus* ,*Eurotium*, in which *Candida* species dominated
+ Reduced fungal diversity in these population.

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?

Malessezia
Predominant in skin flora. Abundant in CF patients, chronic rhinosinusitis
….which role?



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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2 Service d'Exploration Fonctionnelle Respiratoire, Service de Rhumatologie, Service de Gastrologie Adulte, Laboratoire de Parasitologie-Mycologie, CRCM Pédiatrique, Gastroentérologie et Nutrition Pédiatriques, Unité d'Hépatologie, CHU Bordeaux, 33000 Bordeaux, France

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4 Genoscreen Society, 59000 Lille, France

* Correspondence: raphael.enaud@chu-bordeaux.fr

Your next reading…

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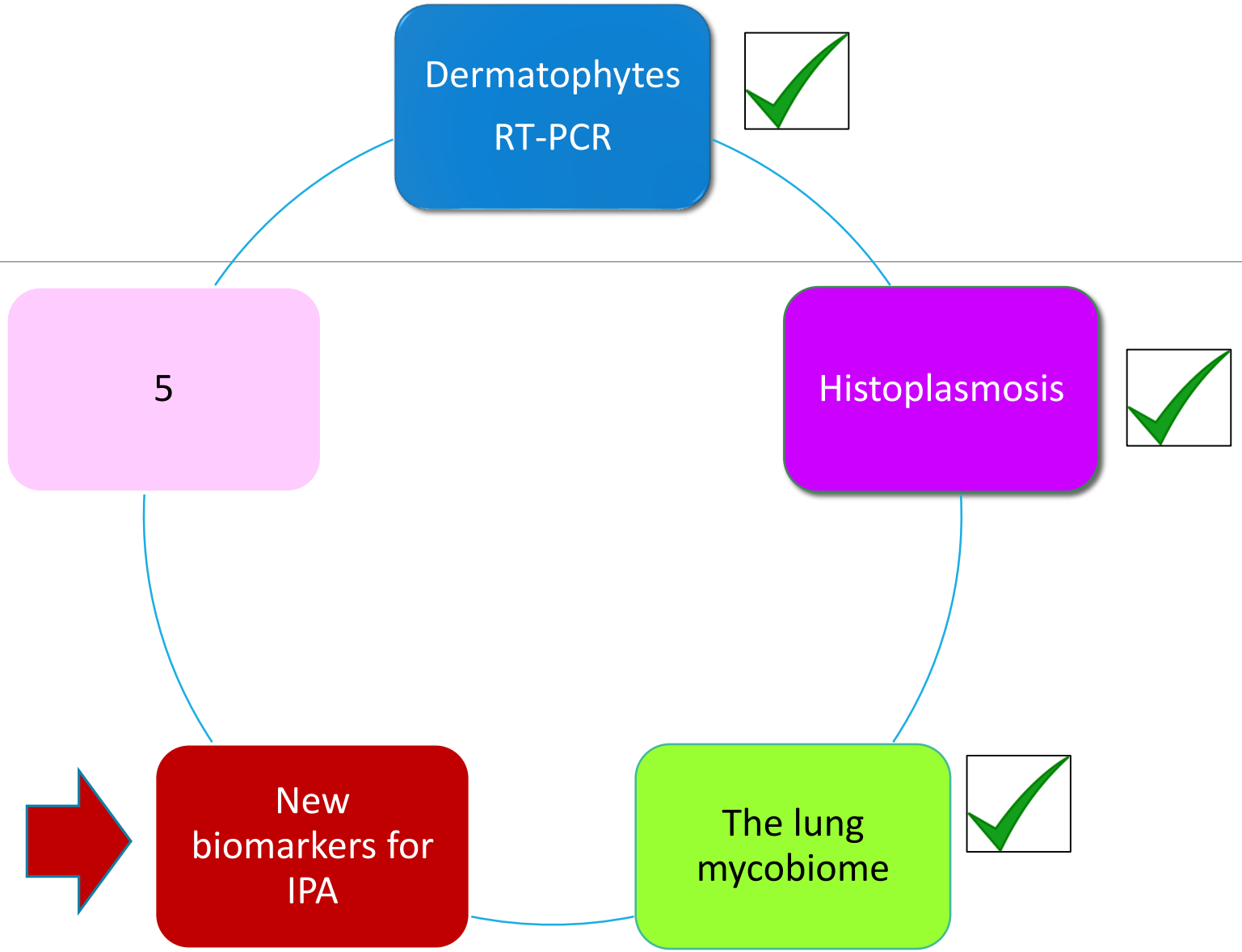
Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
New biomarkers for IPA
5

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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]*

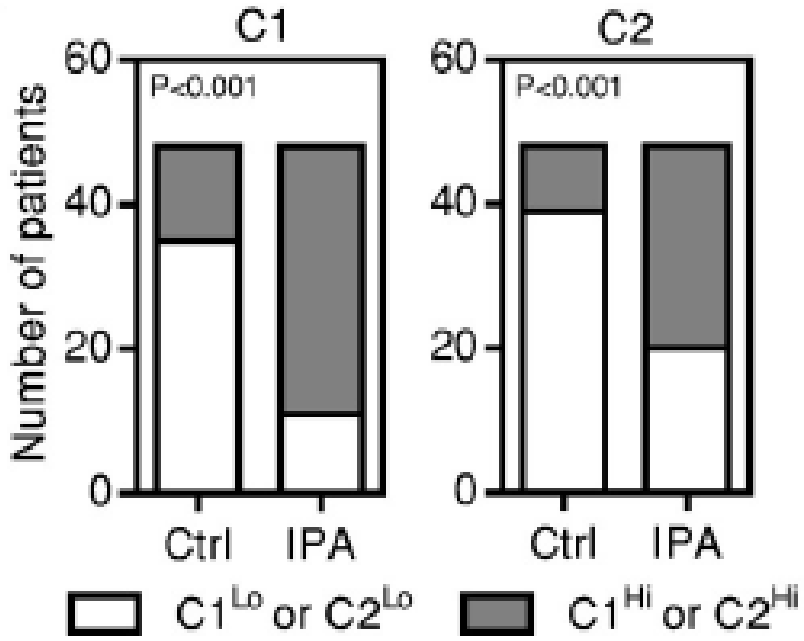
MATERIELS AND METHODS

- 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

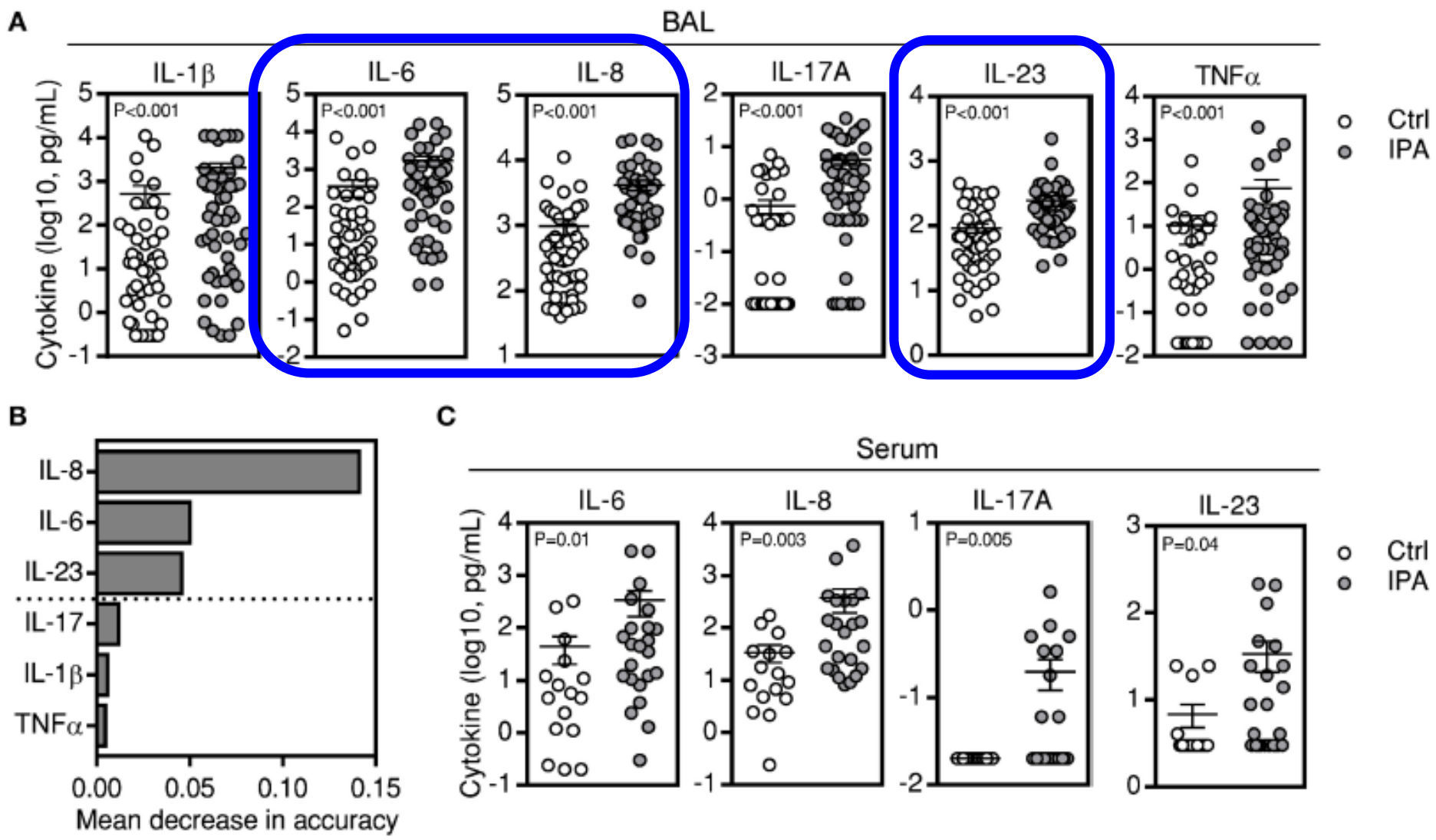
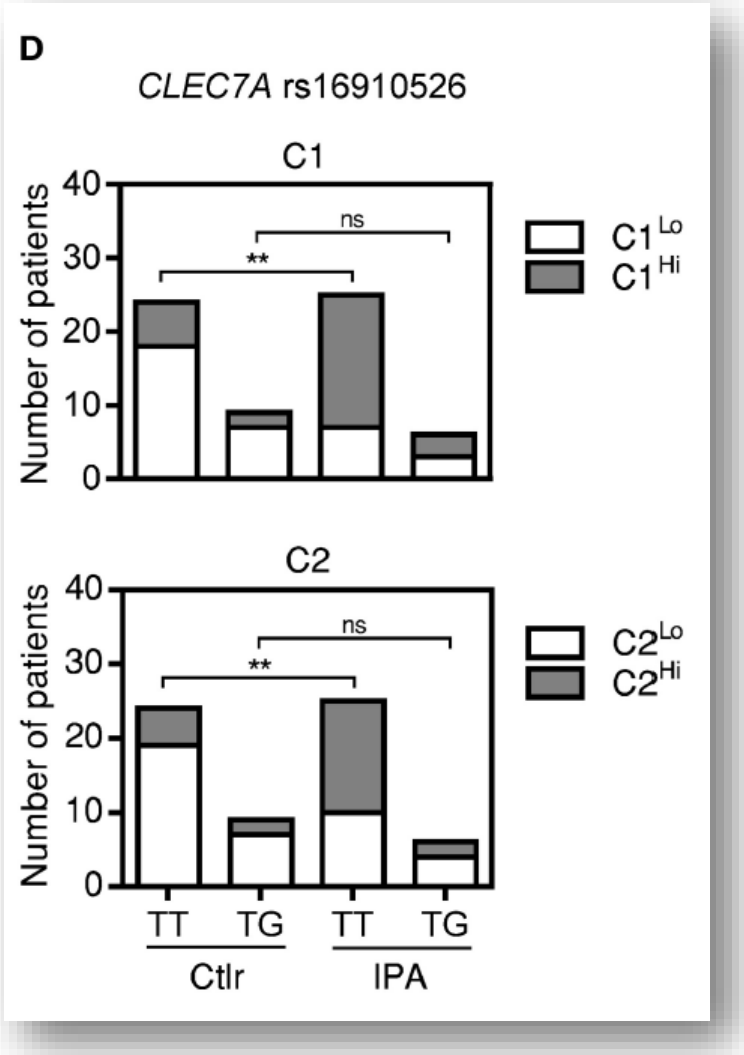

A
BAL
IL-1β
IL-6
IL-8
IL-17A
IL-23
TNFα
Cytokine (log10, pg/mL)
P<0.001
Ctrl
IPA
B
IL-8
IL-6
IL-23
IL-17
IL-1β
TNFα
0.00
0.05
0.10
0.15
Mean decrease in accuracy
C
Serum
IL-6
IL-8
IL-17A
IL-23
Cytokine (log10, pg/mL)
P=0.01
P=0.003
P=0.005
P=0.04
Ctrl
IPA

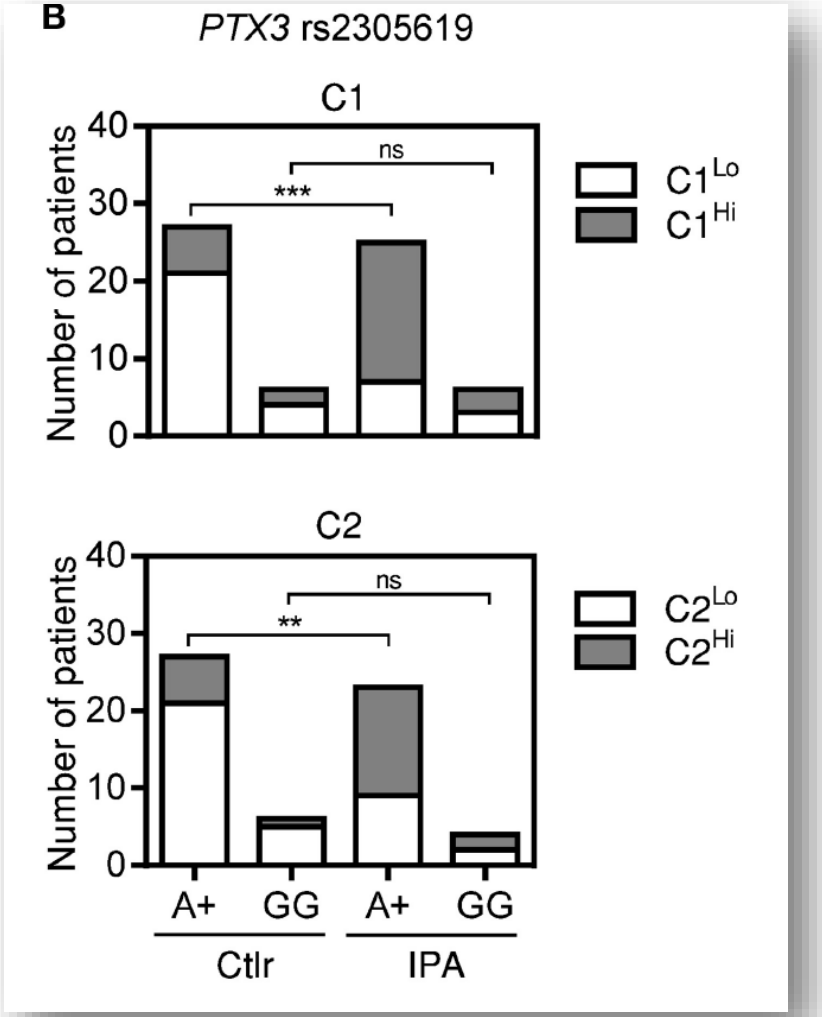
TABLE 2 | Performance of BAL cytokines as diagnostic biomarkers for IPA.

Cytokine	Cut-off†	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	NRI
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

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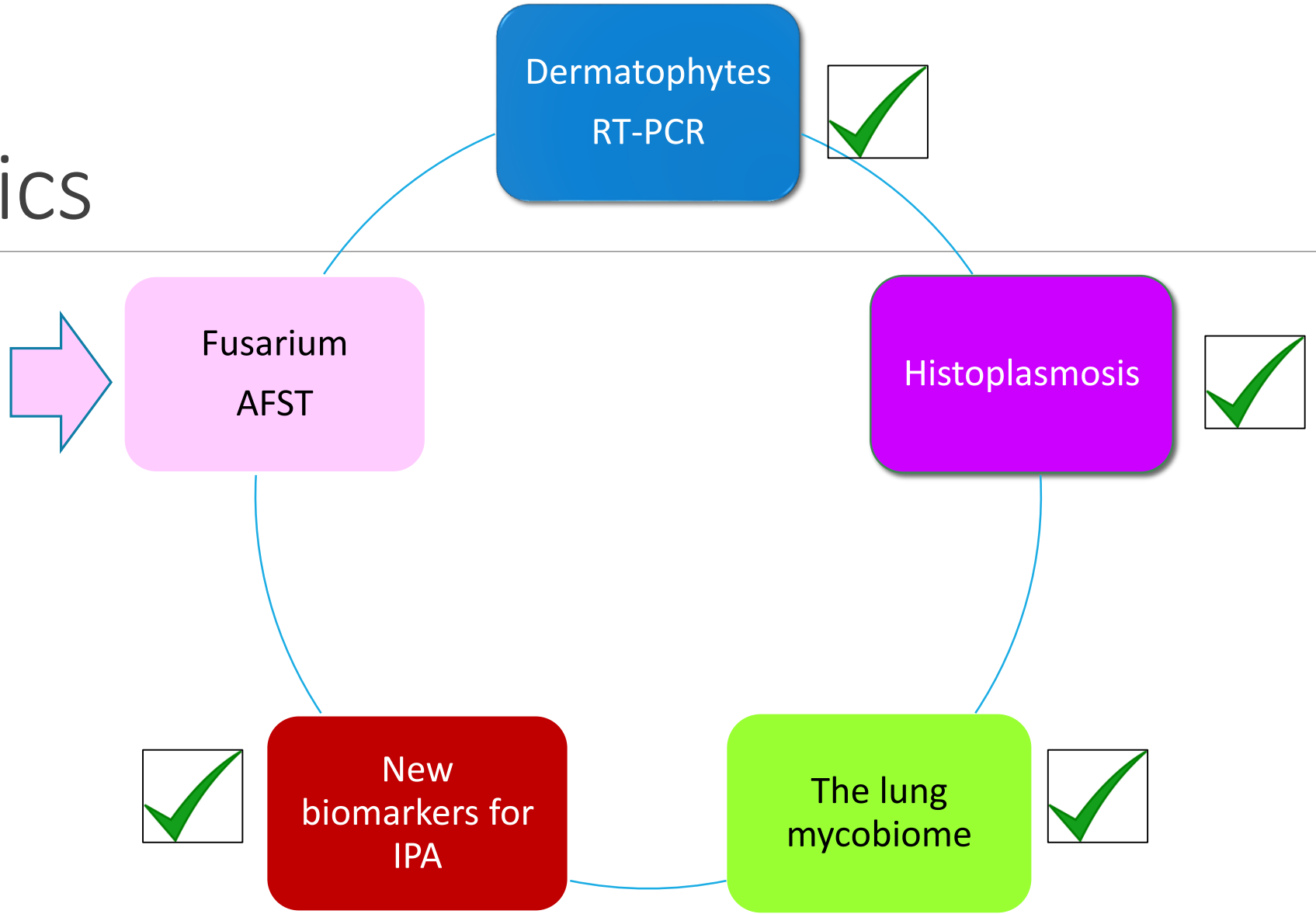
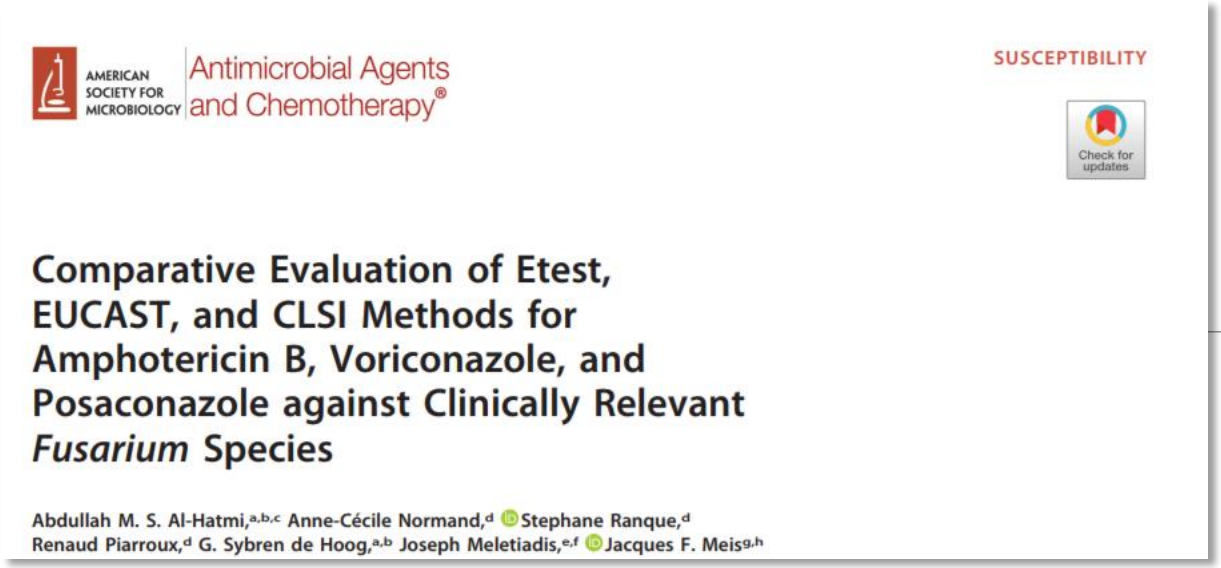

Dermatophytes
RT-PCR
Histoplasmosis
The lung mycobiome
New biomarkers for IPA
Fusarium
AFST



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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