



Moleculaire Diagnostiek van Schimmels

wanneer is het tijd om er mee te starten?

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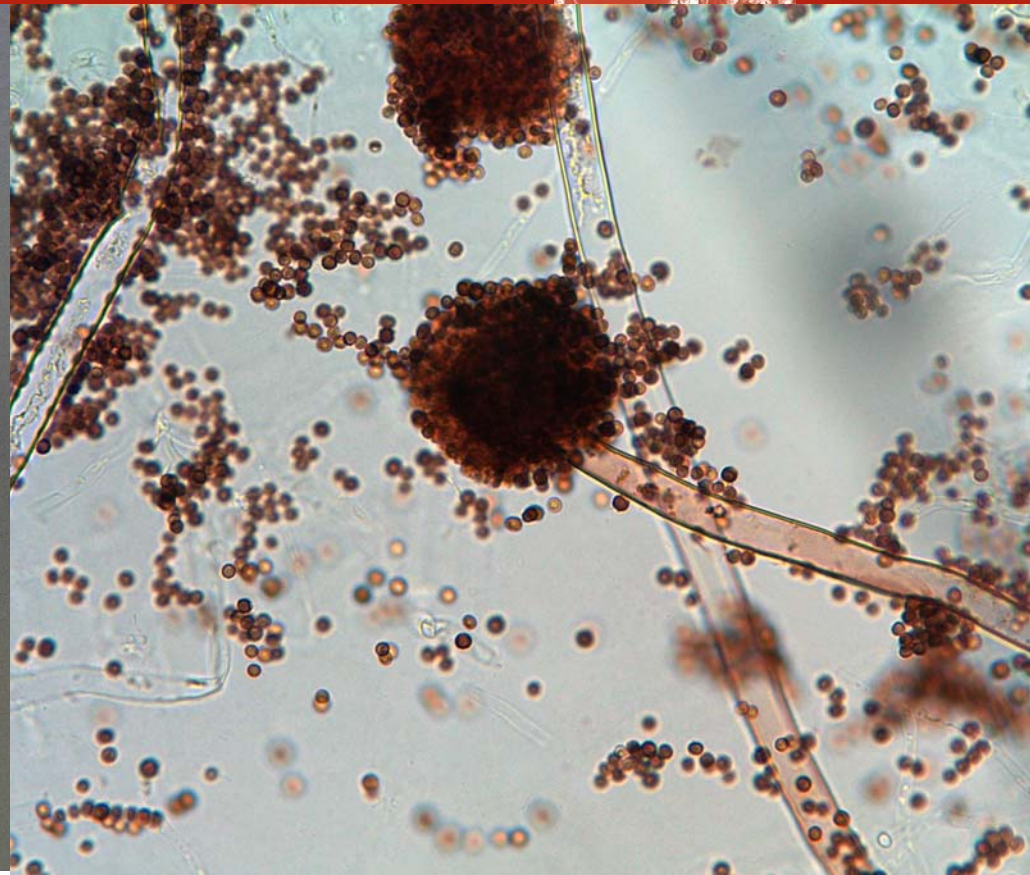
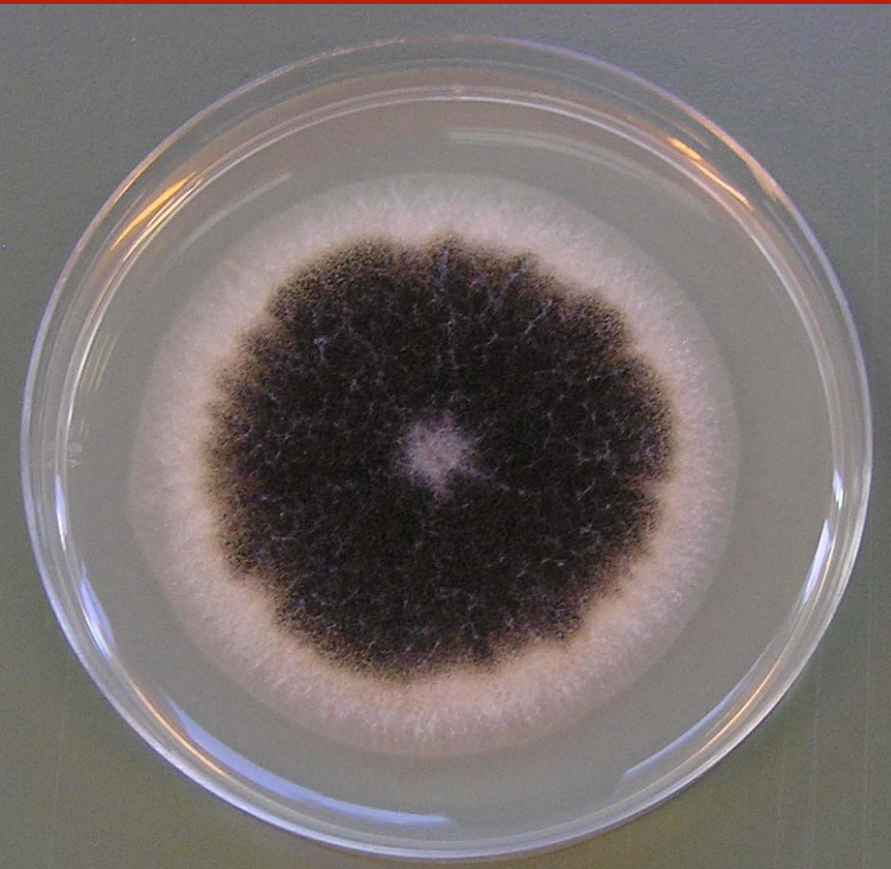
1. Identificatie
2. Detectie

Determinatie



Aspergillus niger

Determinatie



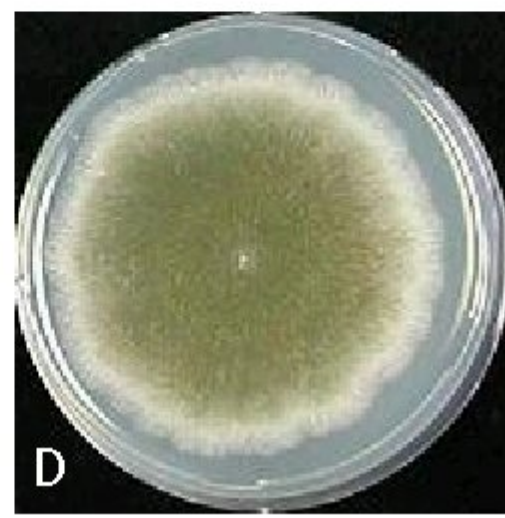
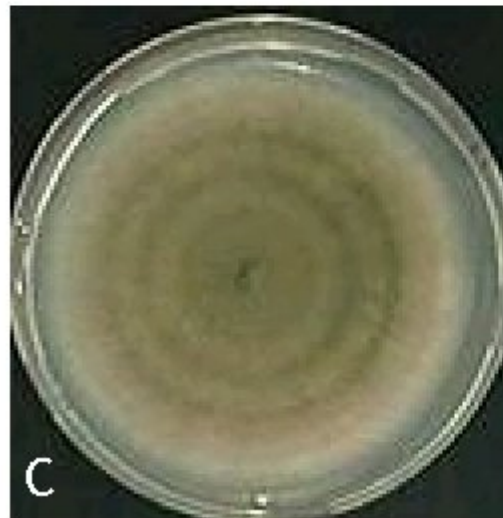
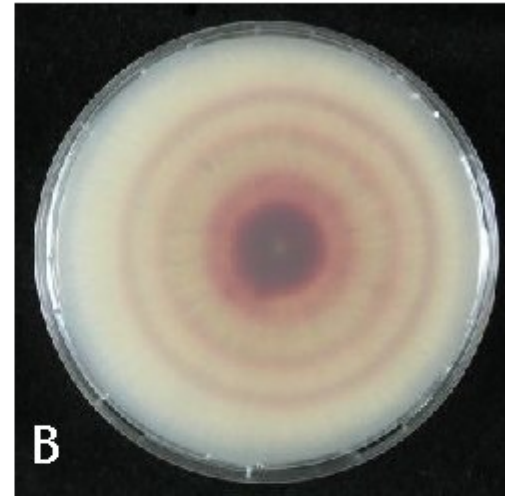
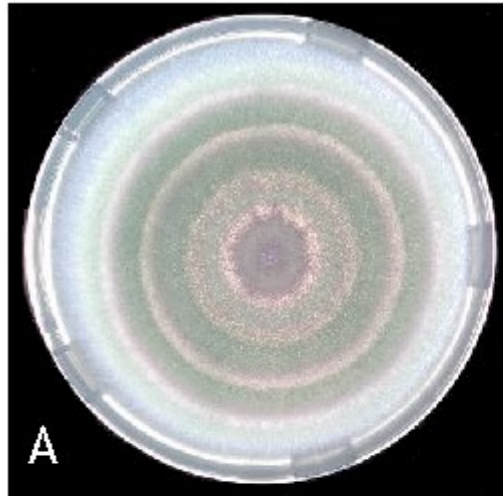
~~Aspergillus niger~~

Aspergillus tubingenensis

Determinatie



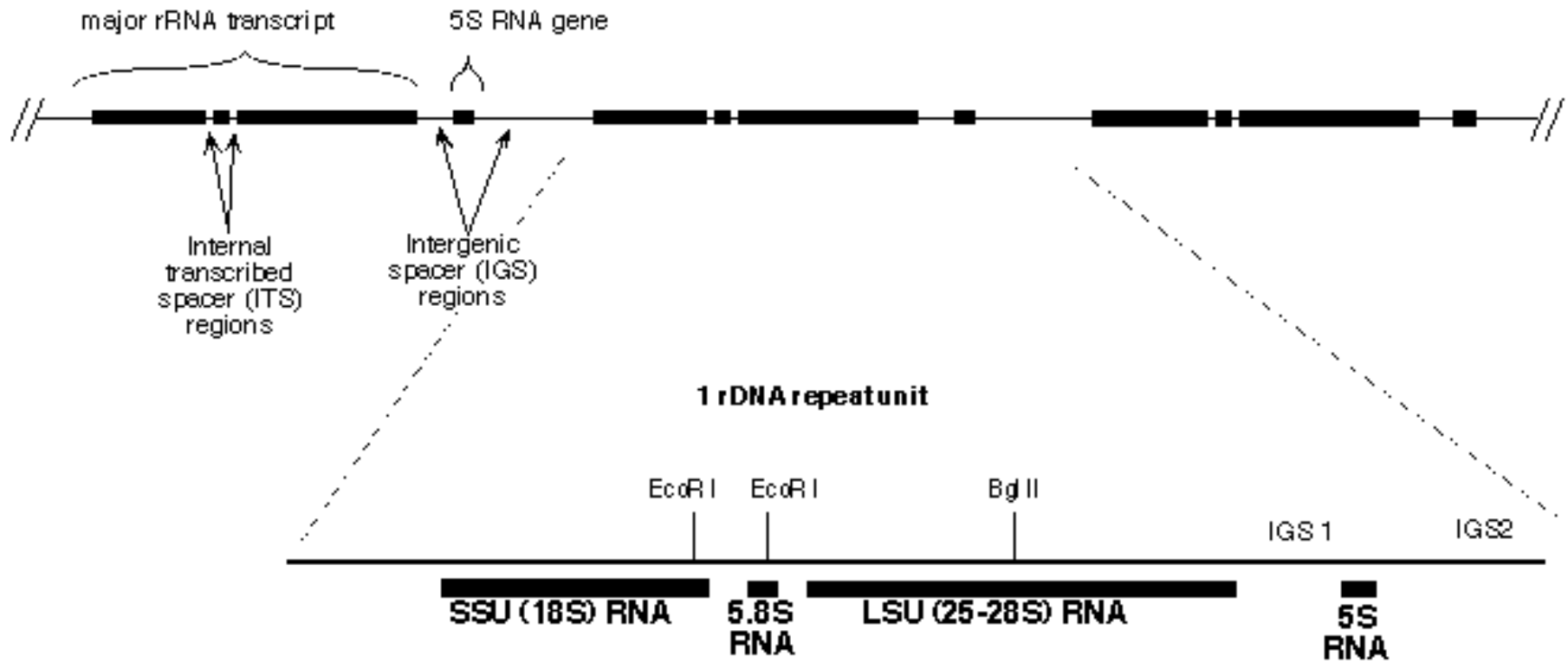
*Aspergillus
nidulans*



Moleculair Mycologische Identificatie “is” al: het unique ribosoom



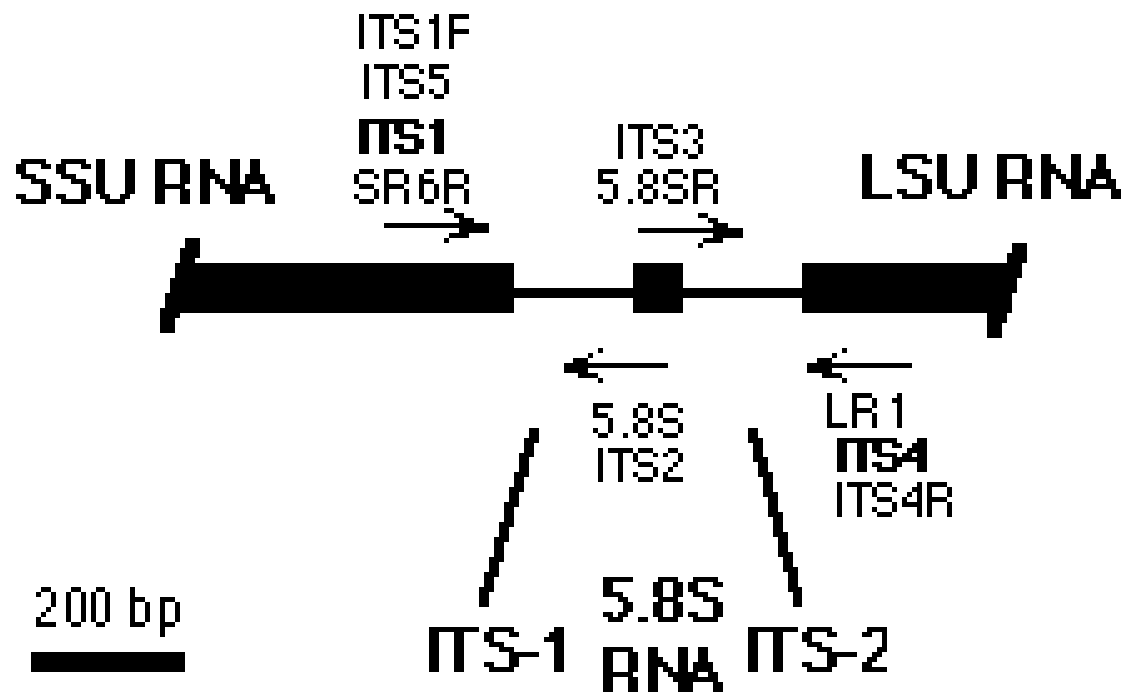
Fungal ribosomal gene clusters



Internal transcribed spacer: Pan Fungal PCR



ITS primers



Primers for routine sequencing are shown in bold

Ribosomaal DNA sequentie analyse



```
> gb|EU664468.1 Aspergillus fumigatus strain 095609 18S ribosomal RNA gene, partial
sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial sequence
Length=556
```

```
Score = 1027 bits (556), Expect = 0.0
Identities = 556/556 (100%), Gaps = 0/556 (0%)
Strand=Plus/Plus
```

```
Query 1 CTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT 60
|||||
Sbjct 1 CTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT 60

Query 61 CCCACCCGTGTCTATCGTACCTTGTGCTTcgggcggggcccgccgttttcgacggccgcccgg 120
|||||
Sbjct 61 CCCACCCGTGTCTATCGTACCTTGTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCCGG 120

Query 121 ggaggccttgcgccccccggccccgcgccccgagAACACCCCAACATGAACGCTGTTCTGA 180
|||||
Sbjct 121 GGAGGCCTTGCGCCCCCGGGCCCGCCCGCCGAGAACCCCAACATGAACGCTGTTCTGA 180

Query 181 AAGTATGCAGTCTGAGTTGATTATCGTAATCAGTAAAACTTTCAACAACGGATCTCTTG 240
|||||
Sbjct 181 AAGTATGCAGTCTGAGTTGATTATCGTAATCAGTAAAACTTTCAACAACGGATCTCTTG 240

Query 241 GTTCCGGCATCGATGAAGAAGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC 300
|||||
Sbjct 241 GTTCCGGCATCGATGAAGAAGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC 300

Query 301 GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTG 360
|||||
Sbjct 301 GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTG 360

Query 361 TCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCC 420
|||||
Sbjct 361 TCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCC 420

Query 421 CGGGGGACGGGCCCCGAAAGGCAGCGGGCCACCGCGTCCGGTCTCGAGCGTATGGGGCT 480
|||||
Sbjct 421 CGGGGGACGGGCCCCGAAAGGCAGCGGGCCACCGCGTCCGGTCTCGAGCGTATGGGGCT 480

Query 481 TTGTCACCTGCTCTGTAGGCCCGGCCAAGCGCCAGCCGACACCCAACCTTATTTTCTAA 540
|||||
Sbjct 481 TTGTCACCTGCTCTGTAGGCCCGGCCAAGCGCCAGCCGACACCCAACCTTATTTTCTAA 540

Query 541 GGTTGACCTCGGATCA 556
|||||
Sbjct 541 GGTTGACCTCGGATCA 556
```

Internal transcribed spacer – Pan Fungal PCR Species identification



BLAST *Basic Local Alignment Search Tool* My NCBI [\[Sign In\]](#) [\[Register\]](#)

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▶ NCBI/BLAST/blastn/Formatting Results - 5PTTUH8D01R [Reformat these Results](#) [Edit and Resubmit](#) [Sign in above to save your search strategy]

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Link
EU664468.1	Aspergillus fumigatus strain 095609 18S ribosomal RNA gene, pa	1027	1027	100%	0.0	100%	
EU664467.1	Aspergillus fumigatus strain 095623 18S ribosomal RNA gene, pa	1014	1014	100%	0.0	99%	
AB369897.1	Aspergillus fumigatus genes for small subunit rRNA, ITS1, 5.8S r	1013	1013	99%	0.0	99%	
AB298709.1	Aspergillus fumigatus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS:	1013	1013	99%	0.0	99%	
EU664469.1	Aspergillus fumigatus strain 091701 18S ribosomal RNA gene, pa	1011	1011	98%	0.0	100%	
EF136363.1	Aspergillus fumigatus 18S ribosomal RNA gene, partial sequence	1011	1011	99%	0.0	99%	
EF495242.1	Aspergillus fumigatus strain Ppf10 18S ribosomal RNA gene, part	1011	1011	100%	0.0	99%	
AY373851.1	Aspergillus fumigatus strain SRRC 43 18S ribosomal RNA gene, r	1011	1011	99%	0.0	99%	
AY939790.1	Aspergillus fumigatus strain ATCC 9197 18S ribosomal RNA gene	1011	1011	99%	0.0	99%	
AY214447.1	Aspergillus fumigatus strain UWFP 500 18S ribosomal RNA gene,	1011	1011	99%	0.0	99%	
AY214446.1	Aspergillus fumigatus strain ATCC 16907 18S ribosomal RNA qer	1011	1011	99%	0.0	99%	
AF455542.1	Aspergillus fumigatus isolate wb161 small subunit ribosomal RNA	1009	1009	99%	0.0	99%	

Molecular Identification of Fungal Infections

A hand holding a petri dish with a yellowish fungal culture, overlaid with a DNA double helix and a background of genetic code.

**Do you have an
Unknown Microbe?**

**We developed
the technology
to identify it.**

Molecular Diagnosis of Fungal Infections



Genus/species specific

- 18S rDNA
- 28S rDNA
- ITS
- Mitochondrial cytochrome *b*
- CaMP65 gene (mannoprotein)
- Rnase P RNA gene

Pan-fungal

- 28S rDNA
- ITS

Molecular Diagnosis of Fungal Infections



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Vol. 47, No. 1

Development of an Oligonucleotide Array for Direct Detection of Fungi in Sputum Samples from Patients with Cystic Fibrosis^{∇†}

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Species-Specific Identification of a Wide Range of Clinically Relevant Fungal Pathogens by Use of Luminex xMAP Technology^{∇†}

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Molecular Diagnosis of Fungal Infections: No harmonisation and standardization



Clinical Updates



FUNGAL INFECTIONS

“The lack of specific and rapid diagnostic tests for fungal infections is one of the major impediments to successful management of infected patients.”

EAPCRI



PCR has been used as an aid in the diagnosis of invasive aspergillosis for almost 2 decades. A lack of standardization has limited both its acceptance as a diagnostic tool and multicenter clinical evaluations, preventing its inclusion in disease-defining criteria. In 2006, the European *Aspergillus* PCR Initiative was formed. The aim of the initiative was to provide optimal standardized protocols for the widespread clinical evaluation of the *Aspergillus* PCR to determine its diagnostic role and allow inclusion in disease diagnosis criteria.

Molecular Diagnosis of *Aspergillus* Infections Standardization

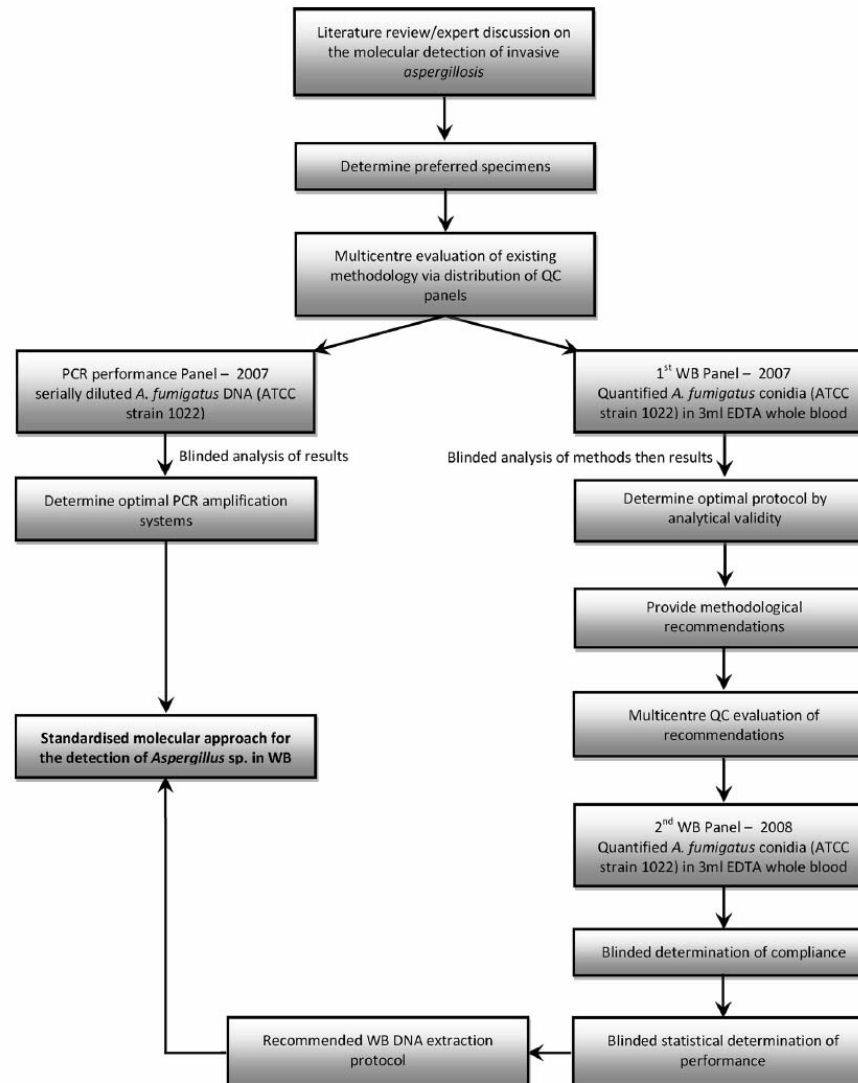


FIG. 1. Summary of the EAPCRI *Aspergillus* PCR standardization process.

Molecular Diagnosis of Aspergillus Infections harmonisation?



TABLE 1. Summary of methods used to test the 2008 EAPCRI WB panel^a

Center	Vol (ml) used	RCL	WCL	Fungal lysis	Method of fungal DNA purification	DNA strategy	Internal control PCR	No. of replicates	Compliant	PCR strategy
2	3	Yes	Yes	B. beating	QIAamp Blood kit (Qiagen)	DNA 1	No	3	No	PCR 1
3	3	Yes	No	B. beating	MagNA Pure LC Total NA kit (Roche)	DNA 2	Yes	3	Yes	PCR 2
4	1–2	Yes	No	Lyticase	Phenol-chloroform	DNA 3	Yes	1	No	PCR 3
5	3	Yes	Yes	Lyticase	DNAeasy Tissue (Qiagen)	DNA 4	No	3	No	PCR 4
6	3	Yes	No	B. beating	MagNA Pure LC DNA kit I (Roche)	DNA 5	No	3	No	PCR 5
7	3	Yes	No	B. beating	ZR Fungal/Bacterial DNA kit (Zymo research)	DNA 6	Yes	3	Yes	PCR 6
8	1.5	Yes	No	B. beating	SeptiFast kit (Roche)	DNA 7	Yes	3	No	PCR 7
9	3	Yes	Yes	B. beating	SeptiFast kit (Roche)	DNA 8	Yes	3	Yes	PCR 7
10	3	Yes	Yes	B. beating	MagNA Pure LC Total NA kit (Roche)	DNA 9	Yes	3	Yes	PCR 1
11	3	Yes	Yes	B. beating	Tissue kit, EZ1 (Qiagen)	DNA 10	Yes	3	Yes	PCR 8
13	3	Yes	Yes	B. beating	High Pure Template PCR kit (Roche)	DNA 11	Yes	3	Yes	PCR 1
14	3	Yes	Yes	B. beating	SeptiFast kit (Roche)	DNA 8	Yes	3	Yes	PCR 9
15	3	No	No	B. beating	QIAamp blood kit (Qiagen)	DNA 12	Yes	3	Yes	PCR 1
16 ^b	3	ND	ND	B. beating	QIAamp DNA minikit (Qiagen)	DNA 13	Yes	3	Yes	PCR 10
17	3	Yes	Yes	Lyticase	DNA Lego kit (Top Bio)	DNA 14	No	3	No	PCR 11
18	3	Yes	No	B. beating	MagNA Pure LC DNA kit I (Roche)	DNA 5	No	3	No	PCR 5
19	3	Yes	Yes	B. beating	GeneXpert (Cepheid)	DNA 15	Yes	3	Yes	PCR 12
20	3	Yes	Yes	B. beating	High Pure Template PCR kit (Roche)	DNA 11	Yes	1	No	PCR 2
21	2	Yes	No	B. beating	SeptiFast kit (Roche)	DNA 7	Yes	3	No	PCR 1
22	3	Yes	Yes	B. beating	High Pure Template PCR kit (Roche)	DNA 11	Yes	3	Yes	PCR 13
23	3	Yes	Yes	B. beating	bioMérieux MiniMag ^c	DNA 16	Yes	3	Yes	PCR 1

Molecular Diagnosis of Aspergillus Infections harmonisation?



TABLE 5. Positivity rates for the two EAPCRI WB panels and PCR performance panel^a

Fungal load (total no. of conidia)	Potential total no. of rRNA copies ^b	No. of copies/ μl^c	Positivity rate (% [no. of PCR tests])		
			PCR panel ($n = 52$)	2007 panel ($n = 61$)	2008 panel ($n = 59$)
>10,000	5.4×10^5	2.7×10^5	94.2 (49)	NT	NT
		2.7×10^4	94.2 (49)	NT	NT
		5.4×10^3	NT	77.0 (47)	NT
		2.7×10^3	92.3 (48)	NT	NT
		5.4×10^2	NT	75.4 (46)	98.3 (58)
1,000	5.4×10^4	5.4×10^2	NT	75.4 (46)	98.3 (58)
500	2.7×10^4	2.7×10^2	94.2 (49)	57.4 (35)	98.3 (58)
100	5.4×10^3	5.4×10^1	NT	34.4 (21)	78.0 (46)
75	4.1×10^3	4.1×10^1	NT	36.1 (22)	67.8 (40)
50	2.7×10^3	2.7×10^1	86.5 (45)	37.7 (23)	74.6 (44)
20–25	$1.1\text{--}1.4 \times 10^3$	$1.1\text{--}1.4 \times 10^1$	NT	29.5 (18)	62.7 (37)
10	5.4×10^2	5.4	NT	27.9(17)	49.2 (29)
		2.7	40.4 (21)	NT	NT
0 ^d	0	0	2.9 (3) ^e	4.9 (6) ^f	11.3 (20) ^g

Molecular Diagnosis of Fungal Infections



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Vol. 48, No. 4

Aspergillus PCR: One Step Closer to Standardization^{∇†}

P. Lewis White,^{1*} Stéphane Bretagne,² Lena Klingspor,³ Willem J. G. Melchers,⁴ Elaine McCulloch,⁵
Bettina Schulz,⁶ Niklas Finnstrom,⁷ Carlo Mengoli,⁸ Rosemary A. Barnes,⁹ J. Peter Donnelly,⁴
and Juergen Loeffler¹⁰ on behalf of the European *Aspergillus* PCR Initiative

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<http://www.eapcri.eu/>

Molecular Diagnosis of Fungal Infections



The MDx Industry in fungal Infections:

Myconostica

D3 Pan-Fungal

Roche MDx Septifast

Philips MDx

Beckman Coulter fungal pathogens

Etc etc.....

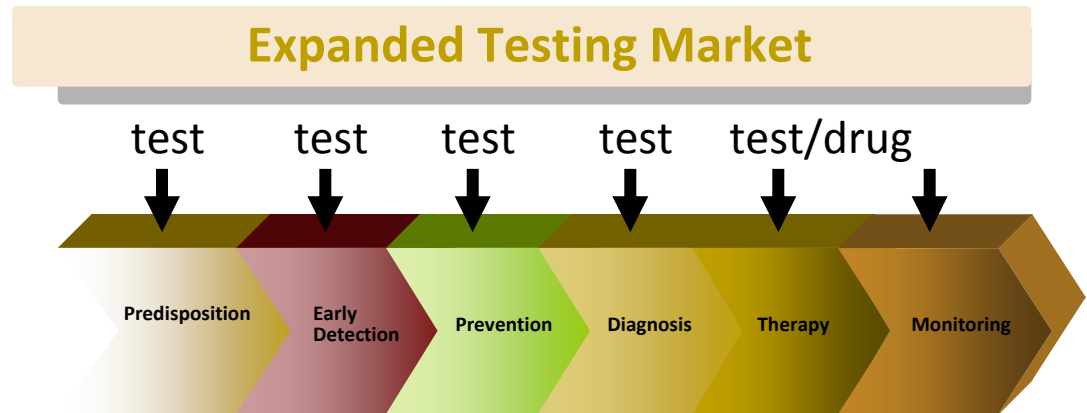
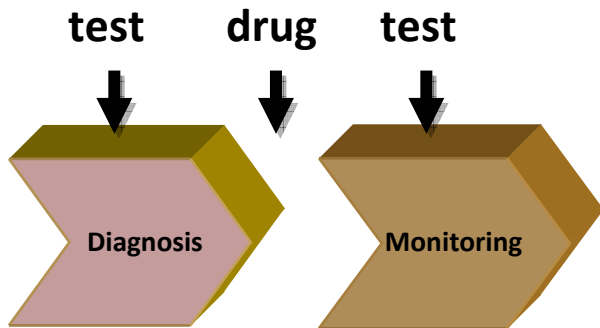
Moleculaire Diagnostiek van Schimmels: Waarom bent u nog niet gestart?



Yesterday

Today & Tomorrow

Traditional Diagnostics



Providing Health Information:

Disease Risk

Drug Choice

Health Status

Therapy Efficacy